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Opioid Induction of Fos in the Female Rat Brain: Modulation by Ovarian Hormones

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ABSTRACT

Opioid induction of Fos in the female rat brain: Modulation by ovarian hormones

Marlene Taube

Previous research has shown that opioid drugs have profound effects on female reproduction. It has also been established that ovarian hormones exert control over opioid peptide synthesis and opioid receptor gene expression. The present thesis investigated functional pathways by which opioids affect the sexual behaviour of female rats, by examining the induction of Fos protein. Ovariectomized, sexually experienced Long Evans rats (n = 48) were implanted stereotaxically with guide cannulae aimed at the right lateral ventricle. One group of rats was treated with estradiol benzoate (10 μg) 48 hr and progesterone (250 μg) 4 hr before testing, another group was treated with EB-alone and a third group was treated with oil. Females were infused with the selective μ-receptor agonist, DAMGO, the selective δ-receptor agonist, DPDPE, the selective κ-receptor agonist, U50-488, or saline. All rats were sacrificed 75 min after the drug infusion. The induction of Fos immunoreactivity was analyzed in the lateral septum, medial preoptic area, ventromedial hypothalamus, medial amygdala, and mesencephalic central gray. The present study found that Fos immunoreactivity was induced differentially by the opioid agonists within the different brain regions, and the hormones either augmented or had no effect on the induction. These data demonstrate that estrogen and progesterone alter the ability of opioid agonists to induce Fos protein within different regions of the female rat brain. These results contribute to the understanding of opioid actions within the female rat brain.

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Opioid drugs such as morphine or heroin have profound effects on reproduction. In the female rat, these include inhibitory effects on sexual behaviour, lutenizing hormone (LH) secretion and estrous cycles (e.g. Plant & Pierce, 1928; Kalra & Kalra, 1984; Weisner & Moss, 1984). However, previous research has established that ovarian hormones exert control over endogenous opioid activity by modulating opioid receptor regulation and gene expression (e.g. Hammer, 1990; Romano, Mobbs, Howells & Pfaff, 1989). The regions of the brain where interactions between ovarian hormones and opioids take place remain to be elucidated. The goal of the present thesis was to identify functional pathways in the brain which are activated by opioids and in which this activation might be modulated by ovarian hormones.

Opioids and reproduction: systemic effects

Opioid receptor agonists and endogenous opioid peptides inhibit LH secretion (e.g. Kalra & Kalra, 1984); this effect may be mediated by an opioid-induced inhibition of gonadotrophin releasing hormone (GnRH)-containing neurons in the medial preoptic area (mPOA) (Bicknell, 1985; Kalra, Allen & Kalra, 1989). Because of their inhibitory effects on LH secretion, endogenous opioid peptides have been considered to be a causal factor of hypothalamic amenorrhea. It has been shown that naloxone administration may play a role in increasing LH secretion in women (e.g. Blankstein, Reyes, Winter & Faiman, 1981; Genazzani, Gastaldi, Petraglia, Battaglia, Surico, Volpe, & Genazzani, 1995). These results suggest that an excessive production of endogenous opioids may play a role in the pathophysiology of women suffering from amenorrhea.

Long term opioid use has effects on a wide range of sexually related phenomena. It inhibits sexual dreams, delays ejaculation and decreases ejaculate volume, while

increasing the likelihood of anorgasmia, amenorrhea, and infertility (as cited in Pfaus and Gorzalka, 1987a). For example, Plant and Pierce (1928) found that female dogs subjected to chronic morphine never came into heat and Myers (1931) found that female rats receiving chronic morphine experienced irregular estrous cycles.

In addition to effects on hormone release and the estrous cycle, opioid agonists have been shown to have direct effects on sexual behaviour in female rodents. In ovariectomized (OVX) female golden hamsters primed with estrogen and progesterone, morphine administration inhibited lateral displacement of the tail (a sensitive measure of female receptivity during copulation) during copulation (Ostrowski, Stapleton, Noble & Reid, 1979). Furthermore, in OVX female rats primed with estrogen and progesterone, proceptive behaviours such as presenting, hopping, darting and ear wiggling are inhibited following morphine injections (Pfaus & Gorzalka, 1987a). Importantly, motor activity was not suppressed by the doses of opioids administered in these studies, indicating that opioids were acting specifically to inhibit sexual behaviour.

Further evidence of the direct effects of opioids on sexual behaviour comes from studies conducted with opioid receptor antagonists. For example, administration of naltrexone, a long acting opioid antagonist, facilitates lordosis behaviour in estrogen-primed female rats (Allen, Renner & Luine, 1985). In other studies, the administration of naloxone, a shorter acting opioid antagonist, did not increase lateral tail displacement in the female golden hamster (Ostrowski, Noble & Reid, 1979; Ostrowski, Noble & Reid, 1981) or lordosis in the female rat (Weisner & Moss, 1984; Lindbloom, Forsberg & Södersten, 1986). However, it was found that naloxone injection given prior to an infusion of β-endorphin, blocked the inhibition of lordosis produced by central infusions

of ß-endorphin (Weisner & Moss, 1984). The findings from Weisner and Moss (1984) suggest that there is no tonic suppression of lordosis by endogenous opioids. However, the longer acting antagonist, naltrexone, was able to facilitate lordosis (Allen et al., 1985), suggesting that this process may not be rapid; but may require the activation of other systems for the endogenous opioid effect to be observed.

Opioid peptide and receptor localization

The systemic effects of opioids on female sexual behaviour do not reveal where opioids are acting in the brain. Therefore, in order to understand which regions of the brain regulate opioid control of female sexual behaviour, it is necessary to review where opioids bind in the brain. Historically, the discovery of opiate receptors (Goldstein, Lowney & Pal, 1971) preceded the discovery of endogenous peptides in the central nervous system (Hughes, Kosterlitz, Fothergill, Morgan & Morris,1975). Since these early discoveries, considerable research has been done to identify opioid receptor subtypes, opioid receptor and peptide gene expression, and the anatomical distribution of opioid receptors in the brain. Opioid receptors have been grouped into three families: mu (μ), delta (δ) and kappa (κ) (Martin, Eades, Thompson, Huppler & Gilbert, 1976) and, more recently, there has been the discovery of an opioid-like receptor (ORL-1) (Kieffer, 1995). Opioid receptors are widely distributed throughout the central nervous system and are particularly dense in limbic, thalamic and hypothalamic and neural areas essential for visceral functions. The current discussion, though not exhaustive, will focus on opioid receptor localization in brain regions thought to be important for reproductive behaviour.

The μ-opioid receptors are found at several levels of the neuraxis including those regions considered most important for sexual behaviour, such as the septum, thalamus,

nucleus accumbens, olfactory tubercule, amygdala, hippocampus (HPC), mPOA, medial preoptic nucleus (mPN), bed nucleus of the stria terminalis (BNST), suprachiasmatic nucleus (SCN), and ventromedial nucleus (VMN), mesencephalic central gray (MCG) (Mansour, Khachaturian, Lewis, Akil & Watson, 1987; Tempel & Zukin, 1987; Mansour, Khachaturian, Lewis, Akil & Watson,1988; Desjardins, Brawer, & Beaudet, 1990). The distribution of mRNA for μ-opioid receptors parallel the distribution of μ-opioid receptors, except in the SCN where only μ-opioid receptors has been found. (e.g. Delfs, Kong, Mestek, Chen, Yu, Reisine and Chesselet, 1994; Mansour, Fox, Burke, Meng, Thompson, Akil & Watson, 1994).

The δ-opioid receptors are also found in brain regions that are considered important for sexual behaviour including the neocortex, amygdala, nucleus accumbens, olfactory tubercule, mPOA, BNST, SCN, VMN and MCG (Mansour et al., 1987; Blackburn, Cross, Hillet & Slater, 1988; Mansour et al., 1988; Desjardins et al.,1990). The pattern of δ-opioid receptor mRNA expression is identical to the pattern of δ-opioid receptors, with the exception of the mPOA and SCN which has δ-opioid receptors, but not δ-opioid receptor mRNA (Mansour, Fox & Watson, 1995).

