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Roles of age, gender and gonadal status in opioid antinociception and ingestive behavior in rats

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ROLES OF AGE, GENDER AND GONADAL STATUS IN OPIOID ANTINOCICEPTION AND INGESTIVE BEHAVIOR IN RATS

by

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Abstract

Roles of Age, Gender and Gonadal Status
In Opioid Antinociception and Ingestive Behavior in Rats

By Anita K. Islam
Mentor: Dr. Richard J. Bodnar

Organismic variables appear to modulate various forms of opioid-mediated behaviors. For instance, morphine antinociception is sensitive to gender and aging differences in that its magnitude is greater in male than in female rats, and is lower in older than in younger female rats. Less is known about alterations in opioid-mediated food intake as a function of age and gender. However, a few studies have observed greater sensitivity of opioid modulation of feeding in male than in female animals, and reduced effectiveness of naloxone inhibition of opioid-feeding with aging in males. The present study systematically examined the roles of age, gender, and gonadal status in morphine antinociception on the tail-flick test, and in two forms of opioid-mediated food intake; deprivation-induced and high-fat feeding. Rats were anesthetized and received either sham surgery or gonadectomy at 3 months of age, and tested in separate groups (n=8-10) at either 6, 12, 18, or 24 mo. of age. Anti- nociceptive efficacy of morphine was assessed at weekly intervals across a dose range of morphine (1-10mg/kg, ip) over a 2 h time course. Following these pain tests the same groups of rats,

at this point, 8, 14, 20+ mo. of age, and an additional 4 mo. group, were evaluated at weekly intervals across a dose range of naloxone (.25-5 mg/kg) over a 2 hr. time course, for their sensitivity to naloxone inhibition of food-deprivation-induced food intake, followed by naloxone inhibition of high-fat intake.

Gender differences emerged with age. Whereas intact males and females displayed similar morphine dose-response curves at 6 mo. of age, aging increased antinociceptive sensitivity to morphine in males and decreased sensitivity in females. Moreover, ovariectomy eliminated the age-related decline in morphine responsivity in females, while castration produced little effect. A dissociation was observed between deprivation-induced intake and high-fat intake in terms of age-related, gender-related, and gonadectomy-related alterations in naloxone hypophagia. High-fat intake was most sensitive to organismic factors in their effects on naloxone hypophagia. Aging increased naloxone inhibition of high-fat intake in intact females, but decreased naloxone inhi- bition in intact males and ovariectomized females. Conversely, aging enhanced naloxone's effects on deprivation-induced intake in males and ovariectomized females. At the highest dose, young intact males were more sensitive to naloxone's inhibitory effects on deprivation intake than were young females.

These results indicate that age and gender interact to alter the efficacy of morphine antinociception and opioid-modulation of food intake in rats, and that ovariectomy but not castration modulates responses of adult rats across the aging process. The ecological relevance of organismic differences in opioid-mediated behavioral process is reviewed in the general discussion.

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My love and gratitude also go to my mother and father for always being there. Without their devoted support and caring, this endeavor would surely not have been possible.

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Glossary of Abbreviated Terms

B-FNA:	beta-funaltrexamine, mu opioid antagonist
CCK:	cholecystokinin
DADL:	delta/mu_1 opioid agonist
DALCE:	$delta_1$ opioid antagonist
DAMGO:	mu opioid agonist
DLF:	dorsal lateral funiculus of the spinal cord
DPDPE:	$delta_1$ opioid agonist
DSLET:	delta/mu_1 opioid agonist
EKC:	ethylketocyclazocine, kappa/mu_1 opioid agonist
ICI174864:	delta opioid antagonist
LC:	locus coeruleus
NalBzOH:	naloxone benzoylhydrazone, $kappa_3$ opioid agonist
Nor-BNI:	nor-binaltorphamine, kappa opioid antagonist
NRM:	nucleus raphe magnus
NRGC:	nucleus reticularis gigantocellularis
NTS:	nucleus tractus solitarius
PAG:	periaqueductal gray
POMC:	proopiomelanocortin
U50488H:	kappa opioid agonist

SPECIFIC AIMS:

The endogenous opioid system has been implicated in a variety of physiological and regulatory responses, with pain inhibition being among the most important opioid function and the most-studied in terms of analyses of the neuroanatomical, neurophysiological, and neurochemical substrates. Antinociception has been observed following exogenous administration of opiates (e.g., morphine) and following opioid receptor subtype agonists selective for mu, delta and kappa receptors. Whereas the general opiate receptor antagonist, naloxone, antagonizes opiate and opioid antinociception, it typically fails to produce decreases in nociceptive thresholds. Thus, examination of variables affecting opioid modulation of antinociceptive processes appears best studied by administration of opioid agonists which activate pain-inhibitory systems.

Evidence has also accumulated in support of a role for endogenous opioid peptides in ingestive behavior. Whereas opiates and opioid receptor subtype agonists stimulate food intake, both general and selective opioid receptor subtype antagonists inhibit different forms of food and water intake. Physiologically high doses of opioid agonists are typically needed to stimulate ingestion, and it is difficult to observe consistent ingestive effects in those situations in which large amounts of food already are being consumed (e.g., deprivation intake, palatable intake). Thus,

examination of variables affecting opioid modulation of ingestive processes appears best studied by administration of opioid antagonists, which inhibit different forms of ingestion.

Organismic factors as age, gender and gonadal status have been shown to affect both opioid-mediated antinociception and opioid-mediated ingestion. Aging has been implicated in opioid antinociception, decreasing morphine's pain-inhibitory effects in female rats, an effect consistent with decreased opioid levels and receptor binding characteristics in older rats. Gender has also been implicated in opioid antinociception, with male animals typically displaying significantly greater magnitudes of antinociceptive effects than female rats. These gender differences in opioid antinociception are in turn differentially modulated by adult gonadectomy and steroid replacement therapy.

Analysis of opioid-mediated ingestive responses as functions of age, gender, and gonadectomy has shown that hyperphagia induced by opioid receptor agonists in young rodents fails to occur in senescent animals. Older male rats are significantly less sensitive to naloxone's anorectic effects upon nocturnal food intake than younger male rats. However, the effects of gender and gonadectomy upon opioid-mediated food intake have been largely unexplored.

It should be noted that gender and gonadectomy effects

upon opioid-mediated behaviors are typically limited to young adult rats, and most studies of aging have typically employed animals of only one gender. Little is known about potential interactions among aging, gender, and gonadal status effects upon opioid-mediated antinociceptive and ingestive responses. Such an interaction was observed for nonopioid antinociception following continuous cold-water swims, which was potentiated in older male rats (Hamm, Knisely and Watson, 1986), and decreased in older female rats (Kramer and Bodnar, 1986). These potentially important interactions among organismic variables have not been systematically examined for opioid-mediated antinociception or ingestion.

Therefore, the present study examined the interactive effects of these three organismic variables on the two forms of opioid-mediated behavior. First, morphine antinociception was examined by assessing changes in tail-flick latencies in rats as a function of age (6,12,18, and 24 months), gender (male and female) and gonadal status (intact, gonadectomized) across a dose range of morphine (1, 2.5, 5 and 10 mg/kg) and over a time course (30-120 min). Second, this study examined the influence of age, gender, and gonadal status on the hypophagic effects of naloxone (0.25-5 mg/kg) over a 2 h time course of intake. Since the analysis of opioid receptor subtype antagonists has revealed different receptor mediation as a function of ingestive

situation, two feeding paradigms were employed. The first paradigm involved food deprivation, which represents a regulatory challenge, and the second involved high-fat intake, which represents a preference in the absence of a need state.

The following background topics are reviewed to provide an underlying conceptual basis for this research: 1) characterization of the endogenous opioid peptides and receptors, 2) aging, gender, and gonadectomy effects upon opioid peptides and receptors, 3) description of opioid pain-inhibitory systems, 4) opioid agonist and antagonist effects upon ingestion, 5) aging, gender and gonadectomy effects upon opioid-mediated antinociception, 6) aging, gender and gonadectomy effects upon opioid-mediated ingestion, and 7) a rationale for the present studies.

1. Opioid Peptides and Opioid Receptor Subtypes:

A. Opioid Peptides: Opioid peptides are derived from one of three distinct precursor molecules: i) proopiomelanocortin (POMC), ii) proenkephalin, and iii) prodynorphin, all sharing a common opiate-active core (Tyr-Gly-Gly-Phe) (see review: Sherman, Akil and Watson, 1989).

i) POMC: The carboxyl terminus of POMC contains the opioid peptide, beta-endorphin and its precursor beta-lipotropin (B-LPH: Eipper and Mains, 1978; Mains, Eipper and Ling, 1977). In the brain, POMC-derived peptides are located in two distinct cell groups in the brain: a) the arcuate and

periarcuate nuclei of the hypothalamus and b) the caudal part of the nucleus tractus solitarius (NTS) (Khachaturian, Lewis, Schafer and Watson, 1985; Watson, Akil, Richard and Barchas, 1978; Bloom, Rossier, Battenberg, Bayon, French, Henricksen, Siggins, Segal, Browne, Ling and Guillemin, 1978). POMC cells in the arcuate nucleus innervate the preoptic area, the amygdala, septum, bed nucleus of stria terminalis, medial-basal hypothalamus, temporal cortex, periventricular thalamus, the periaqueductal gray (PAG), nucleus raphe magnus (NRM), nucleus reticularis gigantocellularis (NRGC), nucleus tractus solitarius (NTS), nuclei reticularis lateralis, parabrachialis and ambiguus, and the dorsal motor nucleus of the vagus nerve (Guillemin, Ling and Burgus, 1976; Khachaturian et al., 1985).

ii) Proenkephalin: The proenkephalin precursor includes leu-enkephalin, met-enkephalin, met-enkephalin-Arg-Phe and met-enkephalin-Arg-Gly-Leu (Kimura, Lewis, Stern, Rossier, Stein and Udenfriend 1980; Comb, Herbert and Crea, 1982). Enkephalinergic perikarya are found as interneurons at most levels of the neuraxis (Hökfelt, Elde, Johansson, Terenuis and Stein, 1977; Khachaturian, Lewis, Hollt and Watson, 1983; Sar, Stumpf, Miller Chang and Cuatrecasas, 1978).

iii) Prodynorphin: The prodynorphin precursor is cleaved to produce three leu-enkephalin-containing peptides: alpha and beta-neo-endorphin, dynorphin A and dynorphin B (Goldstein, Fischli, Lowney, Hunkapiller and Hood, 1981;

Kangawa, Minamino, Chino, Sakakibara and Matsuo, 1981; Seizinger, Hollt and Herz, 1981; Suda, Tozawa, Tachibana, Demura and Shizume, 1982). Immuno-reactive dynorphin perikarya are found in telencephalic, diencephalic, mesencephalic and brainstem sites as well as in the dorsal horn of the spinal cord (Khachaturian et al., 1985).

B. Opioid Receptor Subtypes: Following the discovery of the opiate receptor in 1973 (Pert and Snyder, 1973; Simon, Hiller and Edelman, 1973; Terenius, 1973), multiple forms of the opiate receptor were subsequently described: mu, kappa and delta (Martin et al., 1976; Lord et al., 1977). Opioid receptor subtypes are heterogeneously distributed throughout the neuroaxis (Goodman, Snyder, Kuhar and Young, 1980; Mansour, Khachaturian, Lewis, Akil and Watson, 1986, 1987). Naltrexone and naloxone are general opioid receptor antagonists (Zukin and Zukin, 1981).

i) Mu receptors: Mu receptors are widely distributed throughout the forebrain, midbrain and hindbrain (Mansour, Khachaturian, Lewis, Akil and Watson, 1988). Selective mu agonists (D-Ala^2, met-Phe^4, Gly (ol) ^{5}-enkephalin, DAMGO: Handa, Lane, Lord, Morgan, Rance and Smith, 1981), and antagonists (beta-funaltrexamine, ß-FNA: Portoghese, Larson, Sayre, Fries, and Takemori, 1980; Takemori, Larson and Portoghese, 1981) have been developed. The mu receptor has been further subcharacterized into mu_1 and mu_2 subtypes, based upon pharmacological and biochemical assays (e.g.,

review by Pasternak and Wood, 1986). The mu_1 binding site is a common high affinity site, binding morphine, ethylketocyclazocine, enkephalin peptides and beta-endorphin with equally high affinity. The mu_2 site selectively binds morphine-like compounds more potently than enkephalins (Wolozin and Pasternak, 1981; Pasternak and Wood, 1986; Clark, Houghten and Pasternak, 1988). Autoradiographic studies demonstrate that mu_1 and mu_2 binding sites have similar, though not identical distributions (Goodman and Pasternak, 1985; Maskowitz and Goodman, 1985a,b). Mu_1 binding is higher in the frontal cortex, striatum, ventral pallidum, nucleus accumbens, medial thalamus, interpeduncular nucleus, median raphe and PAG. Naloxonazine, an irreversible mu_1 antagonist (Hahn, Carroll-Buatti and Pasternak, 1982) has been used pharmacologically to discern mu_1 actions.

ii) Delta receptors: Delta receptors are most dense in the forebrain with less binding observed in the diencephalon and brainstem (Mansour et al., 1988). General delta agonists, D-Ser^2,Leu^5-enkephalin-Thr^6 (DSLET) and D-Ala^2,D-Leu^5-enkephalin (DADL) (Lord et al., 1977; Mosberg, Hurst, Hruby, Gee, Yamamura, Galligan and Burks, 1983a) and antagonists, ICI 174864 (Cotton, Giles, Miller, Shaw and Timms, 1984) have been developed. More recent data indicate the existence of $delta_1$ and $delta_2$ subtypes (Negri, Potenza, Corsi, and Melchirri, 1991). The $delta_1$ receptor subtype has

been characterized by the agonist actions of D-Pen^2, D-Pen^5-enkephalin (DPDPE: Mosberg, Hurst, Gee, Yamamura, Galligan, and Burks, 1983b) and the long-term antagonist actions of D-Ala^2, Leu^5, Cys^6-enkephalin (DALCE: Bowen, Hellewell, Kelemen, Huey, and Steward, 1987; Jiang, Bowen, Mosberg, Rothman and Porreca, 1990). The delta$_2$ receptor subtype has been characterized by the agonist actions of D-Ala^2-deltorphan II (Jiang, Heyman, Sheldon, Koslo and Porreca, 1990) and the antagonist actions of naltrindole (Portoghese, Sultana, Nagase and Takemori, 1988a; Portghese, Sultana and Takemori, 1988b; Sofuoglu, Portoghese and Takemori, 1991).

iii) Kappa receptors: The distribution of kappa opioid receptors include dense binding in the telencephalon, diencephalon, neural lobe of the pituitary and NTS with moderate binding in the brainstem and the dorsal horn of the spinal cord (Mansour et al., 1988). Prototypical kappa agonists (U50,488H: VanVoigtlander, Lahti and Ludens, 1983) and antagonists (nor-binaltorphamine, NOR-BNI: Portoghese, Lipkowski and Takemori, 1987; Takemori, Ho, Naeseth and Portoghese, 1988) have been developed. The kappa receptor has been recently subclassified into K_1, K_2, and K_3 binding sites (Zukin, Eghbali, Olive, Unterwald and Tempel, 1988; Rothman, Bykov, deCosta, Jacobson, Rice and Brady, 1990). These data indicate that U50,488H and NOR-BNI are respective k_1 agonists and antagonists, and the K_3 site has been identified using naloxone benzolhydrazone (NalBzoH) (Clark,

Liu, Price, Hersh, Edelson and Pasternak, 1989; Gistrak, Paul, Hahn and Pasternak, 1989; Paul, Levison, Howard, Pack, Hahn and Pasternak, 1990).

2. Organismic Variables and the Opioid System.

A. Aging and the Opioid System. Aging is associated with generalized decreases in catecholamine levels and increases in serotonin levels, suggesting that aging produces relative imbalances in central neurotransmitters (Barden, Dupont, Labrie, Merand, Rouleau, Vaudry, Boissier, 1981). Age-related changes in opioid peptide levels are variable in pituitary and brain for immunoreactive beta-endorphin and enkephalin. Significant age-related elevations in immunoreactive beta-endorphin occur in plasma and all lobes of the pituitary gland. Increased *in vitro* release of immunoreactive beta-endorphin has also been observed from all lobes of the pituitary in older animals (Forman, Sonntag, Meites, 1981; Forman, Marquis, Stevens, 1985; Missale, Govoni, Croce, Bosio, Spano, Trabucchi, 1983). In contrast, others found that pituitary beta-endorphin function was either unchanged (Gambert, Garthwaite, Pontzer, Hagen, 1980) or decreased (Rogers, Shoemaker, Morgan, Finch, 1985) in aged animals. Such variability may be due to animal strain, gender, definitions of aging, high assay variability, and lack of control for beta-endorphin-secreting pituitary tumors (Rogers et al., 1985). Age-related changes in central beta-endorphin levels show more

consistent declines, e.g., in the hypothalamic arcuate nucleus (55% decline), and in beta-endorphin's major projection areas (50% decline) including the periaqueductal gray and striatum (Barden et al., 1981; Gambert et al., 1980; Rogers et al., 1985). The age-related reductions in beta-endorphin content correlate with marked age-related loss in number of beta-endorphinergic neurons in the arcuate nuclei of female mice (Miller, Joshi, Billiar, Nelson, 1991). POMC mRNA levels are also reduced in aged mice hypothalamus (Nelson, Bender, Schacter, 1988).

Other opioid peptides are also altered during aging. Aging increases dynorphin-like immunoreactivity in the frontal cortex (Jiang, Owyang, Hong, Gallagher, 1989), but produces both increases and decreases in met-enkephalin. Whereas large age-related increases in immunoreactive met-enkephalin have been observed in the anterior pituitary and hypothalamus of rats (Kumar, Chen, Huang,1980; Steger, Sonntag, Van Vugt, Forman, Neites), decreased immunoreactive met- and leu-enkephalin content in the hypothalamus of aged rats has also been observed (Dupont, Savard, Merand, Labrie, Boissier, 1981). Strain differences have been reported, with older mice showing slight elevations of hypothalamic met-enkephalin, but significant losses in striatal and cortical enkephalin (Rogers et al., 1985).

Age-related analysis of whole-brain and regional opioid receptor assays showed selective decreases in ^{3}H-naloxone

binding in the spinal cord, midbrain, striatum, olfactory tubercle and nucleus accumbens, but not in the hindbrain, diencephalon or hippocampus (Neisewander, Nonneman, McDougall, Bardo, 1989). Similar age-related decreases in ^{3}H-etorphine binding have been observed in the frontal poles, hippocampus, striatum and anterior cortex of male rats (Hess, Joseph, and Roth, 1981). Analyses of specific opioid receptor subtypes reveal decreases in ^{3}H-dihydromorphine (mu) binding in whole-brain, hypothalamus, frontal poles, anterior cortex and striatum of aged male rats, and the thalamus and midbrain of aged female rats (Messing, Vasquez, Speihler, Martinez, Jensen, Rigter, McGaugh 1980; Messing, Vasquez, Samaniego, Jensen, Martinex, McGaugh,1981; Piva, Maggi, Limonta, Dondi, Martini, 1987). The age-related declines in mu receptors occur for both high-affinity mu_1 and low-affinity mu_2 binding sites (Ueno, Liu, Ho, and Hoskins, 1988). Whereas an age-related increase in binding affinity has been reported (Messing et al., 1981), this was not subsequently replicated with other radioligands in other brain regions (Hess et al., 1981; Neiswander et al., 1985).

The affinity, but not density, of ^{3}H-ethylketocylazocine binding of kappa receptors is increased in aged mouse brain (Ueno et al., 1988). Kappa binding also increases in discrete brain assays, but only in the amygdala and thalamus (Maggi, Limonta, Dondi, Martini, Piva, 1989).

In contrast, an age-related decline in kappa receptors has been observed by autoradiography of guinea pig brain (Hiller, Fan, Simon, 1992). Finally, aging fails to alter the density and affinity of binding of delta receptors using ^{3}H-(D-Ser^{2}-Leu^{5})-enkephalin-Thr (Ueno et al., 1988). The ability of aged rats to maintain up-regulation in response to chronic naloxone treatment (Neisewander et al.,1989), together with age-related increases in mu and kappa binding affinity, may reflect compensatory responses to the age-related decline in opioid receptors. This suggests that the opioid system, in contrast to other receptor systems, maintains some plasticity with aging (DeBlasi, Cotecchia, Mennini, 1982; Maggi, Schmidt, Ghetti, Enna, 1978; Roth et al, 1984; Govoni, Memor, Saiani, Spano, Trabucchi, 1980).

B. Gender Differences and the Opioid System: In some studies, male rats were found to display higher levels, relative to females, of beta-endorphin in the pituitary and midbrain (Lee, Panerai, Ballabarba, Friesen, 1980; Mueller, 1980), of dynorphin in the pituitary (Molineaux, Hassen, Rosenberg, Cox, 1986), and of met-enkephalin in the pituitary (Hong, Yoshikawa, Lamartinere, 1982). In contrast, others have found that antisera directed against either beta-endorphin or dynorphin fails to display gender-sensitive immunoreactivity (Simerly, McCall, Watson, 1988), and that met-enkephalin immunoreactivity is increased in the periventricular hypothalamus of female rats (Watson,

Hoffman, Wiegand, 1986). Also, in one study, antisera directed against leu-enkephalin produced greater immunoreactivity in female than in male rats, although more selective antisera (which does not cross react with dynorphin) produced denser immunoreactivity in males (Simerly et al., 1988).

Estrous cycling produces differential changes in hypothalamic beta-endorphin with increases in the arcuate nucleus and median eminence during proestrous and decreases in the preoptic suprachiasmatic region during diestrous (Knuth et al., 1983). Administration of estradiol benzoate lowers baseline plasma and pituitary levels of beta-endorphin in intact females, as well as attenuating the stress-induced release of beta-endorphin (Mueller, 1980).

Both males and females display age-related declines in the number of mu-opioid receptors, albeit in different ways. The differential densities of high- and low-affinity binding sites in thalamus, midbrain and cortex of female rats are replaced by a steady, intermediate level of binding (Messing et al., 1980b). In contrast, although male rats display age-related declines in the number of mu-opiate receptors in frontal cortex, the affinity shows an age-related increase (Messing et al., 1980a).