The κ-opioid receptors are found across regions that are regarded as important for sexual behaviour such as the neocortex, nucleus accumbens, olfactory tubercule, pyramidal and molecular layers of the HPC, VMN, amygdala, mPOA, BNST, median eminence, SCN and MCG (Mansour et al., 1987; Tempel & Zukin, 1987; Mansour et al., 1988; Desjardins et al., 1990). κ-opioid receptor mRNA has been found in each of these regions and, in addition, in the substantia nigra, spinal cord, and ventral tegmental area (VTA) (Mansour et al., 1994).

Finally, as mentioned, a new opioid receptor-like receptor, (ORL-1) has been discovered (Kieffer, 1995; Nothacker, Reinscheid, Mansour, Henningsen, Ardati, Monsma, Watson & Civelli, 1996). The endogenous ligand for this receptor is called orphanin FQ (Nociceptin). Orphanin binding sites are also found in brain regions involved in the control of sexual behaviour, including the lateral septum (LS), several hypothalamic nuclei, hippocampal formation, basolateral and medial amygdala (MEA), MCG, several cortical regions, anterior olfactory nucleus, ventral forebrain, substantia nigra, and the spinal cord (Neal, Manosur, Reinscheid, Nothacker, Civelli, Akil, & Watson, 1999). ORL-1 mRNA is expressed in similar brain regions.

Hormonal regulation of opioid systems

Research has shown that different hormonal environments in the female rat can regulate changes in opioid receptor density as well as opioid peptide gene expression. For example, Hammer and his colleagues have shown that the density of μ-opioid receptors in the mPOA varies with the estrus cycle; in female rats, the density of μ-opioid receptors was found to be highest during met- and diestrus as compared to proestrus. Conversely, levels of β-endorphin, which binds with equal affinity to both μ-receptors and δ-receptors, have been found to be low during proestrus and high during estrus in the mPOA (Knuth, Sikand, Casanueva, Havlicek & Friesen,1983). It has also been shown that estrogen treatment decreases β-endorphin content and reduces the expression of proopiomelanocortin mRNA in the hypothalamus (Wardlaw, Thoron, & Frantz, 1982; Wilcox & Roberts, 1985). Effects of estrogen on proopiomelanocortin mRNA and β-endorphin content were partially restored following the coadministration of progesterone expression (Wilcox & Roberts, 1985; Hammer & Bridges, 1987). These results suggest

that progesterone may act to disinhibit the effect of estrogen on proopiomelanocortin mRNA (Wilcox & Roberts, 1985) as well as the effect of estrogen on β-endorphin content (Hammer & Bridges, 1987).

The effects of a variety of different hormone regimens on the number of μ-receptors in the mPOA have also been examined in OVX rats. Mateo, Hijazi, and Hammer (1992) found that administration of estrogen in combination with progesterone did not increase mPOA μ-receptor labeling measured three hours following progesterone administration. Similarly, females maintained on a Silastic estrogen capsule followed by progesterone injections did not show increased binding three hours later. mPOA μ-receptor binding did, however, increase 27 hours after progesterone injection. This indicates that the induction of changes in mPOA μ-receptor density is long lasting and takes time to develop.

Levels of μ-opioid receptor density have also been studied in normal cycling female rats and have been shown to vary with the phase of the estrous cycle. For example, Maggi, Dondi, Rovati, Martini, Piva and Limonta (1993) found that the μ-opioid receptors were elevated in the hypothalamus at various stages of the estrous cycle. Hammer (1990) observed that the number of μ-opioid receptors was minimal on the morning of proestrus, which immediately precedes the period prior to sexually receptivity in the female rat. It was also shown that μ-opioid receptors increased on the morning of estrus as well as on days 1 and 2 of diestrus. These phases correspond to the period when female rats are no longer sexual receptive. Thus, it would appear that the increase in μ-

opioid receptors seen on the morning of estrus and on days 1 and 2 of diestrus contribute to the decrease in sexual receptivity.

Estrogen has been shown to have a variety of effects on opioid gene expression and receptor synthesis. For example, estrogen produces long-lasting increases in proenkephalin (PE) mRNA in the ventrolateral aspect of the VMN two hours following treatment (Romano, Harlan, Shivers, Howells & Pfaff, 1988; Romano, Mobbs, Howells & Pfaff, 1989). This short latency suggests a direct effect of estrogen on enkephalinergic cells. When Silastic estrogen capsules were removed, PE mRNA levels declined significantly in the VMN. Thus, these results suggest that PE gene expression is sensitive to fluctuating levels of estrogen (Romano et al., 1988). PE mRNA levels also vary with steroid hormone levels during the rat estrous cycle (Funabashi, Brooks, Kleopoulos, Grandison, Mobbs & Pfaff, 1995) and with estrogen levels corresponding to different levels of sexual receptivity in OVX rats (Lauber, Romano, Mobbs, Howells & Pfaff, 1990). Lauber et al. (1990) found that increases in lordosis displays and PE transcription, events that may be mediated by the VMN, occurred in parallel with the concentration of estrogen. It has also been shown that estrogen increases the content of μ-opioid receptor mRNA levels in the VMH and arcuate nucleus (Quiñones-Jenab, Jenab, Ogawa, Inturrisi & Pfaff, 1997).

Eckersell, Popper & Micevych (1998) have shown that estrogen treatment affects μ-opioid internalization, which is demonstrated by a redistribution of μ-opioid immunoreactivity (μ-opioid-ir) following agonist binding, that results in an increase in the density of immunoreactive processes. Internalization is a process by which the ligand-receptor complex are transported from the plasma membrane into the cytoplasm, where

the ligand is then degraded, and the receptor is returned to the plasma membrane. In this study, OVX rats were injected with estrogen via a subcutaneous cannula. This treatment resulted in an increase in the density of μ-opioid-ir processes in the mPN, BNST and the MEA posterodorsal (pd) 30 min after estrogen infusion. When OVX females were treated with naltrexone prior to estrogen treatment, these increases in the density of μ-opioid-ir processes were not observed. These data suggest that estrogen induces a rapid release of endogenous opioid peptides in the mPN, BNST and MEA.

Opioids and female sexual behaviour: central effects

Opioid administration has been shown to have different effects on female sexual behaviour depending on the brain region stimulated, the opioid receptor subtype and the hormonal state of the animal. In order for female sexual behaviour in the rat to be fully expressed, both estradiol benzoate (EB) and progesterone (P) are necessary (Pfaff, 1980). In ovariectomized (OVX) females, estrogen promotes lordosis, whereas progesterone facilitates subsequent proceptive and solicitation behaviours (for a full review see Pfaff, 1980; Pfaus, 1995). Effects on female sexual behaviour have been found following administration of opioid peptides and their antagonists into the lateral ventricle and the third ventricle in estrogen and progesterone primed rats. Following administration of β-endorphin into the third ventricle, decreases in lordosis were observed (Weisner & Moss, 1984; Weisner & Moss, 1986a; 1986b). Inhibition as well as facilitation of lordosis has been shown following β-endorphin infusions into the lateral ventricle (Pfaus & Gorzalka, 1987b). More recently, Gorzalka, Hedema, Lester and Hanson (1997) reported that a 2 μg dose of β-endorphin facilitated lordosis when it was infused into the lateral ventricle and inhibited lordosis when it was infused into the third ventricle. There was no significant

effect on ear-wiggling frequency (a proceptive behaviour) when the drug was administered into either ventricle. These results are interesting in light of β-endorphin's approximately equal affinity for both μ- and δ-opioid receptors (Paterson, Robson, & Kosterlitz, 1983). Therefore, these different effects may reflect distinct opioid receptor subtype activation in different regions of the brain, or the activation of brain regions that either facilitate (lateral ventricle) or inhibit (third ventricle) lordosis.

Researchers have also shown that central administration of selective opioid agonists and antagonists affect sexual behaviour of the female rat. For example, lateral ventricular infusions of the selective μ-receptor agonist, morphiceptin, produced a dual effect on lordosis in female rats following infusion into the lateral ventricles (Pfaus, Pendleton, & Gorzalka, 1986). A low dose inhibited lordosis whereas a higher dose facilitated it. It was also observed that naloxazone, a high affinity μ_1 receptor antagonist, blocked the inhibition of lordosis produced by the low dose of morphiceptin, but not the facilitation produced by the higher dose. Because naloxazone binds to the μ_1 receptor, these results suggest that morphiceptin activated μ_1 receptors in order to inhibit lordosis. It has also been found that lateral ventricular infusions of a selective δ-opioid receptor peptide, D-Pen^2, D-Pen^5-enkephalin (DPDPE), facilitated lordosis (Pfaus & Gorzalka, 1987b) whereas a selective κ-opioid agonist, dynorphin 1-9, produced only a trend towards facilitation. These effects were prevented by prior administration of naloxone. Overall, these results were the first to show that female sexual behaviour is regulated differently by selective opioid receptor subtypes.

It was subsequently established that effects on female sexual behaviour following opioid administration will differ depending on the hormonal state of the female rat, as

well as on the opioid agonist administrated (Pfaus & Pfaff, 1992). Lateral ventricular infusions of the highly selective μ-opioid receptor agonist, D-Ala$^{\mu}$, MePhe^{4}, Gly-ol^{5}-enkephalin (DAMGO), inhibited sexual receptivity in a dose-dependent fashion in OVX rats primed with estrogen and progesterone. OVX rats primed with estrogen alone, however, did not exhibit these effects. DAMGO also significantly reduced pacing behaviour in the female rat. However, infusions of the highly selective δ-receptor agonist, DPDPE, facilitated sexual receptivity and proceptive behaviours in female rats primed with estrogen alone or estrogen and progesterone. In addition, DPDPE infusions significantly reduced the number of rejection responses displayed by females primed with estrogen alone. Furthermore, the highly selective κ-receptor agonist, U50-488, dramatically facilitated sexual receptivity in rats primed with estrogen alone. In female rats primed with estrogen and progesterone, this facilitation was comparatively modest, though statistically significant. This experiment revealed that opioids affect female sexual behaviour differently depending on the opioid receptor subtype that is stimulated, and on the hormonal priming regimen.

The effects of opioids have been examined following administration into brain regions that, as mentioned earlier, are important for the regulation of female sexual behaviour. These regions include the VMN, mPOA and MCG. Although the effects of opioids administered into other brain regions, including the LS and the MEA, have not been studied, lesioning techniques have been used to elucidate the roles of these regions in female sexual behaviour.

Role of the VMN. One of the sites central to the regulation of female rat sexual behaviour is the VMN. Lesions of the VMN decrease proceptive behaviour and lordosis

(Pfaff & Sakuma, 1979). However, female rats have been shown to recover from the damage caused by this lesion and this recovery can be observed by return of lordosis (La Vaque & Rodgers, 1975; Pfaff & Sakuma, 1979; Richmond & Clemens, 1988). The connections between the VMN and the MCG are essential for lordosis in the female rat (Hennessey, Camak, Gordon & Edwards, 1990).

It has been established that opioid infusions in the VMN can inhibit or facilitate lordosis behaviour. For example, Vathy, Van der Plas, Vincent, and Etgen (1991) found that bilateral infusions of morphine into the VMN inhibited lordosis. This inhibition was blocked by prior infusions of naloxone. However, Sinchak, Hendricks, Baroudi and Micevych (1997) found that administration of orphanin into the VMN facilitated lordosis in a dose-dependent manner in female rats treated with estrogen. This orphanin facilitation in the VMN contrasts with the inhibition observed following μ-opioid receptor activation, implying that orphanin opioids are activating a different population of neurons in the VMN.

Role of the mPOA. Activation of the mPOA facilitates pacing and proceptive behaviours and inhibits lordosis. Following lesions of the mPOA, an increase in sexual receptivity is observed in estrogen and progesterone primed female rats (Hoshina, Takeo, Nakano, Sato & Sakuma, 1994; Veney & Rissman, 1997). Electrical stimulation of the mPOA, on the other hand, inhibits lordosis (e.g. Takeo, Chiba, & Sakuma, 1993; Hoshina et al., 1994). It should be noted, however, that mPOA lesions have little effect if the female is able to pace her copulatory contact with a male rat (Powers & Valenstein, 1971; Whitney, 1986). The role of the mPOA in proceptive behaviours is thought to be mediated by a projection from the mPOA to the VTA (Hasegawa, Takeo, Akitsu,

Hoshina, & Sakuma, 1991).

Infusions of opioids into the mPOA have also been found to have effects on female sexual behaviour. For example, infusions of β-endorphin into the mPOA inhibit lordosis behaviour (Sirinathsinghji, 1985). Furthermore, infusions of the μ-opiate receptor antagonist, ß-funaltrexamine, into the mPOA facilitated lordosis in estrogen primed female rats (Hammer, Dornan, & Bloch, 1989).

Role of MCG. Electrolytic lesions of the MCG have been found to impair lordosis and these lesions are most effective in the dorsal half of the MCG (Sakuma & Pfaff, 1979). The immediate decline in lordosis behaviour contrasts to a gradual decline that has been observed following lesions of the VMN (Sakuma & Pfaff, 1979). As mentioned, the connections between the VMN and the MCG are essential for lordosis in the female rat (Hennessey et al., 1990).

Opioid infusions into the MCG also affect female sexual behaviour. Infusions of naloxone into the MCG facilitate lordosis in EB-primed rats (Sirinathsinghji, Whittington, Audsley & Fraser,1983; Sirinathsinghji,1984), whereas infusions of β-endorphin into the MCG abolish it (Sirinathsinghji et al.,1983). Pretreatment with a lutenizing hormone releasing hormone (LH-RH) antagonist inhibited the facilitation observed following naloxone infusion and abolished the attenuation of lordosis observed following β-endorphin infusions for approximately 12 hours (Sirinathsinghji, et al., 1983; Sirinathsinghji, 1984). This suggests that β-endorphin in the MCG inhibits lordosis by inhibiting LH-RH secretion.

Role of the LS and MEA. As mentioned, two other brain regions involved in the regulation of female sexual behaviour are the LS and MEA. Lesions of the LS facilitate

lordosis behaviour in female rats (Yamanouchi & Arai, 1990). Yamanouchi and Arai (1990) conducted four types of transections of the dorsal, ventral or posterior inputs and outputs of the septal area in female rats. They found that the ventrolateral efferents were responsible for sending the inhibitory signal for lordosis. In the case of the amygdala, different lesions of nuclei in the amygdaloid complex have different effects on female sexual behaviour (Mascó & Carrer,1980). Lesions of the anterior part of the corticomedial nucleus attenuated lordosis, whereas lesions of the posterior part of the lateral amygdaloid nucleus significantly increased lordosis.

The Present Experiment

Overall, research has shown that opioids act to facilitate or inhibit different aspects of female sexual behaviour. These effects depend on the route of administration, the brain regions that are stimulated and the type of opioid receptor subtype that is activated. In addition, the hormonal states of the female rat regulate opioid receptor density as well as peptide gene expression. The present thesis used induction of the immediate early gene *c-fos* in order to identify functional pathways in the brain where opioid activation is modulated by hormones, specifically estrogen and progesterone. In this experiment the effects of three opioid ligands selective for μ (DAMGO)-, δ (DPDPE)-, and κ (U50-488)-opioid receptors were investigated in females subjected to one of three different hormone regimes, EB and P (EB + P), EB alone or OIL alone. The ligands were administered to the females in the lateral ventricle and Fos induction was examined in the LS, mPOA, VMN, posterior-dorsal MEA (MEApd) and MCG.