C. Gonadectomy and the Opioid System: In male rats, castration reduces central met-enkephalin (Hong et al., 1982; Dupont et al., 1980) and eliminates the gender

difference in mesencephalic beta-endorphin levels (Lee et al., 1980). The latter effect is reinstated by treatment of the castrated rats with testosterone propionate (Forman et al., 1985). In female rats, ovariectomy eliminates the gender-sensitive increases in leu- and met-enkephalin immunoreactivity (Simerly et al., 1988; Watson et al., 1986). Additionally, ovariectomy alters circulating levels of both met-enkephalin (Dupont et al., 1985; Hong et al., 1982) and dynorphin A and B (Molineaux et al., 1986).

3. Endogenous Opioid Antinociceptive System:

The study of how supraspinal loci sensitive to opioid antinociception interact with other supraspinal and spinal loci sensitive to opioid antinociception has involved the analysis of neuroanatomical, neurophysiological and neurochemical substrates. Fields and Basbaum (1978) initially described a nociceptive modulatory pathway originating in the midbrain periaqueductal gray (PAG) and projecting to the substantia gelatinosa of the spinal cord via an excitatory link with serotonergic neurons in the medullary nucleus raphe magnus (NRM). Basbaum and Fields (1984) subsequently proposed a role for reticular structures lateral to the PAG and NRM, which receive projections from the PAG and also project to the spinal cord, as being important in this pain inhibitory system. These include the locus coeruleus (LC), the nucleus reticularis gigantocellularis (NRGC), reticularis magnocellularis

gigantocellularis, nuclei raphe pallidus, and paragigantocellularis lateralis and ventralis (Beitz, Mullett and Weiner, 1983; Mantyh, 1983). Further, reciprocal connections between the LC and the medial medulla exist, suggesting that spinal noradrenergic projections are also important in pain inhibition (Moore and Bloom, 1979; Ennis and Aston-Jones, 1987; Clark and Proudfit, 1991; Nygren, Olson and Sieger, 1977).

Electrophysiological studies have further implicated the activation of the rostral ventromedial medulla (RVM), which includes the NRM and NRGC, in the mediation of opioid antinociception elicited from the PAG (Lovick, West and Wolstencroft, 1978; Pomeroy and Behbehani, 1979; Mohrland and Gebhart, 1980a; Sandkuhler and Gebhart, 1984; Zorman, Hentall, Adams and Fields, 1981). Recently, Fields and colleagues (1991) have introduced a neurophysiological classification system involving cells in the RVM which respond differentially to nociceptive inputs by either increasing ("on-cells") or decreasing ("off-cells") their activity. Off-cells are activated by morphine and have a pattern of discharge consistent with a role of RVM output pain inhibitory neuron, whereas on-cells are suppressed by morphine and have a discharge pattern suggestive of facilitation of nociception (Fields, Bry, Hentall and Zorman, 1983; Vanegas, Barbaro and Fields, 1984; Barbaro, Heinricher and Fields, 1986; Cheng, Fields and Heinricher,

1986; Fields, Barbaro and Heinricher, 1988; Barbaro, Heinricher and Fields, 1989; Fields, Heinricher and Mason, 1991).

Small amounts of morphine or endogenous opioids injected directly into various regions of the PAG of rats, cats, and monkeys produces strong antinociceptive effects (Malick and Goldstein, 1977; Pert and Yaksh, 1974; Tsuo and Jang, 1964; Yaksh, Yeung and Rudy, 1976, Yaksh and Rudy, 1978a). Moreover, opiate and opioid antinociception in the PAG is reversed by the opiate receptor antagonist naloxone. Subsequent mapping studies indicated that antinociception can also be elicited following microinjections of morphine into either the NRM, NRGC and nuclei reticularis paragigantocellularis and reticularis paragigantocellularis lateralis of the RVM (Takagi, Satoh, Akaike, Ahibata and Kuraisi, 1977; Akaike, Shibata, Satoh and Takagi, 1978; Azami, Llewelyn and Roberts, 1982; Dickenson, Oliveras and Besson, 1979; Levy and Proudfit, 1979; Vasko, Pang and Vogt, 1984) as well as following intrathecal administration into the spinal cord (see review: Yaksh, 1981). These findings suggest that the aforementioned brain structures and pathways underly morphine and opioid antinociception. The following two sections review opioid modulation of spinal and supraspinal antinociception.

A. Spinal System: Intrathecal administration of opiates and opioid analogues produce a dose-dependent

antinociception which can be blocked by naloxone (Yaksh and Rudy, 1978b; Yaksh, 1981). This suggests that the antinociception is mediated by the direct action of opiates on spinal cord opioid receptors. The modulation of spinal opioid antinociception has been delineated with the development of selective agonists and antagonists for specific opiate receptor subtypes. These studies have found that intrathecal administration of mu-selective agonists elicits antinociception which is blocked by beta-funaltrexamine, but not by naloxonazine, indicating a mu_2 mechanism of action (Paul, Bodnar, Gistrak and Pasternak, 1989). Delta and kappa agonists produce a predominantly spinal antinociception which is blocked by selective antagonists for these receptors (Yaksh, 1984a,b; Porreca, Mosberg, Hurst, Hruby and Burks, 1984; Porreca, Heyman, Mosberg, Omnaas and Vaught, 1987; Wuster, Schulz and Herz, 1980; Heyman, Mulvaney, Mosberg and Porreca, 1987, Heyman, Vaught, Raffa and Porreca, 1988). Thus, mu_2, delta and kappa receptors have been implicated in spinally mediated opioid antinociception.

B. Supraspinal Antinociception: With the recent development of selective agonists and antagonists for particular receptor subtypes, the involvement of specific opioid receptors in the mediation of supraspinal opioid antinociception has been investigated. This research has demonstrated that the mu_1 receptor plays an important role

in supraspinal opioid antinociception. Microinjections of morphine, DAMGO (mu) and DSLET (delta, mu_1) into the PAG, LC, NRM and NRGC elicits antinociception which can be blocked by naloxonazine (Bodnar, Williams, Lee and Pasternak, 1988). The results of some investigations support a supraspinal role for delta receptors (Jensen and Yaksh, 1986a; Porreca et al., 1984, 1987). DPDPE, a $delta_1$-selective agonist produces antinociception following ventricular administration (Porreca et al., 1987). However, DPDPE fails to alter nociceptive responses following microinjection into the PAG, LC, NRM or NRGC (Bodnar et al., 1988). Kappa receptors do not appear to participate in supraspinal antinociception. Supraspinal administration of selective kappa receptor ligands fails to elicit antinociception (Friedman, Jen, Chang, Lee and Loh, 1981; Chavkin, James and Goldstein, 1982; Fang, Haws, Drasner, Williamson and Fields, 1989). Ethylketo-cyclazocine (EKC), one of the original prototypical kappa agonists, produces antinociception following intra- ventricular administration, but not following administration into either the PAG or LC. Indeed, EKC pretreatment into these structures interferes with the subsequent development of morphine antinociception. EKC's actions upon morphine antinociception is that of a partial mu_1 agonist because intraventricular EKC antinociception is blocked by naloxonazine and because simultaneous administration of EKC into the PAG and LC

produces a naloxonazine-sensitive antinociception (Bodnar, Paul and Pasternak, 1991).

Much evidence supports the participation of both serotonergic and noradrenergic systems in the mediation of supraspinal opioid antinociception. As mentioned above, the RVM receives input from the LC (Moore and Bloom, 1979). Clark and Proudfit (1991) have proposed that supraspinal noradrenergic modulation of spinal antinociception acts through a descending projection from the LC which is activated by RVM neurons. Studies focusing on the role of these two neurotransmitters in the mediation of supraspinal antinociception have revealed that systemic or intrathecal administration of serotonergic or noradrenergic antagonists attenuates the antinociception elicited by stimulation of the PAG, or systemic and intracerebral injections of morphine (Yaksh, 1979; Yaksh and Wilson, 1979; Tseng and Tang, 1989; Hammond and Yaksh, 1984; Proudfit and Hammond, 1981). Moreover, intrathecal administration of serotonergic and noradrenergic antagonists attenuates the antinociception elicited from stimulation or microinjection of morphine into the RVM (Jensen and Yaksh, 1986b; Hammond and Yaksh, 1984; Kuraishi, Harada, Satoh and Takagi, 1979; Barbaro, Hammond and Fields, 1985; Satoh, Akaike, Nakazawa and Takagi, 1980).

4. The Role of Opioids in Ingestive Behavior

In addition to the antinociceptive actions of opiates and opioid peptides, opioids modulate consummatory behavior.

Although chronic administration of morphine to morphine-dependent animals has been found to stimulate food intake (Martin, Eades, Thompson, Huppler, 1976) Holtzman's (1974) observation that naloxone produces equivalent decreases in deprivation-induced food intake as d-amphetamine, provides strong evidence for opioid involvement in ingestion. The effective use of agonists in opioid control of ingestion appears to depend upon feeding parameters and the previous drug history of the animal. Whereas morphine stimulates food intake under spontaneous conditions, it reduces food intake following deprivation (Sanger and McCarthy, 1980). That sedation might account for acute decreases in intake is supported by the enhancement of intake following repeated mu-receptor agonist administration (Woods and Leibowitz, 1985). Dose-dependent increases in food intake have been observed following administration of beta-endorphin, enkephalins and dynorphin (e.g., Grandison and Guidotti, 1977; Jaloweic, Panksepp, Zolovick and Najam, 1981; Morley and Levine, 1983a,b; Tepperman and Hirst, 1983). Food intake is also increased following selective opioid agonists for mu, delta and kappa receptors (e.g., Gosnell, Levine and Morley, 1986a,b; Morley, Levine, Grace and Kneip, 1982; Morley, Levine, Kneip, Grace, Zeugner and Shearman, 1985; Stanley, Lanthier and Leibowitz, 1989).

Opioid agonists appear to modulate macronutrient intake, with most studies suggesting a role in fat intake

(Marks-Kaufman, 1982; Marks-Kaufman and Kanarek, 1980, Shor-Posner, Azar, Filart, Tempel and Leibowitz, 1986; Romsos, Gosnell, Morley and Levine, 1987), and others suggesting a role for baseline diet preferences (Gosnell, Krahn and Majchrzak, 1990). These agonist studies led to the postulation that the kappa receptor is most involved in stimulation of food intake (Morley et al., 1985; Levine, Morley, Gosnell, Billington and Bartness, 1985; Cooper, Jackson and Kirkham, 1985).

The evaluation of general opioid antagonists in food intake revealed stereoselectivity for the (-) stereoisomer (Lowy, Starkey and Yim, 1981), and reliable inhibitory effects across a number of species and under a variety of intake situations (see review: Levine et al., 1985). General opioid receptor antagonism decreases both food and water intake in deprived rats (e.g., Brands, Thornhill, Hirst and Gowdey, 1979; Jaloweic et al., 1981; Lowy, Maickel and Yim, 1980), nocturnal intake (Brands et al., 1979; Jaloweic et al., 1981; Cooper, 1980), glucoprivic intake induced by 2-deoxy-D-glucose (2DG) and insulin (Lowy et al., 1980; Levine and Morley, 1981; Rowland and Bartness, 1982; Ostrowski, Rowland, Foley, Nelson and Reid, 1981), sweet and salty intake (Cooper, 1983; Lynch and Libby, 1983) and hyperphagia following tail-pinch stress (Lowy et al., 1980; Morley and Levine, 1980; Antelman and Rowland, 1981). Opioid antagonists also decrease intake in genetically-obese

(Margules, Moisset, Lewis, Shibuya and Pert, 1978; Recant, Voyles, Luciano and Pert, 1980; McLaughlin and Baile, 1984a,b) and diabetic (Levine, Morley, Brown and Handwerger, 1982) rats. In contrast, chronic opioid antagonist administration produces only transient decreases in intake and body weight (Brands et al., 1979; Jaloweic et al., 1981; Margules et al., 1979; Mann, Pasternak, Hahn, Curreiri, Lubin and Bodnar, 1988; Millan and Morris, 1988), although some irreversible antagonists have produced more long-lasting effects (Gosnell, Grace and Levine, 1987; Shaw, Mitch, Leander and Zimmerman, 1991; Levine, Grace and Billington, 1991).

The roles of different opioid receptor subtypes in ingestive behavior has been facilitated by the use of selective opioid antagonists. Mu receptors have been implicated in the modulation of free feeding since the mu-selective antagonist, B-FNA, and the mu_1 antagonist, naloxonazine, significantly reduce spontaneous intake (Arjune, Standifer, Pasternak and Bodnar, 1990; Mann, Arjune, Romero, Pasternak, Hahn and Bodnar, 1988; Simone, Bodnar, Goldman and Pasternak, 1985; Ukai and Holtzman, 1988). The kappa-selective antagonist, Nor-BNI, also reduces spontaneous nocturnal intake (Arjune and Bodnar, 1990). Whereas the general delta antagonist, ICI174864, reduces free feeding (Jackson and Sewell, 1985a), it does so at doses that produces motor dysfunction (Long, Petras and

Holaday, 1988). In contrast, the $delta_1$ antagonist, DALCE, fails to alter spontaneous intake (Arjune, Bowen and Bodnar, 1991). Evaluation of hyperphagia following food deprivation reveals significant inhibition by B-FNA (Arjune et al., 1990; Levine, Grace and Billington, 1991) and naloxonazine (Simone et al., 1985), marginal reductions by Nor-BNI (Levine et al., 1990) and lack of effects by $delta_1$ (Arjune et al., 1991) and $delta_2$ (Koch and Bodnar, 1993a) antagonism. Glucoprivic hyperphagia induced by 2DG is significantly reduced by mu and kappa antagonists, but not by mu_1 or delta antagonists (Arjune et al., 1990, 1991; Arjune and Bodnar, 1990; Koch and Bodnar, 1993a). Glucoprivic hyperphagia induced by insulin is significantly reduced by only the mu antagonist, B-FNA (Beczkowska and Bodnar, 1991). Hyperphagia following tail-pinch stress is reduced by mu and mu_1 antagonists, but not by other selective antagonists (Hawkins, Cubic, Baumeister and Bartin, 1992; Koch and Bodnar, 1993b). Hyperphagia induced by brief access to a high-fat diet is suppressed by kappa and mu (Arjune and Bodnar, 1990; Islam and Bodnar, 1990), but not by mu_1 or delta receptor antagonists (Islam and Bodnar, 1990; Arjune, Bowen and Bodnar, 1991). Whereas hyperdipsia induced by water deprivation or complex carbohydrates is reduced only by mu-selective antagonism, sucrose-induced hyperdipsia is reduced by kappa, and secondarily by mu antagonism (Beczkowska, Bowen and Bodnar,

1992; Beczkowska, Koch, Bostock, Leibowitz and Bodnar, 1993). Finally, saccharin intake is reduced only by $delta_2$ antagonism (Beczkowska et al., 1993). Thus, the intake situation (spontaneous versus challenge) and the taste situation (normal versus palatable) are important components in determining which opioid receptor subtype is activated in ingestive situations.

Two forms of food intake were examined in detail in the present study. Deprivation-induced food intake was examined because it is representative of a challenge form of intake. High-fat intake was examined because it is representative of a palatable form of intake. Both forms of intake produce reliable effects following repeated testing, and they are differentially sensitive to opioid receptor subtype antagonism, suggesting differential receptor involvement.

5. Pain-Inhibition and Organismic Variables.

A. Aging Effects. Both basal nociception and opioid antinociception are affected by aging. However, some inconsistencies between studies may be due to variable definitions of "aged" animals, use of bimodal (young versus old) dichotomies, variations in measures of nociception, and/or failure to consistently examine dose-response or time-response curves. Both reductions (Chan and Lai, 1982; Gordon, Scobie and Frankel, 1978) and increases (Hess, Joseph and Roth, 1981; Pare, 1969) in basal pain thresholds have been reported as a function of age. Early studies of

age-related changes in morphine antinociception include decreases (Chan and Lai, 1982; Kavaliers, Hirst and Teskey, 1983) and increases (Saunders, Paolino, Bousquet and Miya, 1974) in the magnitude of effect. A more recent study (Kramer and Bodnar, 1986a) evaluated dose-response and time-response curves for morphine antinociception on both spinally-mediated and supraspinally-mediated tests in four age cohorts of female rats. Aging produced significant decrements in the antinociceptive potency of morphine without significantly altering morphine hyperthermia. Similarly, age-related decrements were observed in the antinociceptive response following 2DG administration (Kramer, Sperber and Bodnar, 1985). Girardot and Holloway (1985) reported that the opioid-mediated antinociception following acute intermittent cold water swims (ICWS) failed to differ between mature and aged male rats. However, chronic exposure to ICWS produced marked impairments in the ability of older rats to either adapt to swim stress antinociception or to develop morphine cross-tolerance. Moreover, opioid antinociception elicited by inescapable shock delivered to the forepaws is reduced in aged male rats (Hamm and Knisely, 1985). It should be noted that not all opioid-mediated antinociceptive manipulations decline with age. Antinociception elicited by either beta-endorphin or abrupt food deprivation fail to produce significant age-related changes (Romero and Bodnar, 1987; Hamm and Knisely,

1986).

B. Gender Effects: Gender differences in the responsiveness of rats to nociceptive stimuli have been observed, with females displaying significantly lower shock thresholds than males (Marks and Hobbs, 1972; Pare, 1969). Detection, but not nociceptive thresholds, show minor variations across the female estrous cycle, with the greatest sensitivity occurring during periods of greatest estrogen activity (Drury and Gold, 1978). The magnitude of antinociception following systemic or intracerebroventricular morphine is significantly lower in female than in male rats (Badillo-Martinez, Kirchgessner, Butler and Bodnar, 1984; Chatterjee, Das, Banerjee and Ghosh, 1982; Kepler, Kest, Kiefel, Cooper and Bodnar, 1989). Morphine antinociception appears most effective during the late diestrous phase of the female estrous cycle (Banerjee, Chatterjee and Ghosh, 1983). Modulation of opioid antinociception by Neuropeptide FF (inhibition) and Neuropeptide FF antisera (facilitation) is significantly greater in male than in female deer mice (Kavaliers and Innes, 1992).

A similar pattern of gender differences has also been observed for antinociception resulting from exposure to opioid-mediated environmental stressors. The opioid-mediated antinociception following restraint is significantly higher in male than in female deer mice (Kavaliers and Innes,

1992). Adult female rats display significantly smaller magnitudes of antinociception following ICWS; this effect occurs independently of either basal pain threshold changes, estrous phase, or body weight (Romero and Bodnar, 1986). Indeed, the opioid mediation of ICWS antinociception appears to be dependent on gender, with naloxone attenuating ICWS antinociception in male but not female rats (Romero, Kepler and Bodnar, 1988).

However, not all forms of opioid antinociception are sensitive to gender differences. Whereas male rats display greater antinociception following central administration of the mu-selective agonist, DAMGO, gender differences are not observed for the antinociceptive dose-response functions produced by either DAMGO or the delta/mu_1-selective agonist, DSLET (Kepler et al., 1991).

Interactions between aging and gender have been observed for the nonopioid-mediated antinociceptive response following continuous cold-water swims (CCWS). The magnitude of CCWS antinociception is significantly greater in young male rats than in young female rats (Romero and Bodnar, 1986). Whereas aging produces significant reductions in the magnitude of CCWS antinociception in older female rats (Kramer and Bodnar, 1986b), it produces significant potentiations in the magnitude of CCWS antinociception in older male rats (Hamm and Knisely, 1986).

C. Gonadectomy Effects: The gender differences in basal

nociception are eliminated either when female rats are androgenized, or male rats are castrated (Beatty and Fessler, 1976). Gender differences in magnitude of morphine antinociception are diminished following castration in male rats or testosterone treatment in female rats (Chatterjee et al., 1982; Pinsky, Sheldon and LaBella, 1975). Both castration in male rats and ovariectomy in female rats significantly reduces the magnitude of both ICWS and CCWS antinociception relative to same-sex sham-operated animals (Romero, Kepler, Cooper, Komisaruk and Bodnar, 1987). Steroid replacement therapy with either testosterone proprionate or estradiol benzoate reverses these antinociceptive deficits in gonadectomized animals of both genders without altering antinociceptive magnitude in intact animals. Testosterone is more effective than estradiol in reinstating antinociception to the level of intact animals (Romero et al., 1988).

Again, not all forms of antinociception are affected by gonadectomy, including that produced by the mu-selective opioid agonist, DAMGO, the delta-selective opioid agonist, DSLET, the alpha$_2$ noradrenergic receptor agonist, clonidine, and the muscarinic agonist, pilocarpine (Kepler et al., 1991; Kiefel and Bodnar, 1992).

6. Organismic Variables and Opioid-Mediated Ingestion.

A. Aging Effects: There is a paucity of data concerning the effect of aging on the neurophysiological and chemical

mechanisms modulating ingestive behavior in animals and humans. Older animals eat a greater portion of their food during the daytime than do younger animals (Peng, Jiang and Hsio, 1980). Loss of appetite with resultant decreases in food intake and body weight frequently occurs in late life in humans and animals (see review: Morley and Silver, 1988). Numerous reasons for these changes have been postulated in humans, including psychological and social factors, increased susceptibility to disease, greater medication use, and reduced activity and basal oxygen consumption (resulting in lower metabolic needs). Also, abnormalities in food intake in the elderly may be due in part to a reduction in the hedonic properties of food resulting from diminution in the acuity of the chemical senses. Thus, addition of flavor enhancers to foods has been found to be effective in increasing appetite in anorexic elderly individuals, presumably by counteracting these chemosensory losses (e.g., Schiffman, 1979).

Efforts have also been make to link disturbances in food intake in the elderly to deficits in those neurochemical systems demonstrated to be involved in feeding in young animals (see Morley and Silver, 1988; and Blundell, 1988 for reviews). Aged male rats are 100 times less sensitive to the inhibitory effects of naloxone on nocturnal intake than younger rats, and are also less responsive to the stimulatory actions of the kappa opiate agonist,

butorphanol tartrate (Gosnell, Levine and Morley, 1983). Old mice also are less responsive to morphine's and ketocyclazocine's stimulatory effects upon ingestion, as well as to naloxone's inhibition of intake (Kavaliers and Hirst, 1985). These studies reveal age-related parallels between decreases in opioid feeding and changes in endogenous opioid functioning.