Method

Animals

Female and Male Long-Evans rats were purchased from Charles River Canada, Inc., St. Constant, Québec, weighing approximately 250 g at the start of the experiment. Prior to surgery, the females were housed in pairs in Plexiglas cages (19" x 10.5" x 8") in a colony room maintained on a reversed 12:12/hr light/dark cycle (lights off at 6:00 am) at approximately 21°C. Food and water were available ad libitum. Animals were handled daily for 5 min each by the experimenter for one week prior to gonadal surgery. Sexually experienced Long-Evans males that were housed in pairs in Plexiglas cages in the same colony were used for copulatory sessions throughout the study.

Surgery

Ovariectomy. Prior to the OVX, all females (n = 48) were anesthetized with a mixture of ketamine hydrochloride (50 mg/ml; Ayerst Veterinary Laboratories, Guelph, Ontario) and xylazine hydrochloride (4 mg/ml; Bayer Inc., Etobicoke, Ontario) mixed at a ratio of 4:3 ml, respectively. This mixture was injected intraperitoneally (ip) in a volume of 0.90 ml/kg of body weight. The OVX was performed via bilateral lumbar incisions. In each female, the ovary was identified and, subsequently, ligated and excised. Incisions in the muscle wall were sutured. The initial surgical opening was closed by three or four wound clips. Wound clips were left on for a minimum of 10 days and were removed after the incisions had healed properly. The incisions were treated with Proviodine (10%) for 2-3 days after surgery.

Cannulation. Females were anesthetized with sodium pentobarbital (65 mg/kg), which was administered at 70% of their body weight, prior to cannulation. Before the

somnotol was injected (ip), females were given a subcutaneous injection of atropine (0.4 mg/ml; Sabrex, Boucherville, Q.C.) All females were food deprived prior to cannulation surgery.

Following a sexual experience session (see next section) females were implanted stereotaxically with a 5.5 mm long 22-gauge stainless-steel cannula (Plastics One Products, Roanoke, VA) aimed at the right lateral ventricle. (Coordinates: -0.2 mm posterior to bregma, -1.75 mm lateral to the midline, and -2.6 mm ventral from dura with the incisor bar set at 5 mm above the horizontal plane passing through the interaural line; Pellegrino, Pellegrino & Cushman, 1979). The guide cannula was fixed in place with dental acrylic cement that was molded to four skull screws. Cannulae were occluded with cannula blockers (Plastics One Products, Roanoke, VA), which extended approximately 0.2 mm beyond guide tips. Blockers were removed when infusions were being performed and were replaced immediately after.

Angiotensin II has been shown to reliably induce thirst following injections into the lateral ventricles (Epstein, Fitzimmons, & Rolls, 1970; Pfaus & Pfaff, 1992). Therefore, all animals received an infusion of 2 μg Angiotensin II into the right lateral ventricle prior to the test day. This was conducted to confirm proper cannula placement. Angiotensin II (Sigma Chemicals) was dissolved in physiological saline at a concentration of 1 μg/μl. Only females which drank within 5 minutes following these infusions were used as subjects in the experiments. Females in the EB + P group also received a saline injection and copulatory session 4 days prior to test day. All injections were made with a 28-gauge stainless steel cannula that protruded 2 mm ventral to the tip of the guide cannula. This injector was connected via plastic tygon tubing to a 10 ul

Hamilton syringe. Drug infusions were made using a motorized microsyringe pump in all experiments.

Drug and Hormone Treatments

Hormone treatment and Sexual Experience. Estradiol benzoate (EB) and progesterone (P) were purchased from Steraloids, Inc., (Wilton, N. H), dissolved in sesame oil (OIL), and injected subcutaneously in 0.1 mL. Sexual receptivity and proceptivity were induced by priming each female with a subcutaneous injection of EB (10μg) 48 hours and P (125 μg) 4 hours prior to the copulation session. In all experiments, females were exposed to 60 min of copulation with sexually experienced males 10 days following surgery. Females were habituated to the bilevel chambers for two days prior to the first copulation test, half an hour each session. Following this initial sexual experience females were placed on one of three hormone treatments. In the first group, females (n = 16) received EB every four days before and after cannulation. Six days after cannulation these females were primed with EB 48 hr and P 4 hr before a saline infusion which was then followed by a copulation session with sexually experienced males. The copulation session was, therefore, approximately one week following the cannulation. Four days following the copulation session, females received EB 48 hr and P 4hr before infusion of a drug (see below). In the second group, females (n = 16) received EB alone for 1 month prior to cannulation and for approximately 10 days after. Then, females were primed with EB 48 hr and OIL 4 hr before the drug infusion. In the third group, females (n = 16) received OIL alone for 1 month prior to cannulation and for approximately 10 days after. As in the EB-alone group, females were primed with EB

48 hr and OIL 4 hr before the drug infusion. Therefore, all three groups received an initial copulation session whereas only the EB and P group had a second session.

Drugs. In all experiments, females were randomly assigned to one of the three hormone conditions as well as one of the three drug treatments or vehicle. The selective μ-opioid receptor agonist [D-Ala^2, N-Me-Phe^4, Gly^5-ol], (DAMGO, Sigma, St. Louis, MO), the δ-opioid receptor agonist [D-Pen^2, D-Pen^5 enkephalin (DPDPE, Research Biochemicals International, Natick, MA), and the κ-opioid receptor agonist (-)-*trans*-1S, 2S-U-50488 hydrochloride (U-50488, Research Biochemicals, Natick, MA) were dissolved in nanopure water to yield the concentration of 2000 ng/μl for each agonist. Each rat received 1 μg of a specified opioid agonist, or saline (0.9%), into the lateral ventricle at a rate of 1 μg/min. Following each infusion, the injector was left in the guide cannula for 2 min to ensure that the drug had properly diffused throughout the brain.

Immunocytochemistry

All females were sacrificed 75 minutes after the drug or saline infusion by an ip injection of 0.9 mL sodium pentobarbitol. They were perfused with ice-cold phosphate-buffered saline (400 ml) followed by ice-cold paraformaldehyde in 0.1 M phosphate buffer (400 ml). Brains were removed, postfixed in fresh paraformaldehyde for 4 h, and stored overnight in 30% sucrose at 4°C. Frozen coronal brain sections (30 μm) were cut on a sliding microtome from each brain through the mPOA, LS, (corresponding to plates 18-21 in Paxinos and Watson [1986]), the VMH and MEA (corresponding to plates 26-32 in Paxinos and Watson [1986]), and the MCG (corresponding to plates 47-50). Sections were washed (3x5 min rinses) in cold 50 mM Tris-buffered saline (TBS) and were incubated at 4°C for 30 min in TBS and 30% w/w hydrogen peroxide (H_2O_2). Sections

were rinsed again (3 x 5 min TBS washes) and then incubated for 90 min at 4°C in 0.05 % Triton TBS and 3% normal goat serum (NGS). Following this sections were transferred directly into a solution containing a rabbit polyclonal antibody raised the N-terminal residues 4-17 of human Fos protein (Oncogene Science; diluted 1:75 000) in 0.05% Triton TBS with 3% normal goat serum at 4°C for 72 h. Sections were then rinsed in TBS (3 x 5 min washes) and transferred into biotinylated anti-rabbit IgG made in goat (Vector Laboratories; 1:200) in 0.05% Triton TBS, with 3% normal goat serum for 1 h at 4°C. Then, sections were rinsed in TBS (3 x 5 min washes) and were transferred into an avidin-biotinylate-peroxidase complex (Vectastain Elite ABC Kit, Vector Laboratories; diluted 1:55.55) for 2 h at 4°C. Following incubation with the ABC reagants, sections were rinsed with TBS followed by a 10 min rinse with 50 mM Tris and a 10 min rinse in 3,3' diaminobenzidine (DAB) in 50 mM Tris at room temperature on an orbit shaker. This was followed by another 10 min incubation of 50 mM Tris, DAB and 3% H_2O_2 (0.1 ml per 100 ml of DAB/Tris buffer, pH 7.8) to catalyze the DAB and 8% nickel chloride (400 µl per 100 ml of DAB/Tris buffer + H_2O_2) to colour the DAB chromagen product blue-black. To stop the reaction, sections were rinsed in TBS and then mounted onto gel-coated slides. They were then dehydrated with ethanols, cleared in Hemo-de, coverslipped, and examined under a microscope.

Histological and Statistical Analyses

Fos-immunoreactive (ir) cells were counted using a Leitz Laborlux microscope (40 X) connected to a digital image analysis system (MI, Imaging Research, Inc., St. Catherines, ON), with the standard dimension of a single cell nucleus set between 2 and 50 pixels. Average numbers of Fos-ir cells were calculated from 3 sections/region/rat

which appeared to contain the largest number of Fos-ir cells (Pfaus, Kleopoulos, Mobbs, Gibbs & Pfaff, 1993; Pfaus, Marcangione, Smith, Manitt & Abillamaa, 1996). Two-way analyses of variance (ANOVA) were used for each region to examine the main effects of drug and hormone and their interaction on the mean number of Fos-ir cells. For each significant ANOVA, post-hoc comparisons of the group mean were made using the Tukey method, $p<0.05$.

Results

Behavioural Observations

Following drug infusions, the behavioural response of each animal was observed. Following infusions of DPDPE or U50-488, females behaved as they had prior to infusions, without any noticeable changes. Following infusions of DAMGO, some females displayed a decrease in general motor activity. However, once the experimenter picked up these animals, they responded.

Fos Induction in the LS

Effect of DAMGO. In the EB + P group, Fos induction was fairly scattered throughout the intermediate (LSI) and ventral (LSV) regions. However, some cells were observed in a band that lined the ventricles. In the EB-alone group, low to moderate amounts of Fos cells were found to be slightly more concentrated in the LSV, near the septohypothalamic nucleus. In the OIL group, Fos cells were more concentrated in the LSV and appeared to be higher in number than in the EB+P or EB-alone treated rats (Figure 1 and 1a).

A two-way ANOVA revealed a significant main effect of drug treatment $F(1, 18) = 22.94$, $p < 0.001$. Post hoc comparisons revealed that drug-treated rats had significantly

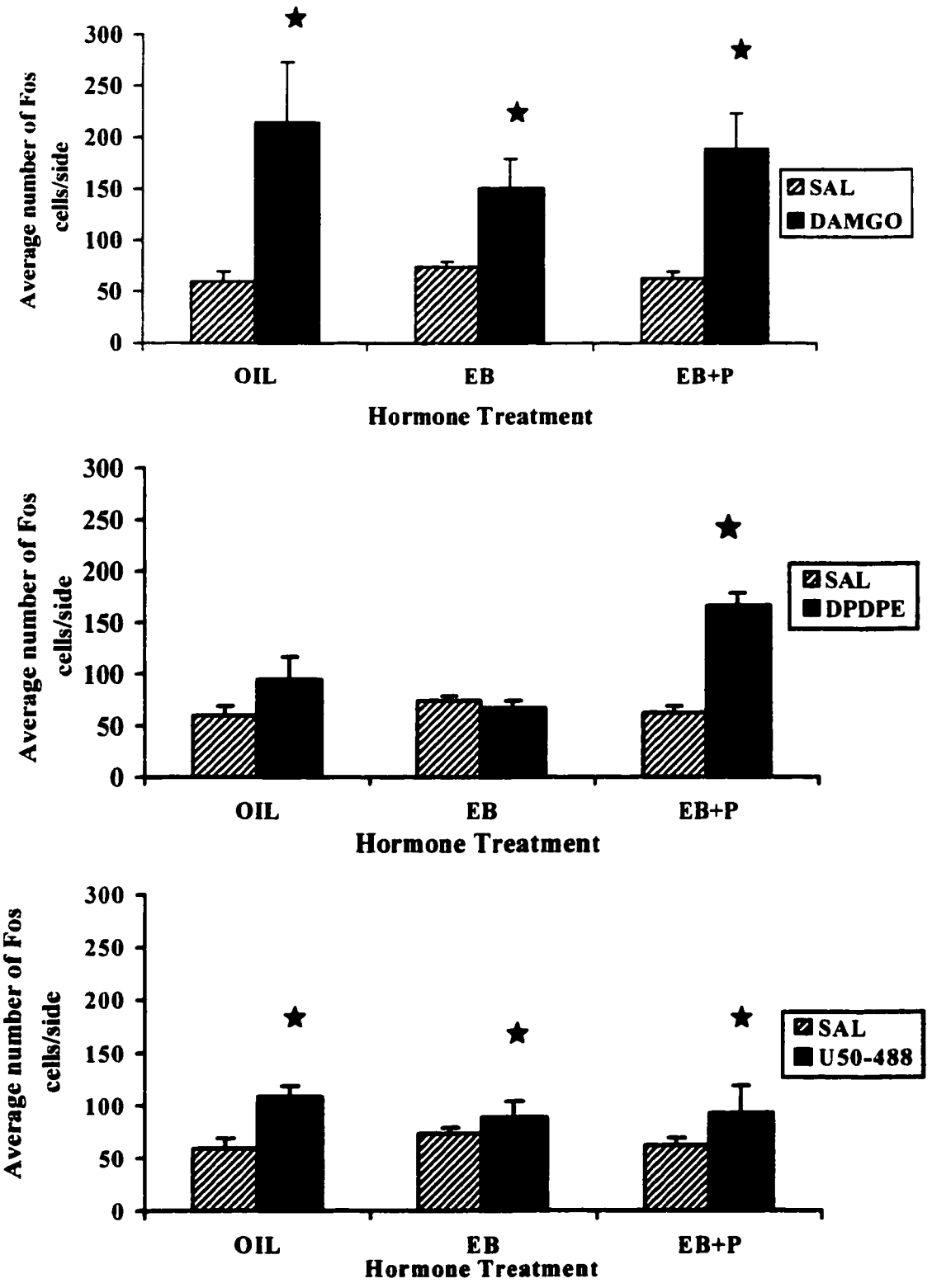

Figure 1. Average number of Fos cells/side in the LS. Solid bars represent means from OVX rats given hormone and drug treatment (n = 4/condition); hatched bars represent means from OVX rats given hormone and saline treatment (n = 4/condition). Vertical lines denote standard errors.

Figure 1a Representative digitized image of the LS taken at 40 X

greater Fos induction than saline-treated rats across all hormone conditions. There was no significant main effect of hormone or significant interaction between hormone treatment and drug treatment.

Effect of DPDPE. In the EB + P condition, high amounts of Fos were concentrated in the intermediate area of the LS (LSI) compared to a low amount observed in the EB-alone group. The OIL group had moderate Fos induction that was somewhat higher than the EB-alone group but lower than the EB + P group. In both the EB-alone and OIL group, the Fos was scattered. However, in the EB-alone group, the scattering was slightly more concentrated in the LSD area, whereas in the OIL group it was more concentrated in the LSV area (Figure 1 and 1a).