Older rodents show increased sensitivity to the satiating actions of cholecystokinin-octapeptide (CCK-8). Since CCK-8 has been postulated to be a physiological antagonist of opioids, the age-related decreases in opioid-mediated ingestion may partly be due to increased CCK activity in older animals (Morley and Silver, 1988). Decreased activity of the opioid feeding system with aging may also be related to zinc deficiencies present in elderly animals. Zinc has been postulated to play a role in the regulation of opioid receptors, and zinc-deficient animals have been observed to be unresponsive to the stimulatory ingestive effects of dynorphin (see review: Morley and Silver, 1988).

B. Gender, Gonadectomy and Opioid-Mediated Ingestion: A sexual dimorphism exists for the set-point level at which male and female mammals maintain their their body mass (cf. Leshner and Collier, 1973). In humans, laboratory rats, and other mammalian species, the male animal eats more and weighs more than its female counterpart, particularly at

maturity (cf. Bell and Zucker, 1971). Gonadal steroids have been demonstrated to play a role in the regulation of food intake and weight gain in rats. Gender differences in baseline food intake and body weight appear to be determined both by organizational and activational effects of gonadal hormones, as well as by non-hormonal (e.g., genetic) factors. Castration in adult male rats and mice results in small decreases in food intake and body weight relative to intact males (Bell and Zucker, 1971; Wright and Turner, 1973).

Removal of ovarian hormones in gonadecomized females results in large increases in food intake and body weight, although not to the level of intact males. The ovariectomized animal overeats until reaching a plateau, then reduces intake to pre-surgery levels, which is sufficient to maintain a stable increased (20-25%) weight (Mook, Kenney, Roberts, Nussbaum and Rodier, 1972; Tartellin and Gorski, 1973). This is apparently accomplished by an increase in meal size with compensatory decrease in meal frequency (Blaustein and Wade, 1976). Carcass analyses indicate that the weight gain represents increases in all carcass constituents (Leshner and Collier, 1973).

Estrogen replacement treatment of ovariectomized female rats produces dose-related decreases in food intake/body weight; progesterone has little effect (Tartellin and Gorski, 1973; Wade, 1972). Alterations in

food intake/body weight in rats correlates with fluctuations in circulating levels of estradiol. In a number of species, food intake and body weight are lowest at estrous and maximum during diestrous (see review: Fantino and Brinnel, 1985). These data suggest that estrogens have an inhibitory effect on body weight, possibly by changing the set-point for level of body fat (Wade, 1972).

Adult female rats drink more of sweet saccharin solutions than do male rats. This gender difference seems to be due to the stimulatory effects of female ovarian hormones, since ovariectomized rats show much lower preference for saccharin than intact females, whereas saccharin preference is not significantly different between castrated and intact male animals (Zucker, 1969). Relative weight gain (difference between palatable-fed versus chow-fed animals) is greater for females than for males. However, there is a greater similarity between males and females fed a supermarket diet than between same sex chow-fed versus palatable diet-fed animals. Male and female rats develop similar obesities (determined by the Lee Index, an indirect measure of body fat content) when offered highly palatable supermarket foods (e.g., marshmallows, peanut butter, sweetened condensed milk). These results suggest that there is no significant gender difference in responsiveness to taste properties (Sclafani and Gorman, 1977).

To date, few studies have examined gender and

gonadectomy differences in opioid modulation of food intake. McLaughlin and Baile (1983) reported that female rats were insensitive to the inhibitory effects of the opiate antagonist, nalmefene, on body weight and water intake. In contrast, male rats showed a decrease in body weight and water intake following nalmefene treatment. Also, relative to male rats, adult female are insensitive to naloxone's effects on food-deprivation induced feeding (Wager-Srdar, Gosnell, Morley and Levine, 1985). While naloxone inhibits nocturnal intake in female rats during late metestrous, diestrous, and proestrous, female rats fail to decrease intake following any dose of naloxone, during estrous (Wager-Srdar, Gannon and Levine, 1987).

Estradiol-treated ovariectomized rats appear less sensitive to the inhibitory effects of naloxone on nocturnal feeding than either vehicle-treated ovariectomized rats, or sham-operated females. Addition of progesterone to the estradiol-treated rats elevates their sensitivity to naloxone, but not to the level of sham animals. Ovariectomized controls are twice as sensitive to naloxone's suppressive effects as sham-operated females (Morley, Levine, Grace, Kneip and Gosnell, 1984). Thus, estradiol appears to decrease, and ovariectomy increase, the sensitivity to naloxone. Given that dynorphin concentrations are decreased in ovariectomized animals, Morley and co-workers (1984) suggested that ovariectomy increases the

sensitivity of opioid receptors involved in feeding, rather than increasing the levels of endogenous opioid peptides. Alternatively, the decreased responsiveness to naloxone produced by estradiol could be mediated by opioid peptides other than dynorphin. Since castration fails to alter the effectiveness of naloxone's inhibition of nocturnal intake, this suggests that testosterone is not significantly involved in the modulation of the opioid feeding system (Wager-Srdar et al., 1987).

7. Rationale for Present Research.

Although opiate drugs have long been known to inhibit pain responses, recent examination of the individual variability in responsiveness to these drugs has led to an investigation of the role of organismic variables in modulating this opioid-mediated function. The ability of these variables to alter antinociceptive responses to opioid and nonopioid stressors has been well-documented (see review: Bodnar et al., 1988). Antinociceptive responses induced by environmental stressors are the most sensitive to age and gender differences. However, previously observed senescent changes in adaptation to stress (Girardot and Holloway, 1985a,b) make it difficult to distinguish among age-related, gender-related and gonadectomy-related changes in antinociception, versus organismic variable effects on stress responses. Therefore, administration of an opiate agonist was selected as the means of producing

antinociception.

Previous research has indicated that morphine antinociception is sensitive to: a) aging, which reduces the efficacy of morphine in female rats (Kramer and Bodnar, 1986) and male mice (Chan and Lai, 1982), b) gender, with greater magnitude of antinociceptive effects in young adult males (Kepler et al., 1989), and c) gonadectomy, which reduces the antinociceptive efficacy of morphine in young adult animals of both genders (Kepler et al., 1989). Thus, it is evident that organismic variables play a role in modulating opioid-mediated behaviors. However, most studies have examined either age-related changes in animals of one sex, or gender differences in only young adult animals. Since there are little data indicating if and how these three variables interact, the first aim of this research was to systematically evaluate alterations in the sensitivity of morphine antinociception in rats as a function of age, gender, and gonadal status, by determining systemic dose-response relationships for morphine antinociception across a time course. Morphine was chosen because it is the prototypical analgesic in clinical use, and a systematic evaluation of its differential sensitivity to age, gender, and gonadal influences could have implications for the therapeutic utilization of analgesics in animals and humans.

As reviewed previously, opiates have multiple behavioral effects in addition to their antinociceptive

properties. Opioid agonists typically increase food and water intake, while opioid antagonists produce consistent inhibitory effects upon many forms of ingestion. Opioid antagonist effects can be observed under circumstances where a clearly-defined regulatory challenge is present, such as the increased intake following food deprivation. Further, opioid antagonists also inhibit intake under conditions in which a need state is absent, for instance, when intake is stimulated by a dietary preference. As previously mentioned, both of these different ecological forms of feeding are inhibited by general opioid antagonists, but differentially modulated by opioid receptor subtypes.

Age-related declines in the hypophagic effects of general opiate antagonists and in the hyperphagic effects of opiate agonists have been observed in male rats (Gosnell et al., 1983). Female rats are less sensitive than males to the hypophagic properties of opiate antagonists (Wager-Srdar et al., 1985, 1987). Finally, the sensitivity of naloxone's inhibition of nocturnal feeding in female rats is decreased by estradiol and increased by ovariectomy (Morley et al., 1984). Therefore, the second specific aim of this research was to systematically evaluate the interactions of age, gender, and gonadal status in altering the sensitivity of naloxone hypophagia upon deprivation-induced feeding and high-fat feeding in rats, by determining systemic dose-response relationships for naloxone hypophagia across a time

course. The examination of these interactive effects upon opioid function across two behaviors will allow evaluation of the nature of such changes, i.e., whether they are either general or specific to a particular behavior.

To this end, separate groups of male and female rats received either sham or gonadal surgery at age three months, to control for age at time of surgery. A cross-sectional design was selected to minimize the number of injection conditions that each animal would otherwise be subjected to in a longitudinal study. All animals were given a minimum of one month to recover from surgery, to allow for full development of gonadectomy-induced changes (e.g., weight, which was monitored during the experimental period). The sham operations allowed for evaluation of gender differences and served as references for assessment of gonadectomy effects. The choice of four age cohorts, based upon previous studies conducted in our laboratory, permitted consideration of the nature of aging effects, if observed, i.e., whether they be gradual and progressive, or abrupt alterations. Note that menopausal age in the female Sprague-Dawley rat is reached between 450-540 days.

Although central drug injections decrease the effects of pharmocokinetic variables, the use of a peripheral route of administration of drugs in this long-course study was neccesitated by the short-lived patency of intracerebroventricular cannulae, and the risk involved in

subjecting older animals to surgery. To decrease potential confounding by tolerance effects, each subject received a maximum of one injection separated by at least a one week interval between ascending dose conditions. Dose-response and time-response (across a two-hour time course) of drug actions were evaluated in order to yield both peak and total (duration) effects, both in terms of magnitude and potency.

The tail-flick response, which is a spinally-organized reflex to radiant heat pain, was measured since this is an index of rodent nociception that has an excellent correlation with clinical pain reports in humans, and appears to be less influenced by motor impairments than the supraspinally-mediated jump response to electric shock. Taking measurements of a second physiological response to assess specificity of age/gender effects upon pain inhibition was deemed unneccesary since previous studies on opioid analgesia in our laboratory found a dissociation between age/gender effects upon analgesic and hypothermic responses following analgesic manipulations.

GENERAL METHODS

Subjects and Surgery: Albino Sprague-Dawley rats (60 days of age, Charles River Laboratories, Wilmington, MA) were housed individually in wire mesh cages in the Queens College Vivarium, and were maintained on a 12 h light: 12 h dark cycle with rat chow and water available ad libitum. At 90 days of age, two groups of male rats and two groups of female rats were matched on the basis of body weight. All rats were anesthetized with chlorpromazine (3 mg/kg, ip) followed by ketamine (100 mg/ml sterile water/kg body weight, im). One group of male rats was castrated by removal of the testes and testicular fat with a single 2-cm. midscrotal incision. One group of female rats was ovariectomized by removal of the ovaries and ovarian fat with a dorsal incision (Romero and Bodnar, 1986; Romero et al., 1987, 1988; Kepler et al., 1989). The second groups of male and female rats received sham surgery in which the organs were exposed, but not removed. All rats were allowed a minimum of one month to recover from surgery, and to allow full development of gonadectomy-induced weight changes (Beatty and Fessler, 1977; Marks and Hobbs, 1976) which were monitored during the experimental period.

Gonadal Analyses: After the last experimental session, each animal received an overdose of anesthetic (Euthanasia, H. Schein and Co.) and was sacrificed. With the bladder and urethral region exposed, the seminal vesicles were dissected

in the males, blotted dry, and organ weights determined. The fallopian tubes of females were likewise dissected, dried and weighed (Romero et al., 1987).

EXPERIMENT 1. Age, Gender and Gonadectomy Effects upon Morphine Antinociception.

Organismic variables in the modulation of opiate and opioid-mediated antinociception include aging, gender and gonadectomy factors (see review: Bodnar et al., 1988). Initial evaluation of aging effects upon morphine antinociception revealed variable effects (Chan and Lai, 1982; Kavaliers et al., 1983; Saunders et al., 1974; Spratto and Dorio, 1978; Wallace et al., 1980) which were due to potential confounding factors. Kramer and co-workers (1986) subsequently found that female rats aged 14, 19 and 24 mo. displayed significant reductions in morphine antinociception on the tail-flick and jump tests relative to rats aged 4 and 9 mo., without significant changes in morphine hyperthermia. Whereas age-related reductions were also observed for opioid- and nonopioid-mediated forms of stress-induced antinociception, including 2-deoxy-D-glucose (Kramer et al., 1985), inescapable front-paw and hind-paw shock (Grossman et al., 1982; Hamm and Knisely, 1985) and intermittent cold-water swims (Girardot and Holloway, 1985), antinociception following either beta-endorphin (Romero and Bodnar, 1987) or food deprivation (Hamm and Knisely, 1986) were insensitive to aging. However, whereas female rats displayed age-related reductions in nonopioid continuous cold-water swim antinociception (Kramer and Bodnar, 1986), male rats displayed age-related increases in the same antinociceptive

response (Hamm et al., 1986).

Gender is a second critical organismic variable in modulating morphine antinociception, with males displaying significantly greater magnitudes of effects (Baamonde et al., 1989; Badillo-Martinez et al., 1984; Kavaliers and Innes, 1987; Kepler et al., 1989; Lipa and Kavaliers, 1990). This pattern of effects is also observed for antinociception following stress as well as noradrenergic and cholinergic agonists (Kavaliers and Colwell, 1991; Kavaliers and Innes, 1987; Kavaliers and Innes, 1988; Kiefel and Bodnar, 1992; Romero and Bodnar, 1986). Gender differences in antinociception induced by morphine, clonidine, pilocarpine and stress are altered by gonadectomy (Chatterjee et al., 1982; Kepler et al., 1989; Kiefel and Bodnar, 1992; Romero et al., 1987), and the gonadectomy-induced changes in stress-induced antinociception are reinstated by hormonal replacement therapy (Romero et al., 1988). It should be noted that not all forms of antinociception are sensitive to gender differences, including DAMGO and DSLET (Kepler et al., 1991).

These data suggest that morphine antinociception is sensitive to: a) aging, which results in decrements in responsivity in female rats (Kramer and Bodnar, 1986), b) gender, with greater responses in young adult males than females (Baamonde et al., 1989; Badillo-Martinez et al., 1984; Kavaliers and Innes, 1987; Kepler et al., 1989; Lipa

and Kavaliers, 1990), and c) gonadectomy, which decreases the antinociceptive efficacy of morphine in young adult animals (Chatterjee et al., 1982; Kepler et al., 1989). However, since there are little data indicating how these three variables interact in modulating morphine antinociception, the present study evaluated alterations in morphine antinociception in rats as a function of age (6, 12, 18 and 24 months), gender (male, female) and gonadal status (intact, gonadectomized) across a dose range (1, 2.5, 5 and 10 mg/kg) and time course (30-120 min) on the tail-flick test (D'Amour and Smith, 1941), a measure of rodent nociception that correlates well with human clinical effectiveness (Grumbach, 1966).

Protocol: Experimental testing of groups of sham-operated and castrated male rats and sham-operated and ovariectomized female rats began at one of the following four ages: a) 6 mo., b) 12 mo., c) 18 mo. and 24 mo (Kramer and Bodnar, 1986). The following numbers of rats were tested in each group: a) sham-operated males aged 6 (10), 12 (9), 18 (9) and 24 (5) mo., b) castrated males aged 6 (10), 12 (9), 18 (6) and 24 (8) mo., c) sham-operated females aged 6 (10), 12 (8), 18 (9) and 24 (5) mo., and d) ovariectomized females aged 6 (10), 12 (9), 18 (10) and 24 (12) mo.

All rats were tested on the tail-flick test (D'Amour and Smith, 1941) in which a stimulus source (IITC Company) was mounted 8 cm above the dorsum and 2-8 cm proximal to the

tip of the tail of a lightly-restrained animal. Each tail-flick session consisted of three latency determinations made at 10-sec intervals. In order to avoid tissue damage, a trial was automatically terminated if a response did not occur within 15 sec. Following four days of stable baseline latency determinations, all rats in each of the age groups received five injections: vehicle, and 1.0, 2.5, 5.0 and 10.0 mg/kg doses of morphine (Pennick Laboratories) in ascending order. All doses were administered subcutaneously at a concentration of 1 ml of normal saline/kg body weight. A minimum of seven days elapsed between injection dosages to minimize tolerance effects, a protocol which has been established previously (Bodnar et al., 1978; Kepler et al., 1989). Tail-flick latencies were assessed at 30, 60, 90 and 120 min following each injection. All baseline and experimental manipulations were conducted between 3 and 8 h into the light cycle for all groups.

Data Analysis: Two statistical approaches were utilized to analyze the data in terms of magnitude and potency (ED_{50}) of effect. The magnitude of effect was evaluated using split-plot analyses of variance corrected for repeated measures to assess significant differences among vehicle and individual morphine doses (1, 2.5, 5 and 10 mg/kg) across test times (30-120 min) as functions of age (6, 12, 18 and 24 mo.), gender (males and females) and surgery (sham-operated and gonadectomy). Since significant differences in

baseline vehicle tail-flick latencies have been shown to occur as a function of age, gender and gonadal condition, evaluation of significant differences in antinociceptive magnitude were made across groups as a function of age, gender and gonadal status, independent of basal differences. The Maximal Percentage Effect (MPE) was calculated for peak (30 min) and total (30 + 60 + 90 + 120 min) antinociception using the formula: MPE = (Experimental Score - Vehicle Score / Cut-Off Score - Vehicle Score) x 100 (e.g., Hayes et al., 1978). Analyses of variance upon peak and total MPE for each morphine dose were then performed to assess differences among gender and gonadectomy groups for each age group, and differences across ages within a given gender and gonadectomy group (Dunnett comparisons, $p<.05$).

The potency of effects was evaluated by constructing log dose-response functions by performing linear regression analyses for sham-operated and castrated males and sham-operated and ovariectomized females in each age group. Potency was indicated by the ED_{50} for each peak and total MPE effect. Calculations from the linear regression analyses allowed for the determination of significant differences between slopes and intercepts among gender and gonadectomy groups within given ages, and across ages for a given gender and gonadectomy group.

RESULTS

Table 1 summarizes the ability of the different doses of morphine to elicit antinociception on the tail-flick test across age and surgical groups. A MPE analysis was then performed to assess differences in the magnitudes of morphine antinociception.

Morphine (1 mg/kg) MPE: The peak and total MPE of morphine antinociception increased as a function of age for animals aged 6 (peak: 5.9; total: 15.6), 12 (peak: 7.5; total: 16.7), 18 (peak: 15.1; total: 38.0) and 24 (peak: 12.0; total: 37.9) mo. Significant age-related increases were observed in the peak MPE of morphine in castrated males aged 18 mo. (Figure 1A), in sham-operated females aged 18 and 24 mo. (Figure 1B), and in ovariectomized females aged 18 mo. (Figure 1B) relative to corresponding 6 mo. groups. Significant age-related increases were observed in the total MPE of morphine in castrated males aged 18 mo. (Figure 1C), and in sham-operated and ovariectomized females aged 24 mo. (Figure 1D) relative to corresponding 6-mo. groups. Significant gender-related differences were observed with the peak and total MPE of morphine significantly increased in sham-operated females aged 24 mo. relative to corresponding sham-operated males (Figure 1B,D). Significant gonadectomy-related differences were observed with increases in the peak MPE of morphine in castrated males aged 18 mo. (Figure 1A), and decreases in the peak and total MPE of

TABLE 1. Duration (min) of Morphine Antinociception across Conditions.

GROUP	AGE	MORPHINE DOSE (mg/kg)			
		1	2.5	5	10
Sham Males	6	30	120	120	120
	12	xx	60	120	120
	18	30	120	120	120
	24	90	120	120	120
Castrated Males	6	xx	60	90	120
	12	xx	60	120	120
	18	90	90	120	120
	24	30	60	120	120
Sham Females	6	xx	90	60	120
	12	xx	60	60	120
	18	60	60	120	120
	24	90	90	120	120
Ovariectomized Females	6	xx	90	120	120
	12	30	60	120	120
	18	60	120	120	120
	24	xx	90	120	120

xx denotes a failure to produce significant increases in latencies following that dose of morphine. Significant differences in tail-flick latencies were observed across drug conditions ($p<.0001$), across time ($p<.0001$) and for the interactions between age and gender ($p<.05$), gender and surgery ($p<.028$), age and drug condition ($p<.0001$), gender and drug condition ($p<.0003$), age, gender and drug condition ($p<.04$), gender, surgery and drug condition ($p<.0004$), age and time ($p<.0001$), surgery and time ($p<.0008$), age, gender and time ($p<.006$), drug condition and time ($p<.0001$), gender, drug condition and time ($p<.0001$), surgery, drug condition and time ($p<.021$), age, gender, drug condition and time ($p<.0004$) and gender, surgery, drug condition and time ($p<.0005$).

Figure 1. Alterations in the magnitude (Maximal Percentage Effect: MPE, SEM) of peak (Panels A and B) and total (Panels C and D) antinociception following a 1 mg/kg dose of morphine as a function of age in male (Panels A and C) and female (Panels B and D) rats that underwent either sham-operated surgery (solid bars) or gonadectomy (striped bars). Significant differences in MPE were observed across age groups (peak: $p<.0001$; total: $p<.0001$) and for the interactions between age and surgery (total: $p<.011$) and among age, gender and surgery (peak: $p<.011$).

In this and subsequent figures, the enclosed stars denote significant age differences (Dunnett comparisons, $p<.05$) relative to corresponding rats 6 mo. of age. The open stars denote significant gender or male gonadectomy differences (Dunn comparisons, $p<.05$) relative to sham-operated males in that particular age group. The dark stars denote significant gonadectomy differences (Dunn comparisons, $p<.05$) in female rats.

MAXIMAL PERCENTAGE EFFECT
A. Peak Effects: Males
AGE (months)
MAXIMAL PERCENTAGE EFFECT
B. Peak Effects: Females
AGE (months)
MAXIMAL PERCENTAGE EFFECT
C. Total Effects: Males
AGE (months)
MAXIMAL PERCENTAGE EFFECT
D. Total Effects: Females
AGE (months)

morphine in ovariectomized females aged 24 mo. (Figures 1B,D).