The ANOVA showed a main effect of hormone treatment, $F(2, 18) = 8.06$, $p < 0.01$. Post hoc comparisons revealed that the EB + P treated rats had significantly higher Fos induction compared to EB-alone and OIL-treated rats across all drug conditions. There was also a main effect of drug treatment, $F(1, 18) = 20.83$, $p < 0.001$. Post hoc comparisons revealed that Fos induction in drug-treated rats was significantly higher than saline-treated rats across all hormone conditions. There was also a significant interaction of drug and hormone treatment, $F(2, 18), = 11.14$, $p < 0.001$. Post-hoc comparisons of the interaction means revealed that the EB + P treated rats had a significantly higher amount of Fos than the EB-alone and OIL-treated rats as well as significantly higher amounts of Fos than rats treated with saline.

Effect of U50-488. In the EB + P condition, Fos induction was very low and was observed mostly in the LSV area. The EB-alone and OIL groups also had fairly low levels of Fos induction (Figure 1 and 1a).

The ANOVA indicated a significant main effect of drug treatment, $F(1, 18) = 7.46$, $p < 0.05$, with a significantly higher number of Fos cells in drug-treated rats compared to saline-treated rats, across all hormone conditions. There was no significant main effect of hormone or significant interaction of hormone treatment and drug treatment.

Fos Induction in the mPOAa

Effect of DAMGO. In both the EB + P and EB-alone group, the Fos induction was scattered throughout the mPOAa. However, in the OIL group, there was a slight concentration in the lateral mPOAa. There were much lower amounts of Fos in the OIL group versus the EB + P and EB-alone group (Figure 2 and 2a).

The two-way ANOVA revealed a significant main effect of hormone treatment, $F(2, 18) = 27.85$, $p < 0.0001$. Post hoc comparisons revealed that Fos induction in rats treated with EB + P and EB alone were significantly higher than in rats treated with OIL across all drug conditions. There was also a significant main effect of drug treatment, $F(1, 18) = 199.55$, $p < 0.0001$, with significantly greater Fos induction in drug-treated rats as compared to saline-treated rats across all hormone conditions. There was also a significant interaction between drug and hormone treatment, $F(2, 18) = 35.87$, $p < 0.0001$. Post hoc comparisons of the interaction means revealed that the EB + P treated rats had significantly higher Fos induction than OIL treated rats. Also rats treated with EB alone had higher Fos induction than those treated with OIL. EB + P and EB-alone rats infused with DAMGO had significantly more Fos induction than saline-treated rats.

Effect of DPDPE. In the EB + P treatment there was a high amount of Fos in the lateral part of the mPOAa. In the EB-alone group, there were low to moderate amounts of

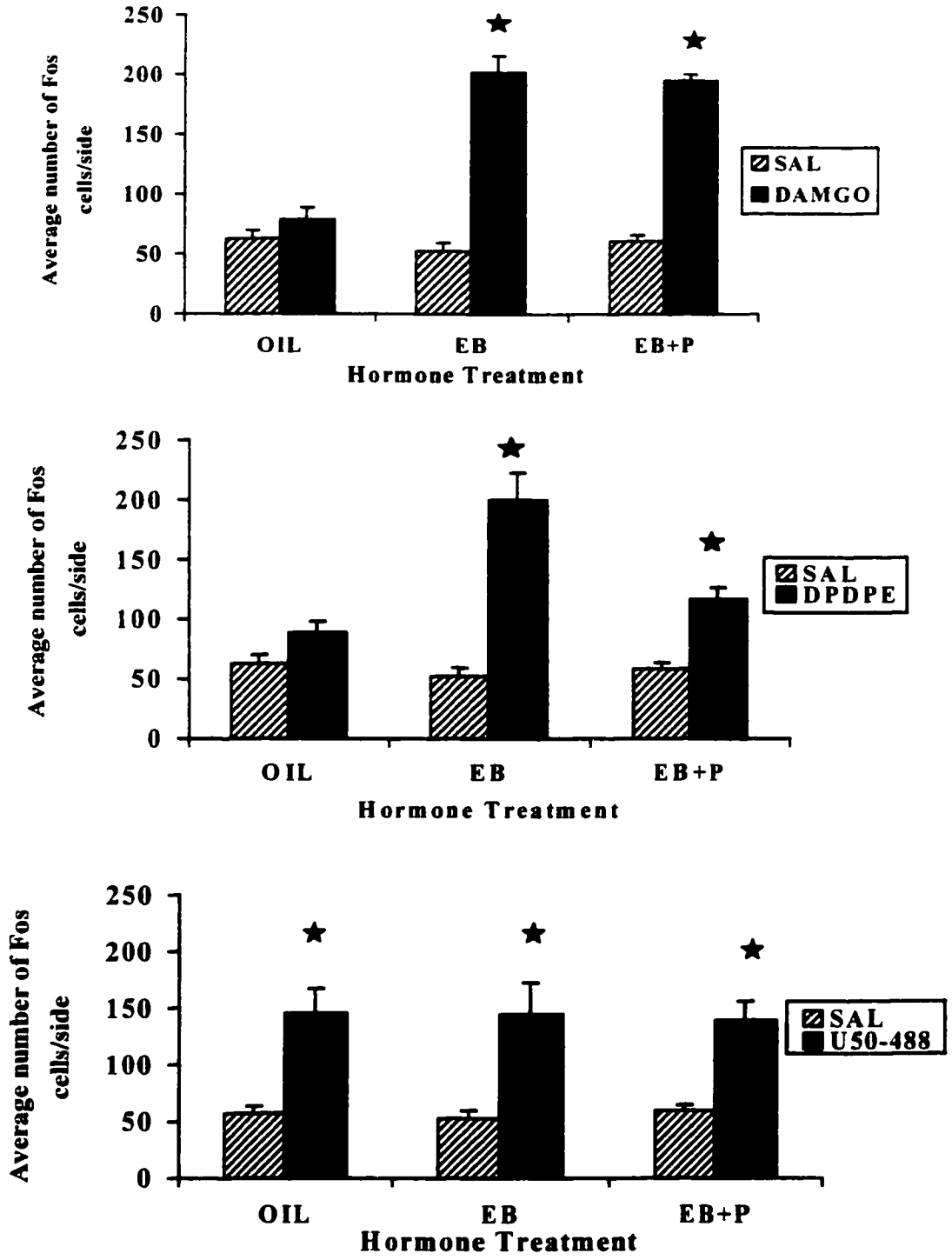

Figure 2. Average number of Fos cells/side in the mPOAa. Solid bars represent means from OVX rats given hormone and drug treatment (n = 4/condition); hatched bars represent means from OVX rats given hormone and saline (n = 4). Vertical lines denote standard errors.

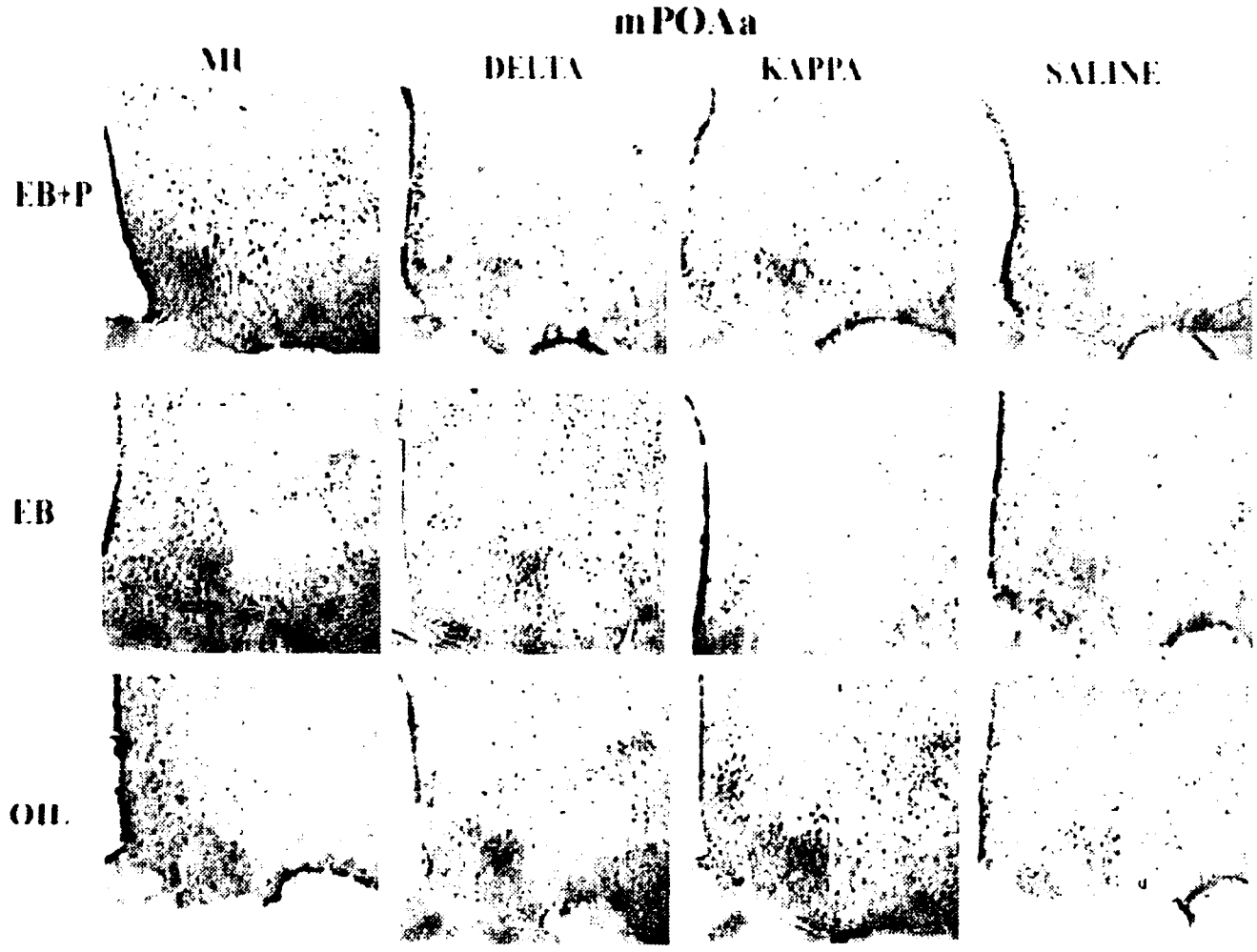

Figure 2a Representative digitized image of the mPOAa taken at 40 X

Fos scattered throughout the mPOAa. There was a slightly greater concentration in the medial aspect. In the OIL group, there were low levels of Fos induction, fairly concentrated near the optic tract (Figure 2 and 2a).

The ANOVA found a significant main effect of hormone treatment, $F(2, 18) = 9.87$, $p < 0.001$, with EB + P treated rats showing greater Fos induction than the EB-alone and OIL-treated rats across all drug conditions. There was also a significant main effect of drug, $F(1, 18) = 63.10$, $p < 0.0001$. Post hoc comparisons revealed that drug-treated rats had a significantly greater amount of Fos induction than saline-treated rats across all hormone groups. There was also a significant interaction between hormone treatment and drug treatment $F(2, 18) = 13.95$, $p < 0.001$. Post hoc comparisons of the interaction means revealed that there was significantly higher Fos induction in EB-alone treated rats compared to EB + P and OIL-treated rats. EB alone and EB + P treated rats that were given DPDPE had significantly more Fos-ir cells than their respective saline groups.

Effect of U50-488. The Fos induction in the EB+P group was scattered and it was seen in low to moderate amounts. In the EB-alone group, the Fos was seen in moderate amounts and it was activated in the dorsomedial part of the mPOAa. In the OIL group there were low levels of Fos that were scattered throughout with a slightly higher concentration in the lateral part (Figure 2 and 2a).

The ANOVA detected a significant main effect of drug treatment, $F(1, 18) = 38.69$, $p < 0.0001$. Post hoc comparisons showed that drug-treated rats had significantly more Fos cells than saline-treated rats across hormone conditions. There was no

significant main effect of hormone treatment or a significant interaction of drug and hormone treatment.

Fos Induction in the mPOAp

Effect of DAMGO. In the EB+P and EB-alone groups, a moderate to high density of Fos cells were concentrated in the mPN region. There were lower amounts of scattered Fos staining in the lateral regions of the mPOAp. In the OIL group the Fos staining was scattered in low amounts. (Figure 3 and 3a).

The ANOVA detected a significant main effect of hormone treatment $F(2, 18) = 7.64$, $p < 0.01$. Post hoc comparisons revealed that the EB + P treated rats had significantly more Fos induction than OIL-treated rats across all drug groups. There was also a significant main effect of drug treatment, $F(1, 18) = 63.72$, $p < 0.0001$. Post hoc comparisons revealed that drug-treated rats had significantly more Fos induction than saline-treated rats across all hormone conditions. There was also a significant interaction of drug and hormone treatment, $F(2, 18) = 9.21$, $p < 0.01$. Post hoc comparisons of the interaction means revealed that EB + P and EB-alone rats had significantly more Fos induction than OIL-treated rats as well as significantly more Fos-ir cells than their respective saline groups.

Effect of DPDPE. In both the EB + P and EB alone groups, Fos induction was fairly concentrated in the mPN, specifically the ventral area of the mPN. There were also high amounts of Fos induction. In the OIL group there were low levels of Fos scattered throughout the mPOA (Figure 3 and 3a).

The ANOVA detected a significant main effect of hormone treatment $F(2, 18) = 4.71$, $p < 0.05$. Post hoc comparisons revealed that the EB-alone treated rats had