Morphine (2.5 mg/kg) MPE: The overall age-related changes in peak and total MPE of morphine antinociception failed to vary systematically. Significant age-related increases were observed in the peak and total MPE of morphine in sham-operated males aged 18 mo. (Figure 2A,C) relative to corresponding 6 mo.-old groups. Significant age-related decreases were observed in the peak MPE of morphine in sham-operated females aged 12 mo. (Figure 2B) relative to corresponding 6-mo.-old groups. Significant gender-related decreases were observed in sham-operated females aged 18 mo. for both the peak and total MPE of morphine, and in ovariectomized females aged 18 mo. (peak MPE) and 24 mo. (total MPE) (Figures 2B,D). Significant gonadectomy-related differences were observed with decreases in the peak MPE of morphine in castrated males aged 18 and 24 mo. (Figure 2A), and in the total MPE of morphine in castrated males aged 18 mo. (Figure 2C).

Morphine (5 mg/kg) MPE: The overall age-related changes in peak and total MPE of morphine antinociception failed to vary systematically. In analyzing individual age-related, gender-related and gonadectomy-related effects, significant differences failed to occur for either the peak or total MPE of morphine at this dose (Figure 3).

Figure 2. Alterations in the magnitude (MPE, SEM) of peak (Panels A and B) and total (Panels C and D) antinociception following a 2.5 mg/kg dose of morphine as a function of age in male (Panels A and C) and female (Panels B and D) rats that underwent either sham surgery (solid bars) or gonadectomy (striped bars). Significant differences in MPE were observed across age groups (peak: $p<.021$; total: $p<.002$), surgery (peak: $p<.039$) and for the interactions between age and gender (peak: $p<.016$; total: $p<.038$) and gender and surgery (peak: $p<.002$; total: $p<.001$).

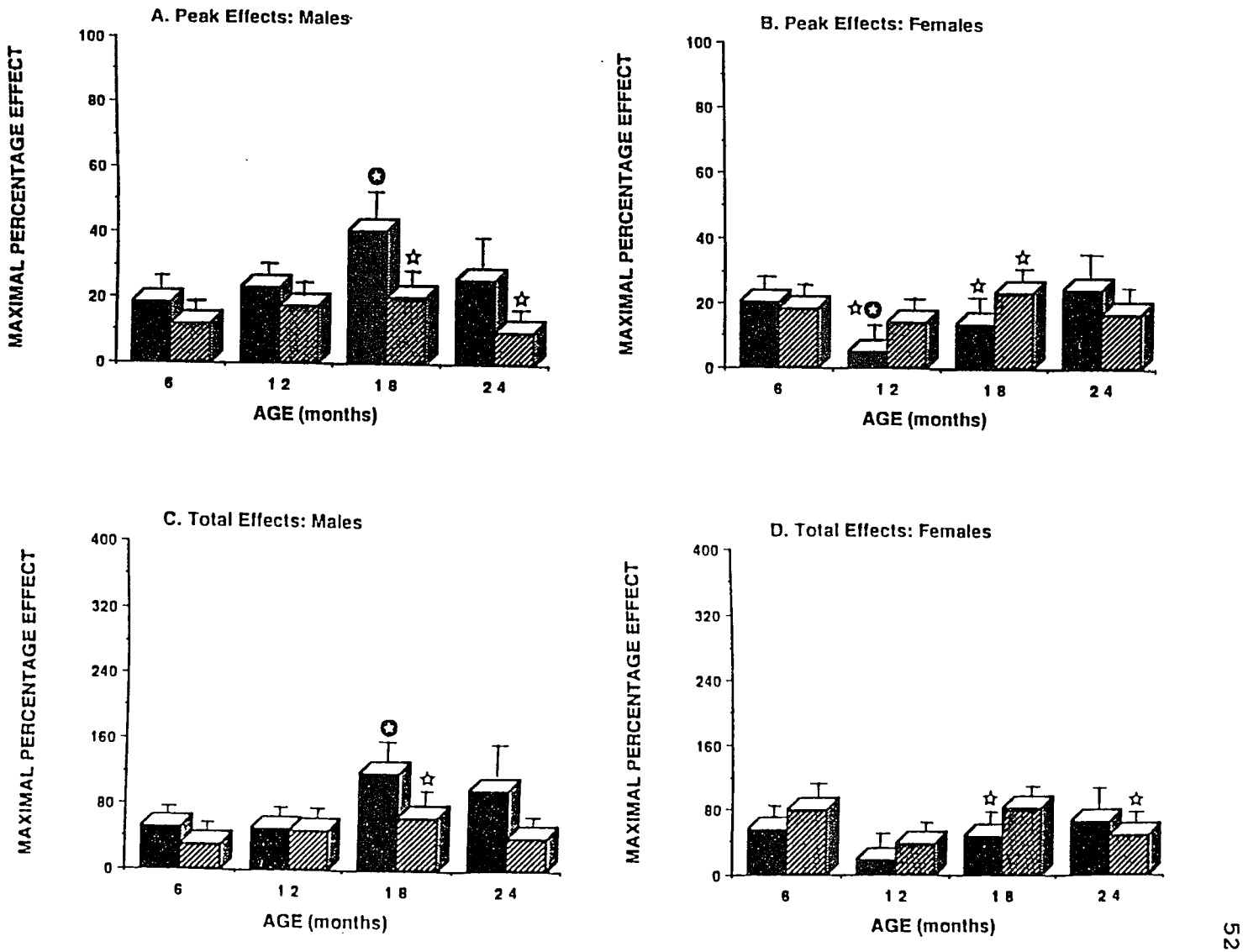
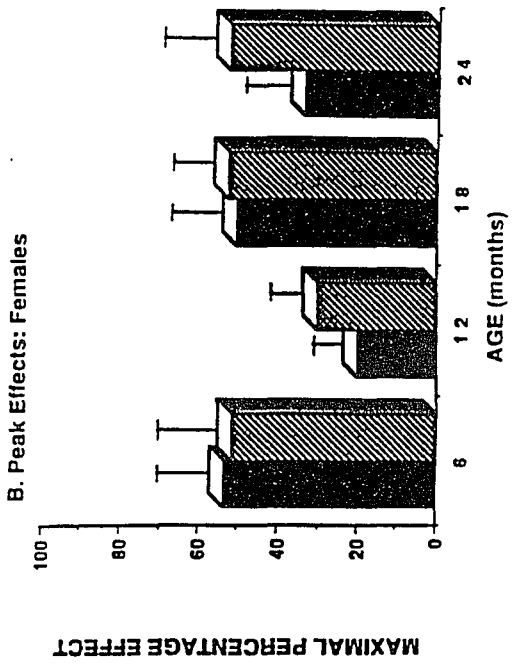
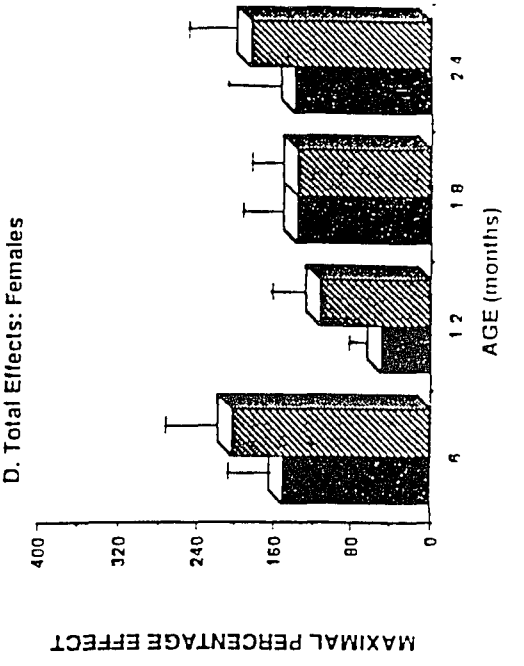
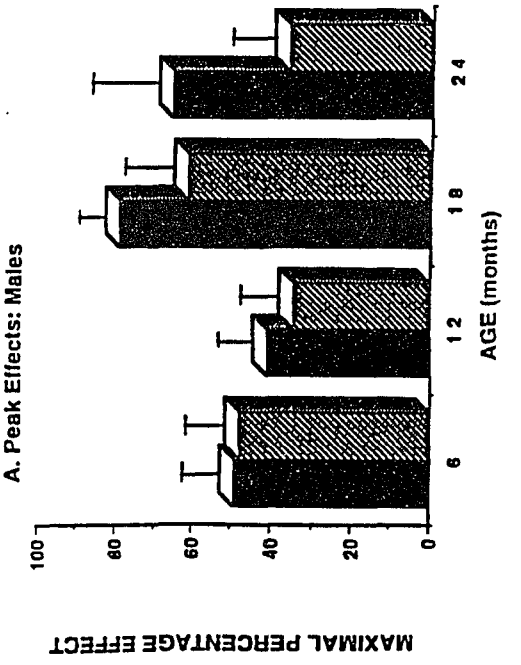
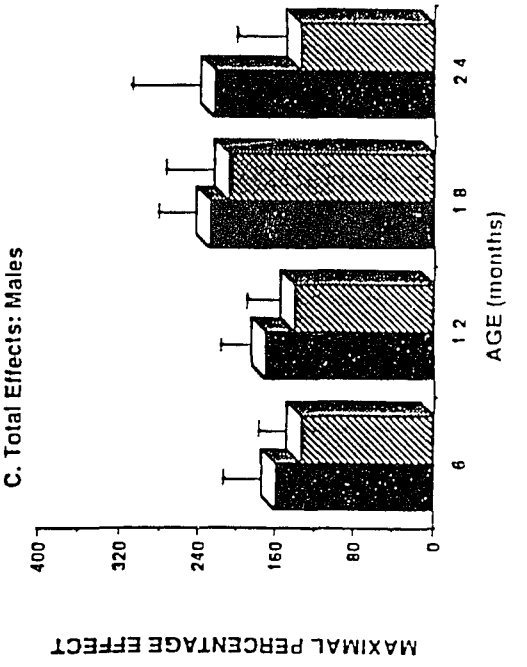

A. Peak Effects: Males
MAXIMAL PERCENTAGE EFFECT
100
80
60
40
20
0
6
12
18
24
AGE (months)
B. Peak Effects: Females
MAXIMAL PERCENTAGE EFFECT
100
80
60
40
20
0
6
12
18
24
AGE (months)
C. Total Effects: Males
MAXIMAL PERCENTAGE EFFECT
400
320
240
160
80
0
6
12
18
24
AGE (months)
D. Total Effects: Females
MAXIMAL PERCENTAGE EFFECT
400
320
240
160
80
0
6
12
18
24
AGE (months)

Figure 3. Alterations in the magnitude (MPE, SEM) of peak (Panels A and B) and total (Panels C and D) anti-nociception following a 5 mg/kg dose of morphine as a function of age in male (Panels A and C) and female (Panels B and D) rats that underwent either sham surgery (solid bars) or gonadectomy (striped bars). Significant differences in MPE were observed across age groups (peak: $p<.003$), surgery (peak: $p<.039$) and for the interactions between age and gender (peak: $p<.016$) and gender and surgery (peak: $p<.002$; total: $p<.026$).


B. Peak Effects: Females
MAXIMAL PERCENTAGE EFFECT
100
80
60
40
20
0
6
12
18
24
AGE (months)


D. Total Effects: Females
MAXIMAL PERCENTAGE EFFECT
400
320
240
160
80
0
6
12
18
24
AGE (months)


A. Peak Effects: Males
MAXIMAL PERCENTAGE EFFECT
100
80
60
40
20
0
6
12
18
24
AGE (months)


C. Total Effects: Males
MAXIMAL PERCENTAGE EFFECT
400
320
240
160
80
0
6
12
18
24
AGE (months)

Morphine (10 mg/kg) MPE: Significant age-related decreases were found in the peak and total MPE of morphine in sham-operated females aged 18 and 24 mo. (Figures 4B,D) relative to corresponding 6-mo.-old groups. Significant gender-related decreases were found in sham-operated females aged 18 and 24 mo. for both the peak and total MPE of morphine (Figures 4B,D). Significant gonadectomy-related differences were found, with increases in the peak and total MPE of morphine in ovariectomized females aged 18 and 24 mo. (Figures 4B,D).

Morphine Dose-Response Functions: Table 2 summarizes the slopes, intercepts and ED_{50} values as functions of age, gender and gonadectomy. Several interesting patterns emerge. First, age-related changes in the morphine dose-response curves vary as a function of gender. Sham-operated males display an age-related decrease in the peak and total ED_{50} of morphine antinociception, whereas sham-operated females display an age-related increase in the peak and total ED_{50} of morphine antinociception. Second, gonadectomy appears to modulate these gender-specific age-related changes in female rats. Ovariectomy eliminated the age-related increases in the peak and total ED_{50} of morphine antinociception observed in sham-operated females. In contrast, castration did not produce systematic effects.

Gonadal Tissue Analyses: Male rats displayed significant changes in body weight as a function of age

Figure 4. Alterations in the magnitude (MPE, SEM) of peak (Panels A and B) and total (Panels C and D) anti-nociception following a 10 mg/kg dose of morphine as a function of age in male (Panels A and C) and female (Panels B and D) rats that underwent either sham surgery (solid bars) or gonadectomy (striped bars). Significant differences in MPE were observed for gender (total: $p<.001$), and for the interactions between age and gender (total: $p<.028$), age and surgery (total: $p<.041$) and gender and surgery (peak: $p<.04$; total: $p<.001$).

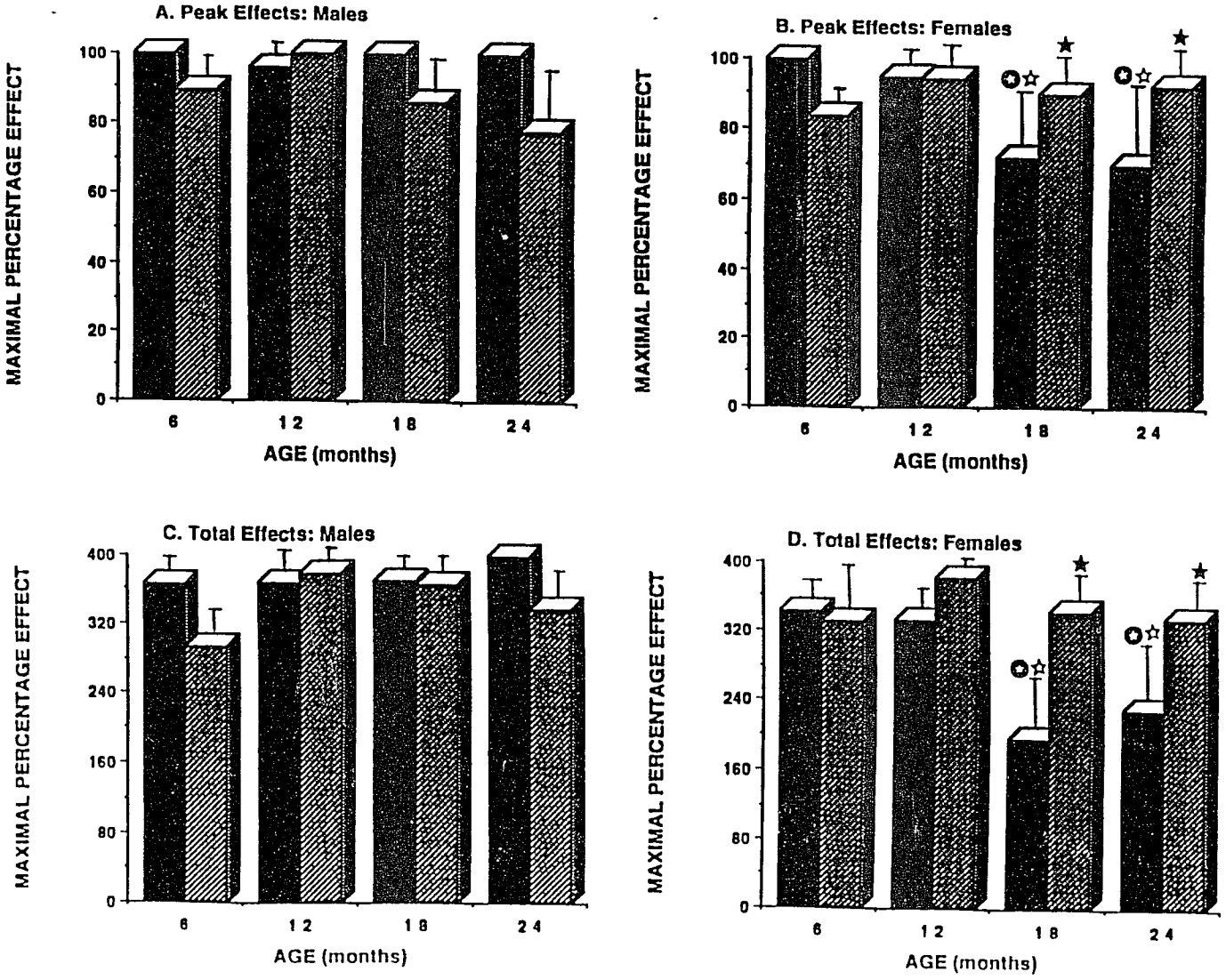

A. Peak Effects: Males
MAXIMAL PERCENTAGE EFFECT
100
80
60
40
20
0
6
12
18
24
AGE (months)
B. Peak Effects: Females
MAXIMAL PERCENTAGE EFFECT
100
80
60
40
20
0
6
12
18
24
AGE (months)
C. Total Effects: Males
MAXIMAL PERCENTAGE EFFECT
400
320
240
160
80
0
6
12
18
24
AGE (months)
D. Total Effects: Females
MAXIMAL PERCENTAGE EFFECT
400
320
240
160
80
0
6
12
18
24
AGE (months)

TABLE 2. Alterations in morphine dose-response function (ED_{50}: mg/kg) for peak and total antinociception on the tail-flick test as functions of age, gender and gonadectomy.

GROUP	AGE (months) 6	12	18	24	Age Sig.
A. Peak Antinociception:					
Sham Males	5.2	5.5	3.8	4.7	p<.02
Castrated Males	5.8	5.6	5.0	6.7	ns
Sham Females	5.2	6.4	6.4	6.7	p<.01
Ovex Females *	5.8	5.9	5.2	5.4	ns
Gender/ Gonadectomy Sig.	ns	ns	p<.02	p<.07	
B. Total Antinociception:					
Sham Males	5.9	5.8	5.0	4.8	p<.02
Castrated Males	7.1	5.9	5.3	6.4	p<.01
Sham Females	6.3	7.3	9.7	8.5	p<.01
Ovex Females	5.8	6.1	5.8	6.0	ns
Gender/ Gonadectomy Sig.	ns	p<.01	p<.01	p<.03	

* In this and subsequent tables, "ovex" female refers to ovariectomized female.

Significant age-related differences in the peak dose-response curves of morphine antinociception were observed for sham-operated males ($p<.02$) and sham-operated females ($p<.01$). Significant age-related differences in the total dose-response curves of morphine antinociception were observed for sham-operated ($p<.01$) and castrated ($p<.01$) males and sham-operated females ($p<.001$). Significant gender-related and gonadectomy-related differences in the peak dose-response curves of morphine antinociception were observed in rats aged 18 mo. ($p<.02$). Significant gender-related and gonadectomy-related differences in the total dose-response curves of morphine antinociception were observed in rats aged 12 ($p<.01$), 18 ($p<.01$) and 24 ($p<.03$) mo.

($p<.001$), but not gonadal status. Both sham-operated and castrated male rats aged 18 (753-807 g) and 24 (678-679 g) mo. weighed significantly more than corresponding animals aged 6 (504-544 g) and 12 (559-620 g) mo. Male rats displayed significant changes in seminal vesicle weight as a function of gonadal status ($p<.001$), but not age. The size of the seminal vesicles was significantly reduced in castrated rats aged 6 (86%), 12 (91%), 18 (74%) and 24 (91%) mo. relative to corresponding sham-operated males. Female rats displayed significant changes in body weight as functions of age ($p<.001$) and gonadal status ($p<.001$). Ovariectomized females of all ages were significantly heavier than corresponding sham-operated females. Older sham-operated (18 mo: 462 g; 24 mo: 441 g) and ovariectomized (18 mo: 583 g; 24 mo: 569 g) female rats weighed significantly more than younger sham-operated (6 mo: 334 g; 12 mo: 332 g) and ovariectomized (6 mo: 413 g; 12 mo: 448 g) females, respectively. Female rats displayed significant changes in fallopian tube weight as functions of gonadal status ($p<.001$) and age ($p<.017$). The size of the fallopian tubes was significantly reduced in ovariectomized rats aged 6 (87%), 12 (88%), 18 (87%) and 24 (88%) mo. relative to corresponding sham-operated females. The weight of the fallopian tubes were significantly larger in 12 mo. old sham-operated females (0.83 g) than in those aged 6 mo. (0.65 g).

DISCUSSION

Previous data have indicated that morphine antinociception is sensitive to: a) aging, with declines in responsivity in female rats (Kramer and Bodnar, 1986), b) gender, with greater responses in young adult males than females (Baamonde et al.,1989; Badillo-Martinez, et al., 1984; Kavaliers and Innes, 1987; Kepler et al., 1989; Lipa et al., 1990), and c) gonadectomy, with decreased responsiveness in young adult animals (Chatterjee et al., 1982; Kepler et al., 1989). The present study was designed to examine the interactions among these three variables in modulating morphine antinociception.

Before proceeding with a discussion of these variables, it is important to examine other potential relevant factors that might explain the obtained results. The first to be considered is that age, gender and/or gonadectomy might influence the absorption, disposition and/or metabolism of morphine independent of morphine's pharmacodynamic actions. It is important to note that, whereas morphine antinociception is significantly altered by age (e.g.,Kramer and Bodnar, 1986), antinociception induced by the opioid peptide, beta-endorphin is not (Romero and Bodnar, 1986). Further, whereas morphine antinociception is sensitive to gender (e.g.,Badillo-Martinez et al., 1984; Kepler et al., 1989), antinociception induced by the enkephalin derivatives, DAMGO and DSLET, are not (Kepler et al., 1991).

One major difference between the opiate, morphine, and these opioid peptides and analogues, is their disposition. Whereas opioid peptides are degraded by aminopeptidases, carboxypeptidases and endopeptidases (e.g., Roques and Fournie-Zaluski, 1986), morphine is metabolized in part into morphine-6-glucuronide, which possesses potent antinociceptive activity in its own right (Abbott and Palmour, 1988; Pasternak, Bodnar, Clark, Inturrisi, 1986; Paul, Standifer, Inturrisi, Pasternak, 1989). It is not known whether aging, gender or gonadectomy alter the rate of disposition of morphine into morphine-6-glucuronide, or into morphine-3-glucuronide, which fails to alter nociceptive thresholds (Pasternak et al., 1986).

A second factor to be considered is age-related, gender-related or gonadectomy-related changes in tail thickness, tail blood flow and tail temperature. Latencies on the tail-flick test appear sensitive to tail temperature at the beginning of testing (Berge, Garcia-Cabrera, Hole, 1988) and may account for some hyperalgesic responses (Tjolsen, Berge, Eide, Broch, Hole, 1988). In the present study, however, female rats displayed significant but quite small (0.2 sec) increases in tail-flick latencies relative to males. Further, although younger rats displayed longer basal latencies than older rats, this increase varied between 0.2 and 0.4 sec. None of these effects appeared to account for the more dramatic changes in morphine

antinociception.

Aging produced significant differences in both the magnitude and potency of morphine antinociception. First, as observed previously (Kramer and Bodnar, 1986), intact female rats displayed significant age-related decreases in morphine antinociception. The ED_{50} of morphine antinociception rose for peak effects in rats aged 12 (24%), 18 (23%) and 24 (30%) mo., and for total effects in rats aged 12 (16%), 18 (55%) and 24 (35%) mo. The differences in the magnitude of effect was especially pronounced following the highest (10 mg/kg) dose, with younger (6 mo.) females displaying maximal effects and older (18 and 24 mo.) females displaying smaller increases (MPE = 70). Interestingly, at the lowest (1 mg/kg) morphine dose, intact older female rats displayed a significantly enhanced response. Second, in contrast to female rats, intact male rats displayed significant age-related enhancement in morphine antinociception with the ED_{50} of morphine declining for peak effects in rats aged 18 (27%) and 24 (10%) mo., and for total effects in rats aged 18 (15%) and 24 (18%) mo. The differences in the magnitude of effect were especially pronounced following a moderate (2.5 mg/kg) dose with older (18 mo.) males displaying significantly greater antinociception relative to younger (6 mo.) males.

The patterns of age-related decreases in morphine antinociception in intact female rats, and age-related

increases in morphine antinociception in intact male rats, are quite similar to antinociceptive effects observed following continuous cold-water swims (CCWS), a nonopioid-mediated stressor. In this paradigm, female rats displayed age-related declines in CCWS antinociception (Kramer and Bodnar, 1986), and male rats displayed age-related increases in CCWS antinociception (Hamm et al., 1986). Thus, gender emerges as an important variable in assessing age differences.

The present data parallel neurochemical differences observed between males and females as a function of age. In non-specific opiate receptor binding studies using ^{3}H-dihydromorphine, age-related reductions in the density of binding sites were observed in the thalamus, midbrain and cortex of female rats with a loss of high and low affinity binding sites replaced by a steady, intermediate level of binding (Messing et al., 1980). In contrast, whereas male rats also displayed an age-related decline in opiate receptor density in the frontal poles, aging produced higher opiate binding affinity in males (Messing et al., 1980).

Gender was found to have an influence in the magnitude and potency of morphine antinociception, although not as one might have predicted from earlier studies in which young males displayed significantly greater magnitudes of effect than young females. This pattern has been observed for morphine antinociception (Baamonde et al., 1989; Badillo-

Martinez et al., 1984; Kavaliers and Innes, 1987; Kepler et al., 1989; Lipa and Kavaliers, 1990), stress-induced antinociception (Kavaliers and Colwell, 1991; Kavaliers and Innes, 1987; Kavaliers and Innes, 1988; Romero and Bodnar, 1986), and antinociception induced by noradrenergic and cholinergic agonists (Kiefel and Bodnar, 1992). In the present study, young (6 mo.) intact males and females displayed similar potencies of morphine antinociception on the tail-flick test for both peak (ED_{50}= 5.17 mg/kg (males); 5.17 mg/kg (females)) and total (ED_{50}= 5.87 mg/kg (males); 6.25 mg/kg (females)) measures. We assessed whether this was either a failure to replicate previous results studying morphine antinociception, or a systematic methodological exception.

Kavaliers and colleagues (Kavaliers and Innes, 1987; Lipa and Kavaliers, 1990) found that male mice displayed significantly greater magnitudes of morphine antinociception than female mice on the supraspinally-mediated hot-plate test. In this study, the gender differences were found to be more pronounced in the dark phase than in the light phase of the light/dark cycle. Thus, species, nociceptive test, and time of testing might account for the different results. Two other studies which indicated that male rats display significantly greater magnitudes of morphine antinociception than female rats did so using either the supraspinally-mediated jump test (Badillo-Martinez, 1984) or the writhing

test, a measure of visceral nociception (Baamonde et al., 1989). Again, differences in the nociceptive test used may account for the different results. The remaining study, from our laboratory (Kepler et al., 1989), found gender differences in morphine antinociception on the tail-flick test, but this was following intracerebroventricular rather than systemic administration of the opiate.

The present data, when considered in conjunction with above-cited results, reveal an interesting pattern in that gender differences in young adult animals is observed for morphine antinociception either when a supraspinal test of nociception is employed or when morphine is administered directly into a supraspinal site. Since morphine acts at both supraspinal and spinal levels of the neuraxis (see reviews: Yaksh, 1981; Yaksh and Rudy, 1978), it is conceivable that the failure to observe gender differences for systemic morphine on a spinally-mediated nociceptive test (Grossman, Basbaum, Fields, 1982; Yaksh, 1981) may be attributed to spinal, gender-insensitive mechanisms. As indicated in the previous section, gender differences did emerge as a function of age with male rats displaying an increased sensitivity to morphine and female rats displaying a decreased sensitivity to morphine.

Gonadectomy appeared to modulate the magnitude of morphine antinociception, especially in female rats and as a function of age. Previous studies of antinociception

induced by morphine, clonidine, pilocarpine and stress revealed gonadectomy effects (Chatterjee et al., 1982; Kepler et al., 1989; Kiefel and Bodnar, 1992; Romero et al., 1987). The present study found that castration marginally altered the magnitude and potency of morphine antinociception, and only did so at one age (18 mo.) and in an inconsistent manner. This is in contrast to the ability of castration to significantly reduce both opioid and nonopioid forms of swim antinociception (Romero et al., 1987), but in agreement with the failure of castration to alter the dose-response functions of central morphine, pilocarpine and clonidine (Kepler et al., 1989; Kiefel and Bodnar, 1992). In contrast, ovariectomy eliminated the age-related reductions in the potency and magnitude of morphine antinociception observed in intact female rats. Whereas aging increased the ED_{50} of morphine antinociception in intact female rats by 23-30% for peak effects and by 16-55% for total effects, ovariectomized rats displayed minor and non-significant age-related changes, with peak effects ranging from a 3% increase to a 11% decrease, and total effects ranging from a 5% increase to a 1% decrease.

Since ovariectomy was performed at 3 mo. of age, this suggests that circulating levels of gonadal steroids (e.g., progesterone, estrogen) may be responsible for the age-related decline in morphine responsivity found in intact females. Met-enkephalin immunoreactivity is increased in the

periventricular hypothalamus of female rats, an effect which is regulated by ovariectomy (Watson, Hoffman, Wiegand, 1986). Further, increased leu-enkephalin immunoreactivity in the brains of female rats is reduced in ovariectomized animals (Simerly et al., 1988). Ovariectomy also alters circulating levels of met-enkephalin (Dupont et al., 1985; Hong et al., 1982) as well as dynorphin A and B (Molineaux et al., 1986). Much, however, needs to be clarified, including the gonadal steroid(s) involved, the opioid peptide or receptor affected, and the locus of action.

These data indicate that such organismic variables as age, gender and reproductive status all interact to modulate rodent responsivity to morphine antinociception. The influence of these variables include: a) the increased sensitivity of male rats and decreased sensitivity of female rats, b) gender differences in opiate antinociception in young animals for supraspinally-mediated nociceptive responses, but not spinally-mediated nociceptive responses, and c) the ability of ovariectomy to mitigate age-related declines in morphine antinociception in female rats.

Experiment 2. Age, Gender and Gonadectomy Effects upon Naloxone Hypophagia: Evaluation of Deprivation-Induced and High-Fat Intake.

The opioid system has been implicated in the control of food and water intake with agonists typically stimulating intake and antagonists typically inhibiting intake (see reviews: Cooper et al., 1985; Levine et al., 1985; Morley et al., 1983). The use of the general opioid receptor antagonists, naloxone and naltrexone, has provided insight into the types of ingestive situations that are mediated by the endogenous opioid system. These antagonists reduce food intake in deprived rats (Brands et al., 1979; Brown and Holtzman, 1979; Cooper, 1980; Frenk and Rogers, 1979; Holtzman, 1974; Jalowiec et al., 1981), in nocturnal intake situations (Brands et al., 1979; Jalowiec et al., 1981), in glucoprivic rats (Lowy et al., 1980), in genetically-obese rats (Margules et al., 1978; McLaughlin and Baile, 1984a,b; Recant et al., 1980) and in diabetic rats (Levine et al., 1982). Further, general opioid antagonists also reduce palatable forms of food intake, including fat (Arjune and Bodnar, 1990; Cooper et al., 1985; Marks-Kaufman and Kanarek, 1990; Marks-Kaufman et al., 1985) and sucrose (Beczkowska et al., 1992; Levine et al., 1982; Siviy and Reid, 1983). The effects of selective opioid receptor subtype antagonists upon different ingestive situations in young rats include reductions in deprivation-induced intake

by mu and mu_1 antagonists (Arjune et al., 1990; Levine et al., 1991; Simone et al., 1985), but not by kappa, delta or $delta_1$ antagonists (Arjune et al., 1991; Levine et al., 1990; A.S. Levine, personal communication). In contrast, high-fat intake is most potently reduced by kappa and mu antagonists, but not by $delta_1$ or mu_1 antagonists (Arjune and Bodnar, 1990; Arjune et al., 1991; Islam and Bodnar, 1990).

Age-related effects on opioid-mediated ingestion include the loss of mu and kappa agonist-induced stimulation of food intake (Gosnell et al., 1983; Kavaliers and Hirst, 1985; Kavaliers et al.,1985) and the decreased sensitivity to naloxone hypophagia at the onset of nocturnal feeding (Gosnell et al., 1983). Ovariectomy increases naloxone's inhibition of nocturnal intake, an effect reversed by estradiol replacement therapy (Morley et al, 1984). In contrast, neither castration nor testosterone treatment alter naloxone-induced inhibition of nocturnal intake (Wager-Srdar et al., 1987).

Since aging, gender and gonadectomy have each been shown to differentially alter either different opioid peptides or different receptor subtypes, it was important to examine whether these variables would affect different intake situations that are mediated by different opioid receptor subtypes. In addition, deprivation-induced intake and high-fat intake constitute different ecological forms of intake, with the former being a regulatory challenge and the

latter a preference in the absence of a need state. Therefore, the present study examined the dose-dependent (0.25-5 mg/kg) effects of systemic naloxone upon deprivation-induced intake and high-fat intake as a function of age, gender and gonadal status. To this end, separate groups of male and female rats that received either sham or gonadal surgery at three months of age, were subsequently tested for their responsivity to naloxone in these intake situations at four, eight, fourteen or twenty (and above) months of age.

Protocols: Following Experiment 1, the same experimental animals, groups of sham-operated and castrated male rats and sham-operated and ovariectomized female rats, were prepared for Experiment 2. These animals had reached the following ages by the beginning of Experiment 2: 8, 14, and over 20 (20+) months of age. An additional 4 mo. group was added, to serve as a "young" adult comparison group. Following the determination of four days of baseline food intakes and body weights to insure stability, all animals were exposed to five injection conditions at 4-6 h into the light cycle in the deprivation protocol: a) vehicle (1 ml saline/ kg body weight, ip), and naloxone (Sigma Chemical Co.) doses of b) 0.25, c) 1, d) 2.5 and e) 5 mg/kg. In the deprivation protocol, food was removed 24 h prior to the injection. At 30 min following the injection, food was reintroduced, and intake was measured 0.5, 1 and 2 h later.

Intake was determined by weighing food pellets prior to and after each condition and adjusting for spillage, which was collected on paper towels under the wire mesh cage. A 1-week interval elapsed between each of the five injection conditions, and body weight and food intake were reassessed to insure that all animals regained their baseline body weight.

Following the deprivation paradigm, all of the same groups of animals were adapted over four days to the placement of a pre-weighed high-fat diet (67% ground laboratory chow + 33% vegetable shortening; 5.5 kcal/g: 11.3% protein, 61.3% fat, 27.4% carbohydrate: Islam and Bodnar, 1990; Mann et al., 1988) in their cages for 2 h beginning 4-6 h into the light cycle. At weekly intervals, all animals were exposed to the five injection conditions: a) vehicle and naloxone doses of b) 0.25, c) 1, d) 2.5 and e) 5 mg/kg. At 30 min following the injection, fresh servings of the high-fat diet were placed in each rat's cage. Intake, adjusted for spillage, was monitored 1 and 2 h after introduction of the diet. The food was then removed from the cage.

Data Analyses: Two statistical approaches were utilized to analyze the data in terms of magnitude and potency (ID_{50}) of naloxone's inhibitory effect. The magnitude of effect was evaluated using split-plot analyses of variance corrected for repeated measures to assess significant differences

among vehicle and individual (mg/kg) naloxone doses for each condition of intake at each test time as functions of age (4, 8, 14 and 20+ mo.), gender (male and female) and surgery (sham-operated and gonadectomy). The ability of different naloxone doses to inhibit a given intake response at each time point was assessed using Dunn comparisons ($p<.05$). To control for weight differences among groups, each intake score was then corrected according to the formula (intake / 100 g body weight) and the amount of food consumed per kg of body weight was reassessed using split-plot analyses of variance and appropriate comparisons. To analyze differences in naloxone effects across age, gender and gonadal status, a percent inhibition score was calculated for each time point at each naloxone dose by using the formula: 1-(naloxone intake / vehicle intake). Split-plot analyses of variance were calculated among age, gender and gonadectomy groups for each naloxone dose.

The potency of effects was evaluated by constructing log dose response functions by performing linear regression analyses for sham-operated and castrated males and sham-operated and ovariectomized females in each age group. Potency was indicated by the ID_{50} for the peak time (1 h) point at which naloxone was exerting effects. Calculations from the linear regression analyses allowed for the determination of significant differences between slopes and intercepts among gender and gonadectomy groups within given

ages, and across ages for a given gender and gonadectomy group.

RESULTS

Body and Gonadal Weights and Basal Intake: Significant age-related increases in body weight relative to 4-month old rats were found in sham-operated males (8, 14 and 20+ month groups), castrated males (14 and 20+ month groups), sham-operated females (20+ month group) and ovariectomized females (8, 14 and 20+ month groups) (Table 3). The body weights of male rats were significantly greater than those of female rats across all age groups. Whereas castrated males failed to differ significantly from sham-operated males in body weight across age groups, ovariectomized females weighed significantly more than sham-operated females in the 8, 14 and 20+ month groups. The seminal vesicle weight of castrated male rats was significantly less (86% reduction) than sham-operated males (Table 4). Whereas castrated males failed to show vesicle weight differences across age groups, the seminal vesicle weights of sham-operated males aged 8 and 14 months were significantly greater than those aged 4 months. The fallopian tube weight of ovariectomized female rats was significantly less (88% reduction) than that of sham-operated females (Table 4). Whereas ovariectomized females failed to show fallopian tube weight differences across age groups, the weights of sham-operated females aged 14 months were significantly greater

TABLE 3. Alterations in body weights (g) following vehicle treatment as functions of age, gender and gonadal status.

AGE (months)		SHAM MALES	CASTRATES	SHAM FEMALES	OVEXS
4	Mean	471.2	460.4	288.8*	345.4
	SEM	16.1	9.5	6.5	9.8
	n	11	10	11	11
8	Mean	562.4+	502.5	325.2*	411.5+#
	SEM	13.4	13.5	10.7	10.6
	n	10	10	10	10
14	Mean	586.5+	547.6+	328.9*	439.2+#
	SEM	19.3	15.8	12.3	17.3
	n	10	9	8	9
20+	Mean	649.9+	697.5+	451.8+*	537.5+#
	SEM	28.9	29.2	21.4	32.6
	n	9	13	17	20

Significant differences in body weight were observed across age groups ($F(3,162)= 74.84$, $p<.0001$), between males and females ($F(1,162)= 282.06$, $p<.0001$), between sham-operated and gonadectomized animals ($F(1,162)= 11.88$, $p<.0007$) and for the interaction between gender and gonadectomy ($F(1,162)= 24.88$, $p<.0001$). +: denotes significant age difference relative to corresponding rats aged 4 mo.; *: denotes significant gender difference relative to same-aged male rats; #: denotes significant gonadectomy difference relative to same-aged and same-gender sham-operated control.

TABLE 4. Alterations in gonadal weights (g) following vehicle treatment as functions of age, gender and gonadal status.

AGE (months)		SHAM MALES	CASTRATES	SHAM FEMALES	OVEXS
4	Mean	0.52	0.07#	0.59	0.07#
	SEM	0.03	0.01	0.04	0.01
	n	11	10	10	10
8	Mean	0.77*	0.11#	0.65	0.11#
	SEM	0.07	0.01	0.04	0.01
	n	10	9	10	9
14	Mean	0.72*	0.06#	0.83*	0.07#
	SEM	0.04	0.01	0.06	0.01
	n	8	9	8	9
20+	Mean	0.62	0.12#	0.60	0.06
	SEM	0.04	0.04	0.06	0.01
	n	9	8	11	8

Significant differences in the weight of the seminal vesicles in males were observed across age groups ($F(3,65)= 3.33$, $p<.015$), between sham-operated and gonadectomized animals ($F(1,65)= 198.75$, $p<.0001$), and for the interaction between age and gonadectomy ($F(3,65)= 2.55$, $p<.048$). Significant differences in the weight of the fallopian tubes in females were observed across age groups ($F(3,65)= 3.03$, $p<.024$), between sham-operated and gonadectomized animals ($F(1,65)= 361.42$, $p<.0001$), and for the interaction between age and gonadectomy ($F(3,65)= 3.47$, $p<.012$). +: denotes significant age difference relative to corresponding rats aged 4 mo.; *: denotes significant gender difference relative to same-aged male rats; #: denotes significant gonadectomy difference relative to same-aged and same-gender sham control. The differences in sample sizes between body weight and gonadal weight analyses reflect the loss of gonadal tissue through either death of the animal or methodological problems connected with removal of gonadal tissue.

than those aged 4 months.

Significant age-related decreases in deprivation-induced food intake relative to 4-month old rats were found in sham-operated males (8, 14 and 20+ month groups), castrated males (8, 14 and 20+ month groups), and ovariectomized females (14 and 20+ month groups) (Table 5). Deprivation-induced intake of male rats at 4 and 14 months was significantly greater than that of corresponding female rats. Whereas castrated and sham-operated males failed to differ significantly, deprivation-induced intake of ovariectomized females at 4 months was significantly greater than that of corresponding sham-operated females (Table 5).

Only female rats displayed significant age-related increases in high-fat intake at 8 and 14 months of age relative to the 4-month group (Table 6).

Naloxone and Raw Deprivation-Induced and High-Fat Intake: Table 7 summarizes the significant effects of naloxone's inhibition of raw intake following food deprivation and exposure to high-fat. Since there were significant differences in body weights and basal intakes among groups, the two intake variables were adjusted as a function of body weight (g intake per 100 g body weight).

Naloxone and Weight-Adjusted Deprivation-Induced Intake: Significant age-related decreases in weight-adjusted vehicle intake occurred in sham-operated and castrated males at 8, 14 and 20+ months, in sham-operated

TABLE 5. Alterations in deprivation-induced food intake (1h, g) following vehicle treatment as functions of age, gender and gonadal status.

AGE (months)		SHAM MALES	CASTRATES	SHAM FEMALES	OVEXS
4	Mean	6.7	7.2	3.7*	5.4#
	SEM	0.5	0.5	0.5	0.2
8	Mean	5.2+	4.4+	4.8	4.5
	SEM	0.4	0.4	0.3	0.1
14	Mean	4.7+	4.6+	3.1*	3.8+
	SEM	0.4	0.1	0.3	0.3
20+	Mean	3.5+	3.6+	3.6	3.0+
	SEM	0.3	0.5	0.5	0.4

Significant differences in basal deprivation-induced food intake after 1 h were observed across age groups ($F(3,162)= 33.70$, $p<.0001$), between males and females ($F(1,162)= 30.33$, $p<.0001$) and for the interactions between age and gender ($F(3,162)= 8.73$, $p<.0001$) and age and gonadectomy ($F(3,162)= 4.27$, $p<.006$). Similar changes in basal deprivation-induced food intake occurred at 0.5 and 2 h as well. +: denotes significant age difference relative to corresponding rats aged 4 mo.; *: denotes significant gender difference relative to same-aged male rats; #: denotes significant gonadectomy difference relative to same-aged and same-gender sham-operated control.

TABLE 6. Alterations in high-fat intake (1h, g) following vehicle treatment as functions of age, gender and gonadal status.

AGE (months)		SHAM MALES	CASTRATES	SHAM FEMALES	OVEXS
4	Mean	7.5	6.9	6.0	7.6
	SEM	0.6	0.7	0.6	0.6
8	Mean	7.1	7.4	9.0+	8.9
	SEM	0.6	0.6	0.9	0.9
14	Mean	9.3	8.0	8.4+	7.2
	SEM	0.8	0.7	0.5	0.6
20+	Mean	6.1	7.6	7.1	6.2
	SEM	0.6	0.9	0.6	0.7

Significant differences in basal high-fat intake after 1 h were only observed across age groups ($F(3,155)= 4.51$, $p<.005$). Similar effects upon high-fat intake occurred at 2 h. +: denotes significant age difference relative to corresponding rats aged 4 mo.; *: denotes significant gender difference relative to same-aged male rats; #: denotes significant gonadectomy difference relative to same-aged and same-gender sham-operated control.

TABLE 7. Summary of analyses of variance data of naloxone's effects upon raw intake following food deprivation and exposure to a high-fat diet.

CONDITION	FOOD DEPRIVATION	HIGH-FAT INTAKE
Age	F(3,162)=37.00, p<.0001	F(3,155)=6.12, p<.0006
Gender	F(1,162)=26.38, p<.0001	ns
Surgery	ns	ns
Drug	F(4,648)=271.93, p<.0001	F(4,620)=369.13, p<.0001
A x G	F(3,162)=13.58, p<.0001	ns
A x S	ns	ns
G x S	ns	ns
A x D	F(12,648)=3.35, p<.0001	ns
G x D	F(4,648)=3.76, p<.005	ns
S x D	ns	ns
A x G x S	ns	ns
A x G x D	ns	F(12,620)=2.88, p<.0007
A x S x D	F(12,648)=1.85, p<.038	F(12,620)=1.94, p<.027
G x S x D	F(4,648)=3.64, p<.006	F(4,620)=2.81, p<.025
A x G x S x D	ns	ns

ns: non-significant (p>.05). Similar effects were observed for deprivation-induced raw intake at 0.5 and 2 h and for high-fat raw intake at 2 h.

females at 14 and 20+ months, and in ovariectomized females at 8, 14 and 20+ months relative to corresponding 4-month old groups. Vehicle intake significantly increased in sham-operated females at 8 months (Table 8). Weight-adjusted vehicle intake was significantly higher in female rats aged 8 and 20+ months than in corresponding males and ovariectomized females. All naloxone doses significantly reduced deprivation-induced food intake in all groups except in sham-operated and castrated males aged 20+ months, in sham-operated females aged 14 months and in ovariectomized females aged 14 and 20+ months. In these latter groups, the lowest (0.25 mg/kg) dose of naloxone was ineffective. (Table 8).

Naloxone and Weight-Adjusted High-Fat Intake: Significant age-related decreases in weight-adjusted vehicle intake occurred in sham-operated males and females at 20+ months and in ovariectomized females at 14 and 20+ months relative to corresponding 4-month old groups. Vehicle intake significantly increased in sham-operated females at 8 and 14 months (Table 9). Weight-adjusted vehicle intake of sham-operated females was significantly higher than either male rats across all ages or ovariectomized females at 8, 14 and 20+ months. All naloxone doses significantly reduced high-fat intake in all groups except for sham-operated males aged 20+ months. In this latter group, the lowest (0.25 mg/kg) dose of naloxone was ineffective.

TABLE 8. Alterations in weight-adjusted deprivation-induced food intake (g, SEM) following vehicle and naloxone as functions of age, gender and gonadal status.

GROUP	NALOXONE DOSE (mg/kg)				
	0	0.25	1.0	2.5	5.0
AGE: 4 months					
Sham Males	1.45	1.10$	0.92$	0.76$	0.72$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
Castrated Males	1.57	0.96$	0.88$	0.56$	0.62$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
Sham Females	1.27	0.73$	0.76$	0.44$	0.70$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
Ovex Females	1.58#	0.91$	0.79$	0.53$	0.46$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
AGE: 8 months					
Sham Males	0.94+	0.65$	0.45$	0.39$	0.32$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
Castrated Males	0.87+	0.63$	0.63$	0.39$	0.30$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
Sham Females	1.50+*	1.02$	0.88$	0.80$	0.58$
	(0.1)	(0.2)	(0.1)	(0.1)	(0.1)
Ovex Females	1.09+#	0.79$	0.49$	0.43$	0.28$
	(0.1)	(0.2)	(0.1)	(0.1)	(0.1)
AGE: 14 months					
Sham Males	0.81+	0.52$	0.40$	0.28$	0.23$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
Castrated Males	0.84+	0.49$	0.45$	0.43$	0.41$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
Sham Females	0.94+	0.78	0.74$	0.42$	0.56$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
Ovex Females	0.88+	0.71	0.50$	0.37$	0.34$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
AGE: 20+ months					
Sham Males	0.54+	0.38	0.27$	0.20$	0.18$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
Castrated Males	0.53+	0.37	0.28$	0.17$	0.13$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
Sham Females	0.82+*	0.44$	0.42$	0.43$	0.35$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
Ovex Females	0.56+#	0.39	0.29$	0.26$	0.15$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)

The weight adjustments in deprivation-induced food intake were based upon the formula: g intake per 100 g body weight. $: denotes significant drug difference relative to corresponding vehicle treatment; +: denotes significant age difference relative to corresponding rats aged 4 mo.; *: denotes significant gender difference relative to same-aged male rats; #: denotes significant gonadectomy difference relative to same-aged and same-gender sham-operated control. Significant differences in weight-adjusted deprivation-

induced food intake after 1 h were observed across age groups ($F(3,162)= 59.00$, $p<.0001$), between males and females ($F(1,162)= 7.43$, $p<.007$), between sham-operated and gonadectomized animals ($F(1,162)= 5.05$, $p<.026$), among vehicle and naloxone doses ($F(4,648)= 246.02$, $p<.0001$) and for the interactions between age and gender ($F(3,162)= 6.62$, $p<.0003$), gender and gonadectomy ($F(1,162)= 5.03$, $p<.026$), age, gender and gonadectomy ($F(3,162)= 2.91$, $p<.036$), age, dose and gonadectomy ($F(12,648)= 1.96$, $p<.025$) and gender, gonadectomy and dose ($F(4,648)= 2.62$, $p<.034$). Similar effects were observed for intakes at 0.5 and 2 h.

TABLE 9. Alterations in weight-adjusted high-fat intake (g, SEM) following vehicle and naloxone as functions of age, gender and gonadal status.

GROUP	NALOXONE DOSE (mg/kg)				
	0	0.25	1.0	2.5	5.0
AGE: 4 months					
Sham Males	1.38	0.81$	0.54$	0.41$	0.25$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
Castrated Males	1.35	0.77$	0.59$	0.61$	0.39$
	(0.2)	(0.1)	(0.1)	(0.1)	(0.1)
Sham Females	1.87*	1.14$	1.03$	0.73$	0.83$
	(0.2)	(0.2)	(0.2)	(0.2)	(0.4)
Ovex Females	1.87	0.87$	0.48$	0.39$	0.37$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
AGE: 8 months					
Sham Males	1.17	0.82$	0.65$	0.51$	0.43$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
Castrated Males	1.35	0.71$	0.60$	0.53$	0.47$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
Sham Females	2.33+*	1.55$	1.16$	0.91$	0.71$
	(0.2)	(0.2)	(0.1)	(0.1)	(0.1)
Ovex Females	1.90#	0.88$	0.38$	0.36$	0.34$
	(0.2)	(0.1)	(0.1)	(0.1)	(0.1)
AGE: 14 months					
Sham Males	1.47	0.88$	0.56$	0.49$	0.33$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
Castrated Males	1.36	0.95$	0.61$	0.73$	0.54$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
Sham Females	2.34+*	1.16$	1.23$	0.98$	0.94$
	(0.2)	(0.1)	(0.3)	(0.2)	(0.1)
Ovex Females	1.50+#	1.02$	0.91$	0.59$	0.64$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
AGE: 20+ months					
Sham Males	0.95+	0.81	0.57$	0.42$	0.33$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
Castrated Males	1.18	0.88$	0.54$	0.46$	0.37$
	(0.3)	(0.1)	(0.1)	(0.1)	(0.1)
Sham Females	1.56+*	0.80$	0.68$	0.72$	0.53$
	(0.1)	(0.1)	(0.1)	(0.2)	(0.1)
Ovex Females	1.19+#	0.73$	0.53$	0.32$	0.26$
	(0.2)	(0.1)	(0.1)	(0.1)	(0.1)

The weight adjustments in high-fat intake were based upon the formula: g intake per 100 g body weight. $: denotes significant drug difference relative to corresponding vehicle treatment; +: denotes significant age difference relative to corresponding rats aged 4 mo.; *: denotes significant gender difference relative to same-aged male rats; #: denotes significant gonadectomy difference relative to same-aged and same-gender sham-operated controls.

Significant differences in weight-adjusted high-fat intake after 1 h were found across age groups ($F(3,155)= 5.14$, $p<.002$), between males and females ($F(1,155)= 22.93$, $p<.0001$), between sham-operated and gonadectomized animals ($F(1,155)= 9.67$, $p<.002$), among vehicle and naloxone doses ($F(4,620)= 344.57$, $p<.0001$) and for the interactions between gender and gonadectomy ($F(1,155)= 18.13$, $p<.0001$), age and dose ($F(12,620)= 2.77$, $p<.001$), gender and dose ($F(4,620)= 13.54$, $p<.0001$), age, gender and dose ($F(12,620)= 2.56$, $p<.003$) and age, gonadectomy and dose ($F(12,620)= 2.12$, $p<014$). Similar effects were observed for intakes at 2 h.

Analysis of Age, Gender and Gonadectomy Effects: As indicated above, there were significant age-related, gender-related and gonadectomy-related differences in both raw and weight-adjusted intake following both food deprivation and high-fat ingestion. Thus, while comparisons of naloxone effects for a given group could be made with the corresponding vehicle score, the age-, gender- and gonadectomy-induced changes in basal intake precluded analyses of naloxone effects across groups. Therefore, naloxone effects upon deprivation-induced intake and high-fat intake were analyzed by transforming the weight-adjusted intakes to a percent inhibition of control intake by using the formula: 1 - (naloxone dose intake / vehicle intake). The percent inhibition scores at each time point were then used in assessing age, gender and gonadectomy effects across naloxone doses in analyses of variance for each form of intake. Then, regression analyses were used to assess whether age, gender or gonadectomy effects altered the ID_{50} of naloxone's dose-response curve for each form of intake.

Age, Gender and Gonadectomy Effects upon Naloxone-induced Inhibition of Deprivation-induced Intake: Age, gender and gonadectomy failed to alter naloxone's inhibition of deprivation-induced intake at doses of 0.25, 1 and 2.5 mg/kg. However, at the 5 mg/kg dose of naloxone, sham-operated males displayed significantly greater inhibition after 0.5 h at 8 and 14 months of age relative to 4 months

of age (Table 10). Male rats also displayed significantly greater inhibition after 0.5 and 1 h than female rats at 14 months of age (Figure 5, Table 10A). Finally, significant increases in inhibition were noted in castrated males (0.5 h) and ovariectomized females (0.5-1 h) at 4 months of age relative to corresponding sham-operated controls (Figure 5, Table 10A).

Regression analyses revealed that the potency of naloxone's inhibition of deprivation-induced intake was less in sham-operated female rats (ID_{50}= 10.7 mg/kg) relative to ovariectomized females (ID_{50}= 0.8 mg/kg) or sham-operated males (ID_{50}= 4.5 mg/kg). In turn, naloxone's potency appeared to be greater in castrated males (ID_{50}= 1.9 mg/kg) relative to sham-operated males (Table 11A).

Age, Gender and Gonadectomy Effects upon Naloxone-induced Inhibition of High-Fat Intake: At the 0.25 mg/kg dose of naloxone, age-related decreases were noted in sham-operated males (20+ months: 1 h), castrated males (14 months: 2 h; 20+ months: 1-2 h), sham-operated females (8 months: 2 h) and ovariectomized females (14 months: 1-2 h; 20+ months: 2 h). At the 1, 2.5 and 5 mg/kg doses of naloxone, age-related decreases were noted in castrated males (14 months: 2 h) and ovariectomized females (14 months: 1-2 h) (Figure 6, Table 12). Whereas sham-operated females aged 20+ months displayed significantly greater inhibition than corresponding sham-operated males following

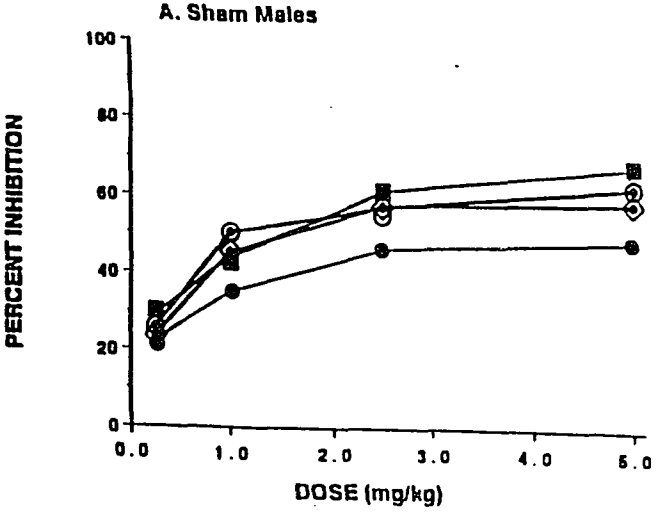
TABLE 10. Alterations in naloxone's inhibition of deprivation-induced intake (0.5 and 2 h) as functions of age, gender and gonadal status.

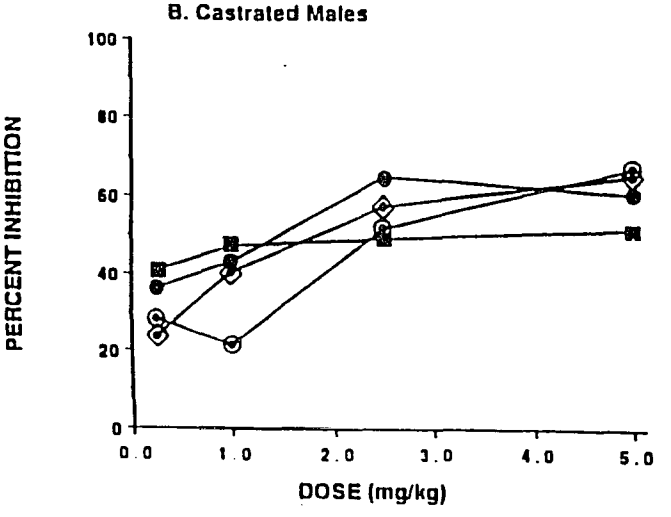
GROUP	NALOXONE DOSE (mg/kg) 0.25	1.0	2.5	5.0
A. DEPRIVATION-INDUCED INTAKE: 0.5 h:				
4 months				
Sham Males	12.3	27.4	34.4	34.6
Castrated Males	37.0	39.3	62.2	60.5#
Sham Females	40.3	29.0	54.7	37.8
Ovariectomized Females	33.7	46.8	63.1	71.2#
8 months				
Sham Males	28.4	52.5	62.4	66.3+
Castrated Males	28.8	30.4	61.1	71.5
Sham Females	40.4	40.8	43.1	61.4
Ovariectomized Females	37.9	64.8	64.3	73.5
14 months				
Sham Males	34.1	48.6	62.2	71.6+
Castrated Males	43.9	62.6	54.8	53.1
Sham Females	29.3	34.8	47.6	41.3*
Ovariectomized Females	20.7	43.2	54.7	61.4
20+ months				
Sham Males	33.2	42.9	54.2	55.4
Castrated Males	15.0	39.5	56.5	65.3
Sham Females	48.2	49.6	45.2	55.1
Ovariectomized Females	8.0	28.0	39.2	73.1
B. DEPRIVATION-INDUCED INTAKE: 2 h:				
4 months				
Sham Males	23.3	37.5	50.4	54.0
Castrated Males	37.0	34.1	61.0	54.9
Sham Females	33.4	44.5	61.6	45.0
Ovariectomized Females	38.2	42.8	62.9	68.3
8 months				
Sham Males	21.4	24.7	43.9	58.7
Castrated Males	20.1	24.9	56.6	60.9
Sham Females	32.6	43.4	51.7	67.9
Ovariectomized Females	29.2	56.0	56.1	68.5
14 months				
Sham Males	33.9	44.2	65.6	69.6
Castrated Males	32.1	39.0	50.9	49.3
Sham Females	39.9	49.6	62.4	58.7
Ovariectomized Females	26.7	41.9	53.8	59.4
20+ months				
Sham Males	32.7	48.0	63.3	56.1
Castrated Males	27.4	41.2	60.1	66.3
Sham Females	31.5	40.8	44.7	53.2
Ovariectomized Females	19.5	32.8	48.0	73.9

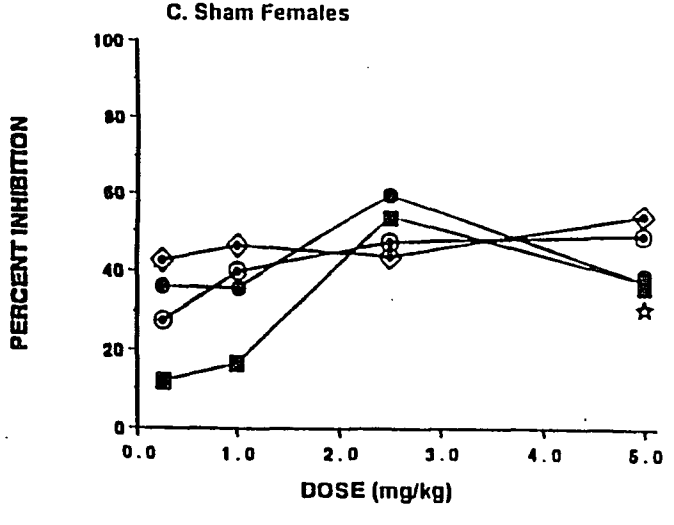
Significant differences in naloxone's inhibitory effects

failed to occur at either 0.5, 1 or 2 h across age, gender and gonadectomy groups following naloxone doses of 0.25, 1 and 2.5 mg/kg. Significant differences in naloxone inhibition were found at 0.5 h following the 5 mg/kg dose as functions of age ($F(3,162)= 3.04$, $p<.031$) and gonadectomy ($F(1,162)= 10.03$, $p<.002$). +: denotes significant age difference relative to corresponding rats aged 4 mo.; *: denotes significant gender difference relative to same-aged male rats; #: denotes significant gonadectomy difference relative to same-aged and same-gender sham-operated controls.

Figure 5. Alterations in the inhibitory actions of naloxone upon deprivation-induced intake after 1 h as functions of gender (males: Panels A and B; females: Panels C and D), gonadectomy (sham-operated: Panels A and C; gonadectomized: Panels B and D) and age (4 months: closed circles; 8 months: open circles; 14 months: closed squares; 20+ months: open diamonds). The naloxone effects in this and the subsequent figure are expressed as percent inhibition which was calculated by the formula: 1- (naloxone intake / vehicle intake). Significant differences in naloxone inhibition were observed at 1 h following the 5 mg/kg dose as functions of gonadectomy ($F(1,162)= 7.91$, $p<.0062$) and the interaction between gender and gonadectomy ($F(1,162)= 5.60$, $p<.019$). Significant age-related differences are denoted by solid stars (Dunnett comparisons, $p<.05$). Significant gender-related and gonadectomy-related differences are respectively denoted by open stars and enclosed stars (Dunn comparisons, $p<.05$).


A. Sham Males
PERCENT INHIBITION
100
80
60
40
20
0
0.0
1.0
2.0
3.0
4.0
5.0
DOSE (mg/kg)


B. Castrated Males
PERCENT INHIBITION
100
80
60
40
20
0
0.0
1.0
2.0
3.0
4.0
5.0
DOSE (mg/kg)


C. Sham Females
PERCENT INHIBITION
100
80
60
40
20
0
0.0
1.0
2.0
3.0
4.0
5.0
DOSE (mg/kg)

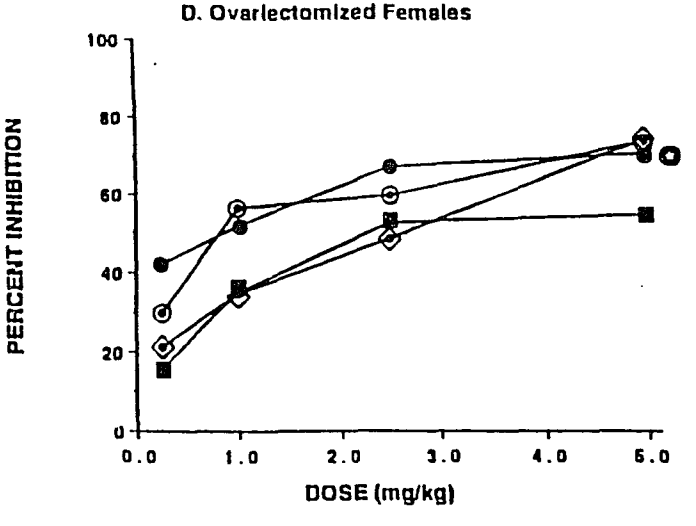
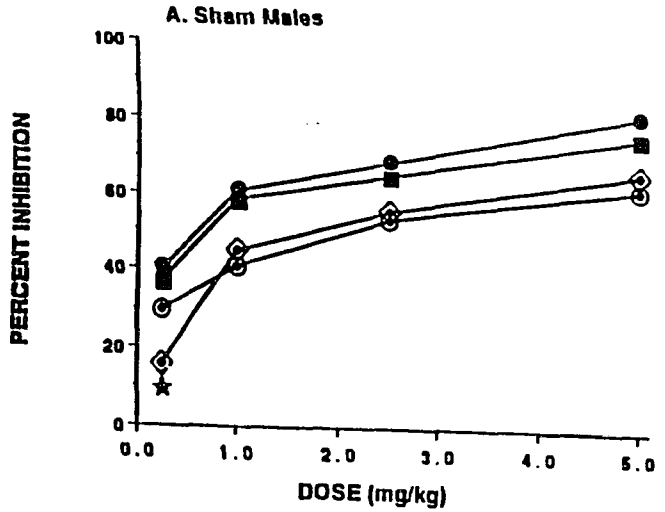

D. Ovariectomized Females
PERCENT INHIBITION
100
80
60
40
20
0
0.0
1.0
2.0
3.0
4.0
5.0
DOSE (mg/kg)

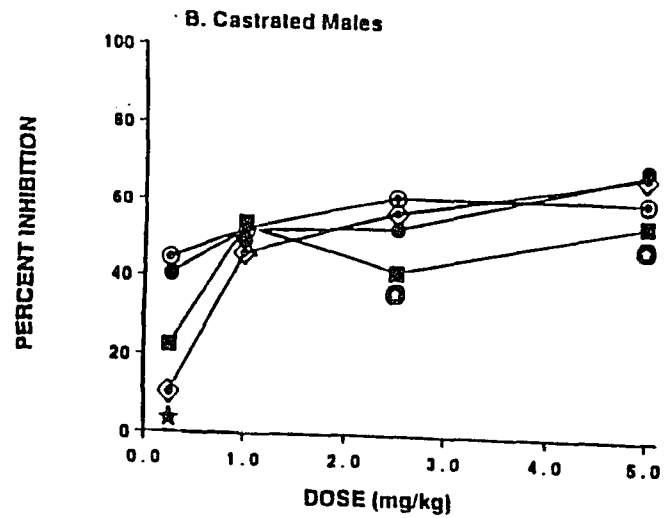
TABLE 11. Alterations in naloxone dose-response function (ID_{50}: mg/kg) for deprivation-induced and high-fat intake (1 h) as functions of age, gender, and gonadal status.

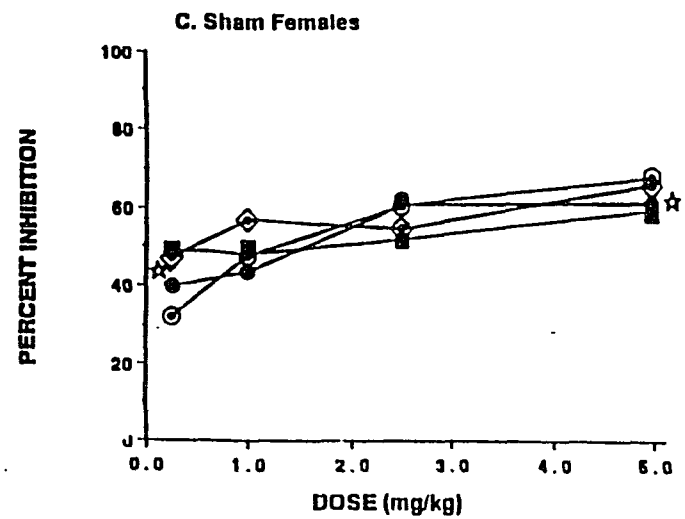
GROUP	AGE (months) 4	8	14	20+	Age Sig.
A. Deprivation Intake:					
Sham Males	4.5	2.3	2.1	2.8	n.s.
Castrate Males	1.9	3.0	3.5	2.6	n.s.
Sham Females	10.7	3.2	5.4	3.6	n.s.
Ovex Females	0.8	1.5	3.5	2.6	n.s.
Gender/ Gonadectomy sig.	p<.01	n.s.	n.s.	n.s.	
B.High-Fat Intake:					
Sham Males	0.5	2.7	0.9	2.6	p<.01
Castrate Males	1.5	0.7	3.7	2.6	n.s.
Sham Females	1.9	1.9	1.2	0.3	n.s.
Ovex Females	0.3	0.3	2.9	1.1	p<.01
Gender/ Gonadectomy sig.	p<.01	p<.01	n.s.	p<.01	

Significant differences in naloxone's dose-response curve for inhibition of deprivation-induced intake were found only across groups at 4 months of age ($F(6,164)= 3.54$, $p<.003$). Significant differences in naloxone's dose-response curve for inhibition of high-fat intake were found across groups at 4 ($F(6,160)= 4.06$, $p<.0001$), 8 ($F(6,152)= 4.74$, $p<.001$) and 20+ ($F(6,208)= 3.43$, $p<.003$) months of age, and across ages for sham-operated males ($F(6,152)= 3.51$, $p<.003$) and ovariectomized females ($F(6,176)= 7.09$, $p<.0001$).

Figure 6. Alterations in the inhibitory actions of naloxone upon high-fat intake after 1 h as functions of gender (males: Panels A and B; females: Panels C and D), gonadectomy (sham-operated: Panels A and C; gonadectomized: Panels B and D) and age (4 months: closed circles; 8 months: open circles; 14 months: closed squares; 20+ months: open diamonds).


A. Sham Males
PERCENT INHIBITION
100
80
60
40
20
0
0.0
1.0
2.0
3.0
4.0
5.0
DOSE (mg/kg)


B. Castrated Males
PERCENT INHIBITION
100
80
60
40
20
0
0.0
1.0
2.0
3.0
4.0
5.0
DOSE (mg/kg)


C. Sham Females
PERCENT INHIBITION
100
80
60
40
20
0
0.0
1.0
2.0
3.0
4.0
5.0
DOSE (mg/kg)

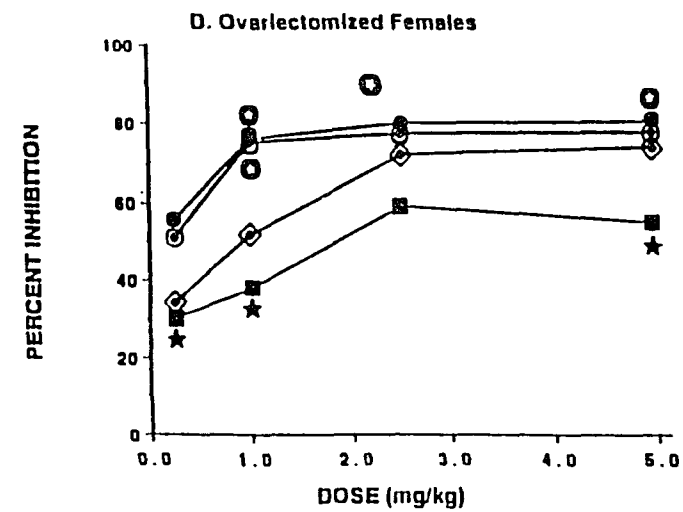

D. Ovariectomized Females
PERCENT INHIBITION
100
80
60
40
20
0
0.0
1.0
2.0
3.0
4.0
5.0
DOSE (mg/kg)

TABLE 12. Alterations in naloxone's inhibition of high-fat intake (2 h) as functions of age, gender and gonadal status.

4 months				
Sham Males	44.2	54.2	67.9	82.2
Castrated Males	47.1	60.2	60.3	70.9
Sham Females	43.3	49.6	57.5	61.5*
Ovariectomized Females	56.6	67.2	75.1	76.6
8 months				
Sham Males	36.5	38.6	50.8	64.5
Castrated Males	32.7	47.0	55.2	62.9
Sham Females	17.6+	39.4	64.5	65.4
Ovariectomized Females	39.5	62.2#	66.1	72.9
14 months				
Sham Males	29.5	52.6	54.5	71.5
Castrated Males	17.9+	32.6+	39.0+	48.2+#
Sham Females	42.0	41.8	54.3	53.1
Ovariectomized Females	21.1+	18.7+#	55.1+	48.1+
20+ months				
Sham Males	22.2	47.5	60.6	67.6
Castrated Males	18.9+	52.2	59.7	68.9
Sham Females	46.7*	53.0	56.7	66.0
Ovariectomized Females	31.9+	52.9	71.2	75.5

Significant differences in naloxone inhibition at the 0.25 mg/kg dose were found across age groups (1 h: $F(3,155)= 3.72$, $p<.013$; 2 h: $F= 5.99$, $p<.0007$), between males and females (1 h: $F(1,155)= 9.83$, $p<.002$) and for the interaction between age and gonadectomy (1 h: $F(3,155)= 3.50$, $p<.017$; 2 h: $F= 2.65$, $p<.05$). Significant differences in naloxone inhibition at the 1 mg/kg dose were found across age groups (2 h: $F(3,155)= 6.40$, $p<.0004$) and for the interaction between age and gonadectomy (1 h: $F(3,155)= 2.92$, $p<.036$; 2 h: $F= 5.28$, $p<.002$). Significant differences in naloxone inhibition at the 2.5 mg/kg dose were found across age groups (2 h: $F(3,155)= 3.22$, $p<.024$), between males and females (1 h: $F(1,155)= 4.71$, $p<.032$; 2 h: $F= 3.87$, $p<.05$) and for the interaction between gender and gonadectomy (1 h: $F(3,155)= 10.16$, $p<.002$; 2 h: $F= 4.11$, $p<.044$). Significant differences in naloxone inhibition at the 5 mg/kg dose were found across age groups (2 h: $F(3,155)= 5.00$, $p<.002$) and for the interaction between gender and gonadectomy (1 h: $F(3,155)= 5.82$, $p<.017$; 2 h: $F= 5.61$, $p<.019$). +: denotes significant age difference relative to corresponding rats aged 4 mo.; *: denotes significant gender difference relative to same-aged male rats; #: denotes significant gonadectomy difference relative to same-aged and same-gender sham-operated controls.

the 0.25 mg/kg dose of naloxone, sham-operated females aged 4 months displayed significantly less inhibition (1-2 h) at the 5 mg/kg dose (Figure 6, Table 12). Castrated males displayed significantly less inhibition (1 h) at the 2.5 and 5 mg/kg doses than corresponding sham-operated controls at 14 months of age (Figure 6, Table 12). Ovariectomized females displayed significantly greater inhibition than corresponding sham-operated females at 4 (5 mg/kg dose) and 8 (1 mg/kg) months of age, but significantly less inhibition at 14 (1 mg/kg) months of age (Figure 6, Table 12).

Regression analyses revealed that the potency of naloxone's inhibition of high-fat intake increased in sham-operated female rats as a function of age, and decreased in sham-operated males and ovariectomized females as a function of age (Table 11B). Whereas sham-operated males and ovariectomized females were most sensitive to naloxone's inhibition of high-fat intake at young ages, sham-operated females were most sensitive at older ages.

DISCUSSION

The present study was designed to determine whether aging, gender and gonadectomy alter the hypophagic properties of naloxone upon food deprivation-induced feeding and high-fat feeding. In this section, first, the effects of aging, gender and gonadectomy upon body weight and food intake under basal and weight-adjusted conditions will be evaluated. Then, aging, gender and gonadectomy effects upon

naloxone-induced inhibition of deprivation-induced and high fat intake will be evaluated.

A. Aging, Gender and Gonadectomy Effects upon Body Weight and Food Intake: Male rats weighed significantly more than female rats across all age groups, confirming previous observations (e.g., Bell and Zucker, 1971; Leshner and Collier, 1973). Whereas castration failed to alter body weight across age groups, older (8-20+ months) ovariectomized females weighed significantly more than their sham-operated counterparts, confirming previous studies (e.g.,Bell and Zucker, 1971; Blaustein and Wade, 1976; Gentry and Wade, 1976; McElroy and Wade, 1987; Tartellin and Gorski, 1973; Wade, 1972). Also consistent with earlier studies (e.g., Brain, 1968; Jakubczak, 1976), aging increased body weight in all groups. However, the rate of increase was slower in sham-operated females and castrated males than in ovariectomized females and sham-operated males.

Food intake differences depended upon both the type of intake and the consideration of basal or weight-adjusted intake. Deprivation-induced feeding decreased with age in sham-operated males, castrated males and ovariectomized females under both basal and weight-adjusted intake conditions. Aging in sham-operated females only reduced weight-adjusted deprivation-induced intake. Thus, older rats appear less capable of responding to deprivation challenges;

this effect occurred in both sham-operated and gonadectomized males and females. Gender differences in deprivation-induced intake were partly dependent upon body weight differences. Young (4 months) female rats ate less than male or ovariectomized rats following deprivation. However, in weight-adjusted analyses, sham-operated females (8 and 20+ months) actually ate more than either sham-operated males or ovariectomized females following deprivation. A different pattern of basal intake effects occurred in the high-fat paradigm. Sham-operated females at all ages consumed greater quantities of fat than males and ovariectomized females in both basal and weight-adjusted conditions, effects which are consistent with "supermarket" diet intake (Sclafani and Gorman, 1977). Aging increased fat intake in sham-operated females aged 8 and 14 months, but decreased fat intake in the oldest (20+ months) group. Thus, hyperphagia following high-fat intake appeared to be less sensitive to aging than deprivation intake, and both forms of intake were dependent upon weight factors. Hence, analyses of naloxone effects were performed on the weight-adjusted data to allow more direct comparisons of aging, gender and gonadectomy differences. This strategy was previously employed in assessing age differences in nocturnal intake (Gosnell et al., 1983). Interestingly, intact female rats showed better adjustment to deprivation and better ability to maintain palatable intake as they aged

than other groups, when intake is adjusted for weight. Also, for age groups of over eight months, weight-adjusted vehicle high fat intake in females was greater than in males and ovariectomized females.

B. Aging, Gender and Gonadectomy Effects upon Naloxone's Inhibition of Deprivation-Induced and High-Fat Intakes: In both basal and weight-adjusted analyses, naloxone (1-5 mg/kg) significantly reduced both deprivation-induced and high-fat intake, confirming previous studies (Arjune and Bodnar, 1990; Brands et al., 1979; Brown and Holtzman, 1979; Cooper, 1980; Cooper et al., 1985; Frenk and Rogers, 1979; Jalowiec et al., 1981; Marks-Kaufman and Kanarek, 1990; Marks-Kaufman et al., 1985). The lowest (0.25 mg/kg) dose failed to inhibit deprivation-induced intake in the oldest sham-operated male, castrated male and ovariectomized female groups, and in sham-operated and ovariectomized females aged 14 months. This dose also failed to inhibit high-fat intake in the oldest sham-operated male group. Whereas age, gender and gonadectomy failed to alter naloxone's inhibition of deprivation-induced intake at lower (0.25-2.5 mg/kg) doses, the highest naloxone dose produced significant age-related inhibition in sham-operated males, and greater inhibition in sham-operated males than in sham-operated females. Gonadectomy significantly increased naloxone's inhibition in young castrated males and ovariectomized females. Differences in naloxone's potency in

inhibiting deprivation-induced intake were only observed in young (4 month) rats, with a rank order of ovariectomized females (ID_{50}= 0.8 mg/kg), castrated males (ID_{50}= 1.9 mg/kg), sham-operated males (ID_{50}= 4.5 mg/kg) and sham-operated females (ID_{50}= 10.7 mg/kg).

In contrast, naloxone's inhibition of high-fat intake differed across a range of doses as a function of age, gender and gonadal status. Whereas sham-operated males and females displayed age-related decreases in naloxone's inhibitory actions on high-fat intake only following the lowest dose, castrated males and ovariectomized females displayed age-related decreases across all naloxone doses. Gender differences in naloxone's inhibition of high-fat intake varied as a function of age, with sham-operated females (20+ months) displaying greater inhibition than same-age sham-operated males following the lowest naloxone dose, and sham-operated females (4 months) displaying less inhibition than same-age males following the highest dose. Castrated males and ovariectomized females displayed less inhibition of high-fat intake than corresponding sham-operated controls at 14 months of age, but ovariectomized females displayed greater inhibition at 4 and 8 (1 mg/kg) months of age. The potency of naloxone's inhibition of high-fat intake tended to increase in sham-operated female rats as a function of age, and decreased in sham-operated males and ovariectomized females as a function of age.

These data indicate that there is a clear dissociation between deprivation-induced intake and high-fat intake in terms of age-related, gender-related and gonadectomy-related changes in naloxone's inhibitory actions. Whereas these variables affected the inhibitory action of naloxone upon deprivation-induced intake at only its highest dose, they altered naloxone's inhibition of high-fat intake across the dose-response curve. This suggests that unlike deprivation-induced feeding, opioid control of high fat intake is altered by these organismic variables, with this system being compromised in older males and ovariectomized females, and enhanced in effectiveness in intact females. Whereas aging increased naloxone's inhibition of deprivation-induced intake in sham-operated males and ovariectomized females, aging decreased naloxone's inhibition of high-fat intake in these same groups. Further, aging increased naloxone's inhibition of high-fat intake, but not deprivation-induced intake in sham-operated females. Hence, there are interactive effects between aging, gender and gonadectomy in determining the extent and direction of change in naloxone's inhibition. Gosnell and co-workers (Gosnell et al., 1983) found that aging decreased naloxone's inhibition of nocturnal intake in male rats, an effect that is consistent with our findings with high-fat intake, but at variance with our findings for deprivation-induced intake. Morley and co-workers (Morley et al., 1984) found that ovariectomy

increased naloxone's inhibition of nocturnal intake in young rats, an effect which is consistent with our findings for deprivation-induced and high-fat intake in young rats. The relative inability of castration to alter naloxone's inhibition of deprivation-induced and high-fat intake is in agreement with the inability of castration to affect naloxone-induced inhibition of nocturnal intake (Wager-Srdar et al., 1987). It appears that whereas testosterone is the gonadal steroid that is most involved in the modulation of opioid analgesia, it plays little role in the opioid regulation of feeding.

The most parsimonious explanation for these data is that deprivation-induced intake and high-fat intake are modulated differentially by the opioid system, and that this differential modulation is selectively affected by the variables of aging, gender and gonadectomy. This is supported by several lines of evidence. First, the degree of naloxone-induced inhibition varies across ingestive situations. In the present study, naloxone was far more effective in inhibiting high-fat intake than deprivation-induced intake irrespective of age, gender and gonadal status, a finding consistent with previous studies (Arjune and Bodnar, 1990; Arjune et al., 1990; Islam and Bodnar, 1990; Levine et al., 1990; Morley et al., 1983). Second, the inhibitory actions of specific opioid receptor subtype antagonists varies as a function of the form of intake.

While the kappa antagonist, nor-binaltorphamine, potently reduces high-fat intake (Arjune and Bodnar, 1990), it marginally reduces deprivation-induced feeding (Levine et al., 1990). Conversely, the mu_1 antagonist, naloxonazine, significantly reduces deprivation-induced feeding (Simone et al., 1985), but fails to affect high-fat intake (Islam and Bodnar, 1990). Finally, the mu antagonist, beta-funaltrexamine, produces equivalent degrees of inhibition of both deprivation-induced intake and high-fat intake (Arjune et al., 1990; Islam and Bodnar, 1990). This would suggest that kappa receptors are more intimately involved in high-fat intake and palatability, while mu, and particularly mu_1, receptors are more involved in deprivation-induced feeding and body weight maintainance. In this model, the greater age-related inhibition of deprivation-induced feeding by high doses of naloxone would be related to the decreased binding characteristics of mu-selective ligands (Messing et al., 1980; Messing et al., 1980; Piva et al., 1987). In contrast, the lesser age-related inhibition of high-fat intake by low doses of naloxone would be related to changes in kappa opioid binding which has displayed variable age-related effects (Hiller et al., 1992; Maggi et al., 1989; Ueno et al., 1988). The inability to localize precisely the site(s) of action at which opioids mediate deprivation-induced intake and high-fat intake, respectively, precludes a definitive mechanism of action by which these organismic

variables exert their effects.

General Discussion

To summarize, the endogenous opioid system influences a wide range of physiological functions, including pain sensitivity and control of dietary intake. Individual variability in the magnitude and direction of opioid control over these functions can be attributed in part to age and gender, with gender differences in opioid-mediated behaviors due partly to the activational influences of circulating gonadal hormones. The two studies in this dissertation evaluated the roles of aging, gender and gonadectomy in morphine antinociception and naloxone's inhibition of deprivation-induced and high-fat food intake in rats. The major findings of these studies include the following. First, the magnitude and potency of morphine antinociception significantly declined as a function of age in female rats, whereas the potency of morphine antinociception significantly increased as a function of age in male rats. This pattern of antinociceptive effects was modulated by gonadal steroids in female rats, since ovariectomy mitigated the age-related changes in females. Second, the ability of the opioid antagonist, naloxone, to reduce food intake following food deprivation was minimally affected by the variables of age, gender and gonadectomy. Only the highest naloxone dose produced significantly greater inhibition in sham-operated males than in females, and greater inhibition

as a function of age in sham-operated males. Finally, organismic influences on naloxone's inhibitory actions on palatable high-fat intake were more pronounced than for deprivation intake. Aging decreased naloxone's inhibition of high-fat intake in sham-operated males and ovariectomized females, but increased inhibition of this form of intake in sham-operated females.

Our findings suggest that organismic variables can modulate the behavioral processes regulated by opioids in different ways and to varying degrees. Most notably, the results of the present study demonstrate that a study of organismic influences on opioid-mediated functions can in turn provide interesting insights into some biological characteristics of gender and age. Interestingly, differences between the sexes in opioid modulation of antinociception and food intake appear to be more notable than differences between young and old animals. Thus, with age there is no overall uniform decline in the opioid-mediated behaviors studied so far. For instance, while the antinociceptive potency of cold-water swim stress is compromised with aging (Kramer and Bodnar, 1986), antinociception resulting from food deprivation and beta-endorphin administration remains intact across the lifespan (Hamm and Knisely, 1986; Romero and Bodnar, 1987). This is consistent with previous studies that found age-related alterations in some but not all endogenous opioid peptides.

By considering interactions among organismic variables in their influence on antinociception and food intake, it was possible to discover that male and female rodents display a divergent course of aging with respect to these physiological functions.

The general discussion to follow will attempt to review issues regarding the possible ecological relevance of the influence of biological factors on the expression of opioid forms of antinociception and food intake. Three separate questions will therefore be addressed: a) what are age, gender, and gonadectomy differences in morphine antinociception telling us about the interaction between organismic variables and antinociceptive processes, b) what are age, gender, and gonadectomy differences in naloxone hypophagia telling us about the interaction between organismic variables and ingestive processes, and c) is there a relationship between opioid control of antinociceptive and ingestive processes? For the first two questions, the discussion will be organized in the following manner: i) species adaptation, ii) gender and adaptation, and iii) aging and adaptation.

A. What do age, gender and gonadectomy differences in morphine antinociception suggest about the interaction between organismic variables and antinociceptive processes?

i. Species and adaptation:

First, it is important to remember that the exogenous administration of an opiate, like morphine, is presumed to exert its antinociceptive effects by simulating activity in endogenous neural pathways, that under natural circumstances would be stimulated by biologically significant triggers such as exposure to aversive or stressful (endogenous or environmental) conditions. As Kavaliers (1988) noted in his recent discussion on the evolutionary and comparative aspects of nociception, most investigations of nociception and antinociception have been conducted with laboratory rats and mice. However, other species of vertebrates such as goldfish, grass frogs and lizards also display opioid antinociception. Indeed, even many species of invertebrates such as Cepaea nemoralis or terrestrial snail (Kavaliers and Tepperman, 1988), Limax maximus or terrestrial slug (Kavaliers and Hirst, 1986), Stagmatoptera biocellata or praying mantis (Zabala, Miralto, Moldonado, Nunez, Jaffe, and Calderon, 1984), Squilla mantis or mantis shrimp (Moldonado and Miralto, 1982), and earthworms (Gesser and Larsson, 1986), display nociception and opioid-mediated antinociception. This is consistent with the discovery that opioid peptides have a wide phylogenetic distribution, and

with the hypothesized importance of the opioid system in modulating the nociceptive and behavioral responses to aversive stimuli. As suggested, these responses to stressful stimuli, behaviors of basic survival to the organism and species, appear to have been present at an early evolutionary stage as well as in mammalian species, which indicates phylogenetic continuity.

Nociception, as well as environmentally-induced and opiate-induced antinoception, have been shown to vary between species and between strains of lab rats and mice. Comparative studies of different populations (subspecies) of deer mice which inhabit distinct geographic areas demonstrate how the biological variable of taxonomic classification influences these responses. Whereas island deer mice exhibit a significantly higher magnitude of restraint stress-induced and mu opiate-induced antinociception than mainland deer mice, the insular animals display lower levels of kappa-induced antinociception than the mainland animals (Innes and Kavaliers, 1987; Kavaliers and Innes, 1987). Kavaliers (1988) speculates that these population differences in opioid activity are subject to natural selection, and represent genetic adaptations to the physical and biological environmental conditions particular to each subspecies.

ii. Gender and adaptation:

Research suggests that, as a part of species survival,

there are natural selection forces to which the individual, based on such organismic characteristics as gender, is subject. Some forms of stress-induced antinoception (intermittent cold-water swims) have been shown to operate via neurochemically distinct pathways in male and female rats (Romero et al., 1988). Recent work has revealed an estrogen-dependent pain-inhibitory pathway unique to the female of the mouse species (Mogil, Sternberg, Kest, Marek, and Liebeskind, 1993). In addition to the population influences on antinociception in the deer mice studied by Kavalier and associates (1987), sex differences were also evident. Adult male deer mice from both the mainland and island displayed greater stress-induced and opiate-induced antinoception than their female counterparts. Male mice also exhibit a significantly greater magnitude of opioid-mediated antinoception in the presence of a predator than females (Kavaliers and Colwell, 1991). Note, antinoception may be advantageous in a situation such as in an encounter with a predator or a territorial intruder, in which responding to noxious stimulation may otherwise disrupt effective defensive and offensive actions. Possibly, gender differences in levels of aggression contribute to the discrepancy between the sexes in patterns of antinociceptive responses.

This research, along with the current and past studies on gender and gonadal differences in morphine

antinociception, suggests that it is likely that males and females evolved distinct endogenous pain inhibitory mechanisms through evolutionary selection. This would enable them to effectively cope with the different types of aversive situations, and resultant painful stimulation, that each sex is more likely to experience. That is to say, just as different species of animals, through millions of years of evolution, have developed specific defense behaviors as adaptations to the perils of their particular environmental niche, so too it may be postulated that male and female organisms of any given species would have evolved specific sets of adaptive mechanisms. These would arise as a response to ecologically relevant aversive stimuli associated with the conspecifics of gender. This is because, as a result of unique physiologies that make them suitable for different roles, males and females do not confront totally similar environments even in the same geographic terrain. It is of note that in humans, it is evident that there are different types of pain that either men or women are more likely to suffer. Women may experience the pains of labor and child-birth. Additionally, migraine headaches, arthritis, and interstitial cystitis are reportedly more common in women, whereas men are more prone so suffer from backache, cluster headaches, and cardiac pain (cf. Touchette, 1993).

While the adaptive significance of gender differences in the antinociceptive response to some forms of stressors

(e.g., cold-water swims) is not immediately obvious, certain situations that are unique to either males or females may clarify the adaptive significance of gender differences in analgesic processes. As examples, the situations of parturition/childbirth and territorial aggression come to mind most prominantly. Both situations are associated with elevations in pain thresholds, and may be considered naturalistic forms of stress-induced antinociception. The latter is a situation in which primarily males become involved, and to which the adaptive response of pain-suppression may be a uniquely male response. Although, note that this has not been tested, and there is some evidence that hormone-dependent aggression is biologically homologous in male and female rats (see Albert, Jonik, and Walsh, 1992). Miczek and colleagues (1982, 1985, 1988) showed that male intruder mice, exposed to repeated attacks and subsequently defeated by resident male mice, exhibit a significant and long-lasting opioid-mediated antinociception that is blocked by naloxone pretreatment and cross-tolerant with morphine.

Whereas males are prone to experience flesh bites from intermale aggressive encounters, in mammalian species females may experience visceral pain resulting from parturition. The work of Gintzler and others (1980, 1984, 1988, 1990) demonstrated that in both rats and humans, there is an antinociception associated with pregnancy. In rats

there is a gradual decrease in responsiveness to aversive stimuli that occurs between 16 and 4 days prior to parturition, and an abrupt increase in pain thresholds that occurs 1 to 2 days before delivery. This is abolished by preadministration of chronic naltrexone. The opioid-mediated antinociception activated by pregnancy appears to be spinally-mediated and involves the dynorphin/kappa opioid receptor system. Thus, the endorphin system has been implicated in the intrinsic mechanisms that modulate maternal thresholds to pain. This is not surprising, given that labor and delivery are experiences that require adaptation to stress.

Short-term antinociception also results from mechanical stimulation of the vagino-cervical area. Komisaruk and colleagues (1977, 1986, 1988) found that cervical probing in rats blocked their withdrawal responses to noxious stimulation, and vaginal (anterior wall) self-stimulation raised detection and tolerance thresholds for painful cutananeous stimulation in humans. Evidently, this form of antinociception involves endogenous spinal opioids, though there is also evidence for spinal glycinergic, alpha-adrenergic, and serotonergic mediation of vaginal-stimulation antinociception. It may be speculated that in rats, cervical probing-induced antinociception serves the function of allowing the female to tolerate the repeated intromissions required for impregnation. Komisaruk suggests

that in humans antinociception resulting from vaginal stimulation may not be properly characterized as a stress-induced antinociception, since the most effective pain reduction appears to occur when the stimulation is perceived by women as pleasurable, not aversive. However, elevation in pain threshold resulting from vaginal stimulation could be classified as a form of stress-induced antinociception under a broader concept of stress that includes non-aversive strong arousal (Selye, 1976).

iii. Aging and adaptation:

Another organismic variable that was considered in the present research is age. Do the changing characteristics of aging organisms, interacting with gender, reflect the influence of selective evolutionary forces? With regard to age effects on opioid-mediated processes, current findings demonstrate that relative to young animals, older males but not females display an increase in the antinociceptive response to opioid administration. Also, past research demonstrated that male but not female rats show an age-related increase in the antinociceptive response to a stressor, cold-water swim (Hamm et al., 1986; Kramer and Bodnar, 1986). It is difficult to speculate why there might be an adaptive gain for males to maintain into an older age, an intact pain-inhibitory system. Indeed, it may be that there is little adaptive significance to the changes seen with aging in antinociceptive and ingestive processes, or in

the opioid control of these processes. That is to say, once the organism has lost its reproductive capacity, its individual survival may be quite irrelevant. Therefore, any age-changes seen in opioid-mediated functions could be nonspecific or secondary to other physiological changes. For instance, as suggested before, the increased potency of morphine antinociception observed in male animals as they aged in the present study may simply have been the by-product of the age-related decline in renal function and consequent reduced efficiency of morphine metabolite excretion. One metabolite of morphine, morphine-6-glucuronide, produces a potent antinociception in its own right (Pasternak et al., 1986; Paul et al., 1989). Gender differences in such physiological age changes has yet to be determined.

iv. Clinical utility and methodological considerations:

The current study has implications for studies of antinociceptive efficacy in human patients, demonstrating the importance of considering the same kind of interaction effects when conducting research with humans. Morphine appears to have greater efficacy in older than in younger male rodents, paralleling the clinical observation of increased sensitivity to morphine in older human patients. However, drugs that relieve pain in older men may not necessarily be as effective in older women. As mentioned before, previous research has suggested that stress-induced

antinociception in rodents operates through neurochemically distinct pathways in males and females. For instance, antinociception induced by intermittent cold-water swims involves the opioid system in male rats but appears to be nonopioid-mediated in females (Romero and Bodnar, 1986). Future studies are needed to determine which of multiple pain-inhibitory pathways can be best exploited in older female organisms.

As mentioned, males and females may be differentially susceptible to specific types of pain. Such differentiation, as functions of organismic factors, indicates that nociceptive reactivity and antinociceptive efficacy are not homogeneous characteristics. Therefore, consideration of organismic variables may also have relevance for the clinical treatment of different forms of pain in men and women, (e.g., peripheral versus central, visceral versus cutaneous-mechanical). Clearly, females need to be included in clinical trials of experimental analgesics. In particular, studies should explore other specific opioid receptor subtype agonists and nonopioid agonists for gender/aging interaction effects. This may eventually lead to new analgesic drugs for women. The results of such research would also have implications for treatment of pain in veterinary medicine.

Some methodological issues should be addressed in future research. Better grounds could be provided for

relating organismic modulation of opioid-mediated behavior to organismic effects on the underlying opioid system, by conducting regional brain assays of structures thought to be involved in the particular function. Ideally, changes in opioid activity, and changes in the specific behavior mediated by opioids, should be assessed within the same animal to allow for stronger inferences about causal relationships. When evaluating alterations in the opioid system as a function of one organismic variable, it will be important to consider the modulatory influences of other organismic variables such as species/subspecies. Also important is the establishment of criteria to exclude animals that have dysfunctions (e.g., beta-endorphin-secreting pituitary tumors) that may independently change responsivity. Furthermore, although the long-term nature of this study necessitated the use of a systemic route of drug administration, central injections are preferable in order to minimize group differences in pharmacokinetic effects. Finally, results from the current study and from previous research in our laboratory suggest that some tests (e.g., jump test) appear to be more sensitive to gender differences in young animals than others (e.g., tail-flick). Therefore, multiple measures of nociception should be included in studies of human pain and analgesia, since organismic variables may affect responsivity to opioid antinociception at spinal and supraspinal levels in a differential manner.

When assessing age effects, the pain test should be a measure that will be minimally sensitive to age-related (e.g., motor) dysfunctions.

B. What do age, gender and gonadectomy differences in naloxone hypophagia suggest about the interaction between organismic variables and ingestive processes?

i. Species and adaptation:

Opioid peptides appear to be involved in regulation of natural feeding behavior in a diverse array of species, (see Levine et al., 1985 and Morley et al., 1983, for reviews). Opioids have even been implicated in the feeding responses of various phylums of invertebrates, including molluscs (e.g., terrestrial slugs, Limax Maximus: Kavaliers, Hirst, and Teskey, 1984, 1985) and arthropods (e.g., American cockroach: Kavaliers, Guglick, and Hirst, 1987). Studies of feeding in invertebrates using selective opiate agonists and antagonists show differential mediation by different receptor subtypes, in a way that parallels opiate actions in mammals (Kavaliers, Rangley, Teskey, and Hirst, 1986). Thus, administration of the kappa agonist U-50,488H to cockroaches elicits food intake, an effect which is partially suppressed by naloxone (Kavaliers et al., 1987). Tail-pinch stress-induced feeding in slugs is inhibited by naloxone (Kavaliers and Hirst, 1986). Kavaliers and Hirst (1987) concluded that opioid influences on feeding have been conserved throughout evolution.

The adaptive significance of opioid modulation of

feeding, a basic physiological function for maintaining homeostasis, comes most dramatically from studies of the triggering of hibernation. It has been suggested that the opioids may be involved in the onset of hibernation (Kromer, 1980; Margules, Goldman, and Finck, 1977; Oeltgen, Walsh, Hamann, Randall, Spurrier, and Myers, 1982), which allows some mammals to survive seasons of low food availability, obviously a very important adaptation in severe climates. Animals become hyperphagic to increase energy stores in preparation for hibernation. The major macronutrient associated with hibernation is fat, due to its promininence in energy conservation. In fact, possibly it is involvement in the control of fat intake that accounts for the role of opioids in hibernation. In support of this, at least one study demonstrated the existence of an opioid sensitive feeding system in a true hibernator, the woodchuck (Nizielski, Morley, Gosnell, Seal, and Levine, 1985).

Unfortunately, no known studies have examined organismic modulation of opioid involvement in this important ecological response. Yet, as we will discuss in the following section, age and gender selectively influence naltrexone inhibition of fat, with fat intake being conserved with aging and females consuming more fat than males. Perhaps females are more fitted to survive a season of hibernation than males, which makes evolutionary sense since females are least expendable for species survival.

A second issue with regard to fat intake is its palatability. Apfelbaum and Mandenoff (1981) found that naltrexone suppressed the hyperphagia induced in rats by exposure to a highly palatable diet, while having little effect on lab chow intake in normal rats. Beta-endorphin is released from the hypothalamus by reward induced by palatable foods (Dum, Gramsch, and Herz, 1983). These findings are consistent with beta-endorphin's role in other situations which also result in hyperphagia: e.g., fasting-induced intake, glucoprivic-feeding, and mild tail pinch-induced feeding. Stressors like food deprivation and tail pinch result in decreased concentrations of hypothalamic beta-endorphin and dynorphin in the rat brain, indicating their release and breakdown (Gambert et al., 1980; Morley and Levine, 1982). Thus, it has been proposed that, in the rat, the endogenous opioid system is not indispensable for control of food intake in a stress-shielded environment, such as when monotonous food is available, but rather, seems to be involved in the hyperphagia provoked either by stressors or by the overabundance of palatable foods, when these events are not within the control of the animal (Apfelbaum and Mandenoff, 1981).

Is the modulatory role of gender with regard to high fat intake related to the nutritional value of fatty food, or to its characteristic of palatability? Although at least one study reported no significant gender differences in

responsiveness to taste properties in food (Sclafani and Gorman, 1977), this same study found that relative weight gain, the difference between palatable-fed and chow-fed animals, is greater for females than for males. Others found that adult female rats drink more of sweet saccharin solutions than do males (Zucker, 1969). The following section addresses the potential biological significance of gender differences in palatable intake versus other forms of feeding such as stress-related.

ii. Gender and adaptation:

That opioids may play a less important role in feeding in females than in males is supported by our finding that young females are not as sensitive to naloxone's inhibition of food intake as males or ovariectomized females. This is consistent with past studies (McLaughlin and Baile, 1983; Morley et al., 1984; Wager-Srdar et al., 1985), and with the observation of higher brain content of opioid peptides in males (e.g., Lee et al., 1980). Gender and gonadal steroid influences on naloxone hypophagia indicate that, as with antinociceptive processes, organismic differences in opioid-mediated food intake may represent an adaptive response to the specific metabolic needs of different physiologies, as well as adaptation to different food habitats. By some accounts, fat is the macronutrient that is most selectively under opioid control (Marks-Kaufman, 1982; Marks-Kaufman and Kanarek, 1980, 1990; Romsos et al., 1987). In the current

study it was observed that opioid modulation of high-fat intake was more sensitive to the influence of organismic variables than was deprivation-induced intake, with intact female rats of all ages consuming more fat than other groups. It may be speculated that this promotes the maintenance of the greater fat constituent of the female body, important for providing sustenance to nursing young. That is to say, possibly high-fat intake is more subject to organismic variability due to different macronutrient requirements of females versus males. By contrast, requirements of different organisms would be similar under conditions of deprivation challenge, i.e., both males and females would need to adopt compensatory hyperphagia post-deprivation. This research supports the hypothesis that one critical role of opioids is to increase the capacity of individuals within a given species to fit their organismic characteristics to the demands of the environment.

iii. Aging and adaptation:

In contrast to the challenge form of intake, intake of a preferred diet undergoes little age-related change, and even shows an increase with age in females. Preserved intake of high-fat with aging is consistent with neurochemical findings of intact kappa receptor concentrations and enhanced binding characteristics over the life course (Maggi et al., 1989; Ueno et al., 1988). As previously mentioned, the kappa receptor is thought to be predominantly involved

in opioid mediation of palatable feeding (Cooper et al., 1985; Morley et al., 1985). The current results, however, are somewhat surprising when considering the senescent decline in chemical senses, and suggest that the hedonic properties of a palatable diet may become less important in regulating its intake in aging organisms.

Neurochemical findings indicate that age-related decline in general opioid receptor binding is greater in females than in males (Messing et al., 1980a,b). However, old females consume more fat than old males, and become more sensitive to naloxone inhibition of deprivation and high-fat intake with age, possibly due to the loss of circulating gonadal hormones. As previously hypothesized, estradiol seems to reduce the sensitivity of the opioid feeding system (Morley et al., 1984). Thus, just as it was proposed that some age-changes seen in opioid-mediated antinociception might be nonspecific or secondary to other physiological changes, in a similar vein, the increased sensitivity of females to naloxone inhibition of fat intake with age, may simply be due to the declining influence of the inhibitory effects of estradiol on opioid function. This, however, may have implications for the development of obesity in older females.

C. Is there any relationship between opioid control of antinociceptive and ingestive processes?

Since these two processes are each modulated by the

opioid system, a number of investigators have conducted research to explore the relationship between them. And, interestingly, it was found that there are certain situations in which both intake of food and pain threshold increase. For instance, in addition to hyperphagia, a 24-hour fast, glucoprivation, and mild tail pinch also produce antinociception in rats. Naloxone antagonizes this antinociception and suppresses the overeating (Bodnar, Kelly, Brutus, and Glusman, 1980; Bodnar, Kelly, Spiaggia, and Glusman, 1978; Hamm and Lyeth, 1984; Levine, Wilcox, Grace, Morley, 1982). In recent studies of the effects of food ingestion on nociception, it was reported that sucrose and saccharin intake alter pain threshold and magnitude of morphine antinociception, and high-fat alters morphine potency. However, there appears to be a lack of consistency with regard to the direction of the influence of carbohydrate and fat intake on pain reactivity and opiate antinociception. Whereas some investigators found that continuous feeding of a sucrose or dextrose/saccharin solution lowered pain threshold in rats (Holder and Bolger, 1988; Roane and Martin, 1990), others found no significant effect of diet on pain thresholds (Kanarek, White, Biegen, and Marks-Kaufman, 1991; Klein and Green, 1988). Moreover, although there are reports that continous sucrose, saccharin, and high-fat feeding enhance potency of morphine antinociception in rats (Roane and Martin, 1990; Marks-

Kaufman, Kanarek, and Delanty, 1988; Kanarek et al., 1991), there are also reports that chronic or brief exposure to sucrose or saccharin reduce the antinociceptive effectiveness of morphine in rats (Bergman, Lieblick, Cohen, and Ganchrow, 1985; Holder, 1988; Klein and Green, 1988), possibly through mechanisms of cross-tolerance.

Thus, while there seems to be little doubt about the existence of a correlation between dietary intake and nociception/antinociception, it is unclear that this has any functional significance. Indeed, when reliable effects are found they tend to be small. Why, indeed, should there be a relationship of relevance between intake of a palatable diet and morphine antinociception, or between food deprivation and pain threshold? After all, there is a considerable dissociation between opiate receptor types and sites that are responsible for analgesia and those that underlie food intake. As reviewed in the introduction of this paper, opioid receptors found to mediate supraspinal opioid antinociception are the mu, mu1, and delta2 receptors, whereas feeding primarily involves the kappa, mu2, and mu1/delta receptor types. Also, the sites that have been implicated in opioid analgesia (e.g., periaqueductal gray, rostral-ventral medulla) are distinct from those found to subserve ingestive behavior (e.g., periventricular nucleus, parabrachial nucleus, nucleus tractus solitarius). Therefore, in the absence of any clear evidence of a

functional association between food intake and antinociception, it may be that they are coincidentally related, covarying together because each involves the release of opioid peptides. Indeed, it appears that not only do endogenous opioids play an important role in the initiation of intake of sweet substances, but opioid function is in turn altered by this intake (Dum et al., 1983). The ingestion of palatable foods apparently activates endogenous opioid activity, which concurrently may diminish responsiveness to aversive stimuli. That this relationship is merely one of co-occurence is supported by the finding that cholecystokinin, a neuropeptide which suppresses feeding possibly by degrading the palatability of food (Ettinger, Thompson, and Staddon, 1986; Gibbs, Young, and Smith, 1973), also appears to act as an antagonist of opiate antinociception (Faris, Komisaruk, Watkins, and Mayer, 1983; Watkins, Kinscheck, and Mayer, 1984).

On the other hand, it would not be entirely correct to dismiss as a purely coincidental association, the rise in plasma level of opioids triggered by food ingestion and the concurrent increase in pain thresholds. Changes in plasma levels of opioids suggest nothing specific about activation of opioid-mediated processes, since release can occur from any number of brain sites, and opioids play different roles in different brain regions. Perhaps it is not wholly unlikely that there is some ecological significance to the

relationship between some forms of feeding, and antinociception. For instance, both deprivation-induced intake and glucoprivic intake can be regarded as compensatory regulatory responses to biological stress. Similarly, the increases in pain threshold resulting from food deprivation and glucoprivation can be regarded as forms of stress-induced antinociception. Like other situations in which it may be more adaptive to suppress pain, and pain behavior, in order to cope most effectively with an environmental crisis (e.g., escaping from a predator, intruding into another male's territory, or falling into cold water and having to cope with a thermoregulatory challenge), it could be speculated that the hungry animal's need to locate food is best served by being unresponsive to pain, in order to take risks that may involve bodily harm, e.g., venturing out and chancing an encounter with a predator.

Possibly, too, it is the reward component of palatable intake that plays a significant role in the alteration of pain threshold. It has been postulated that the role of opioids is to potentiate reward processes "in the service of an appetitive motivational state" (Carr, 1984). Perhaps the concurrent decrease in emotional responsiveness to aversive stimuli helps to accomplish this.

Future research should explore the potential role that organismic variables may play with respect to the

relationship between food intake and antinociception. The site of this interaction also has yet to be determined.

To conclude, the current study demonstrates the importance of considering the influence of biological variables such as age and gender, on physiological functions. Moreover, it shows that studying them within a single design has the advantage of revealing complex interactions. Given that much research has been devoted to uncovering the neural and biochemical mechanisms of antinociception and food intake, now it is important to emphasize research on organismic differences in such physiological/behavioral responses. This pursuit would be consistent with the general shift in focus in biomedical research in recent years. It would reflect the increasing realization that differences due to organismic variability are significant, and important take into account when evaluating disease processes and efficacy of clinical (including drug) treatment.

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