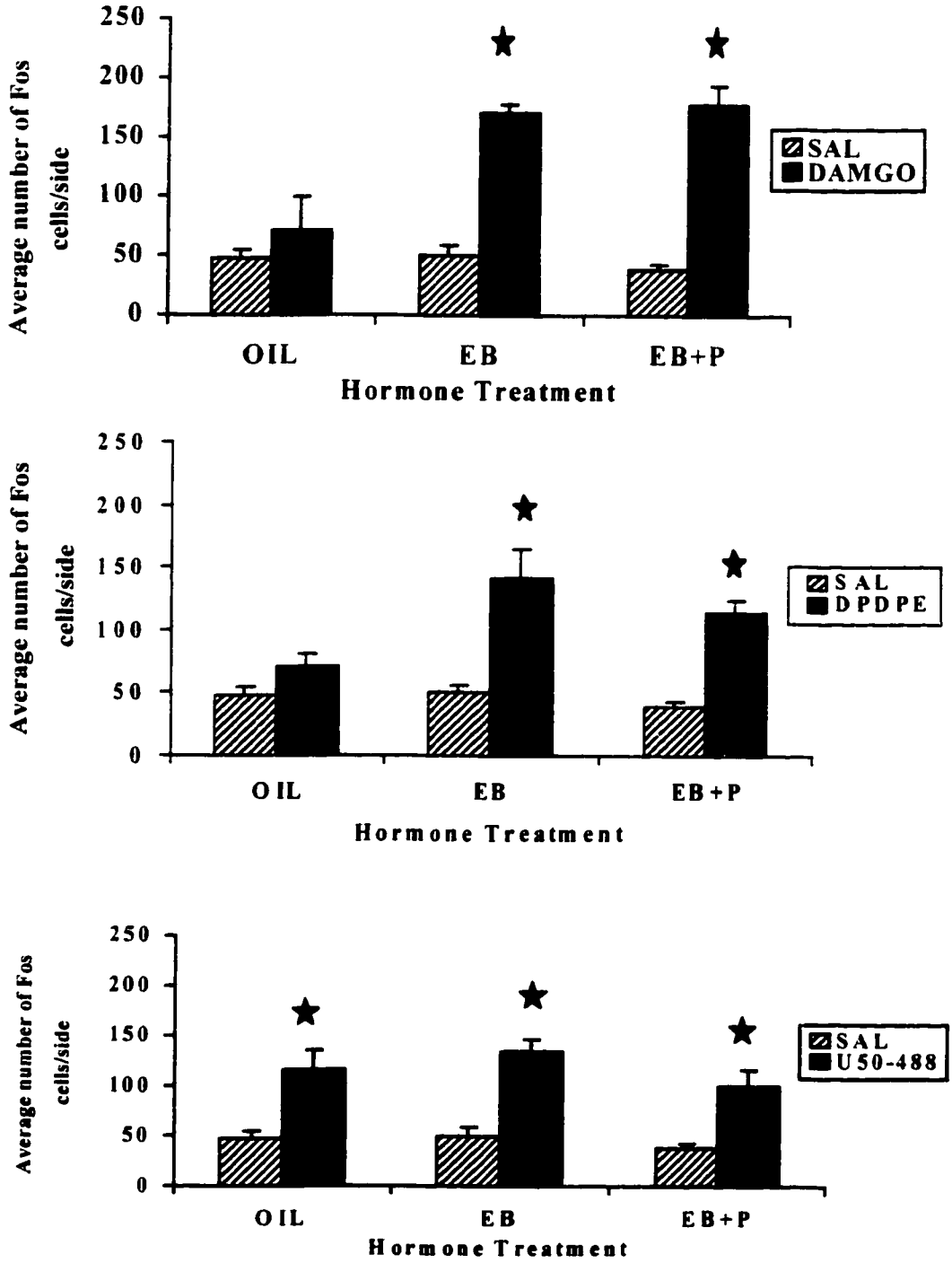

Figure 3. Average number of Fos cells/side in the mPOAp. Solid bars represent means from OVX rats given hormone and drug treatment (n = 4/condition); hatched bars represent means from OVX rats given hormone and saline treatment (n = 4/condition). Vertical lines denote standard errors.

Figure 3a Representative digitized image of the mPOAp taken at 40 X

significantly more Fos induction than OIL treated rats across all drug groups.

There was also a main effect of drug treatment, $F(1, 18) = 43.48$, $p < 0.0001$. Post hoc comparisons revealed that drug-treated rats had significantly more Fos than saline-treated rats across all hormone conditions. There was also a significant interaction of drug and hormone treatment, $F(2, 18) = 4.60$, $p < 0.05$. Post hoc comparisons of the interaction means revealed that EB-alone treated rats had significantly more Fos induction than OIL treated rats. Both EB-alone and EB + P treated rats that were given DPDPE had significantly more Fos cells than their respective saline groups.

Effect of U50-488. In all three hormone treatment groups the Fos induction was scattered throughout the mPOAp and was observed in low amounts. (Figure 3 and 3a).

The ANOVA detected a main effect of drug treatment, $F(1, 18) = 43.46$, $p < 0.0001$, with significantly more Fos induction in drug-treated rats compared to saline-treated rats across all hormone conditions. There was no significant main effect of hormone treatment or hormone and drug interaction on Fos induction.

Fos Induction in the VMNa

Effect of DAMGO. In the EB+P group there were high levels of Fos staining throughout the VMN. In the EB-alone group there were low levels of Fos scattered throughout the VMN. In the OIL group, there appeared to be more Fos induction then the EB-alone group and it was slightly concentrated in the ventrolateral area of the VMN. (Figure 4 and 4a).

The ANOVA revealed a significant main effect of hormone treatment, $F(2, 18) = 31.97$, $p < 0.0001$. Post hoc comparisons revealed that EB+P treated rats had significantly more Fos induction then the EB-alone and OIL-treated rats across all drug conditions.

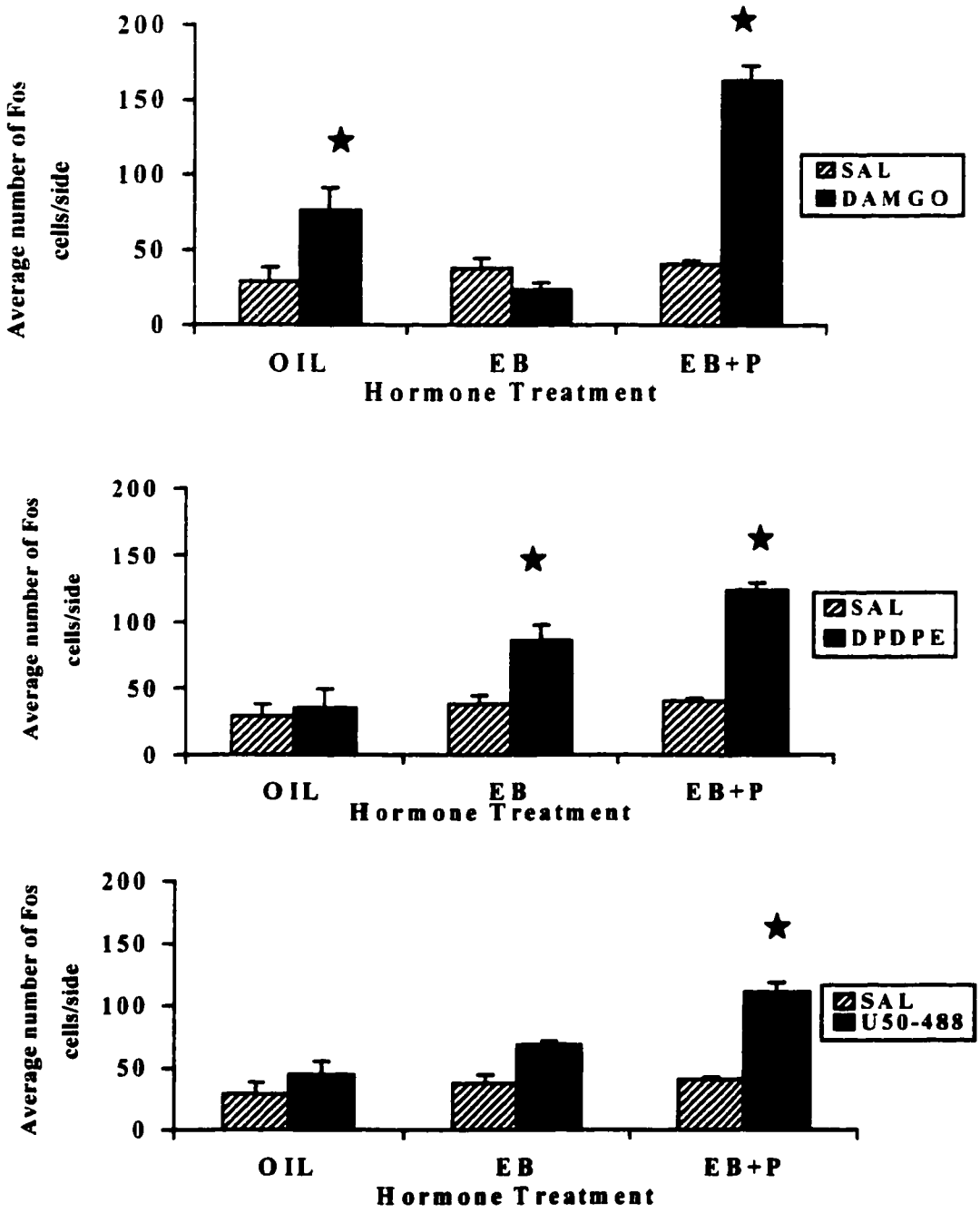

Figure 4. Average number of Fos cells/side in the VMNa. Solid bars represent means from OVX rats given hormone and drug treatment (n = 4/ condition); hatched bars represent means from OVX rats given hormone and saline treatment (n = 4/ condition). Vertical lines denote standard errors.

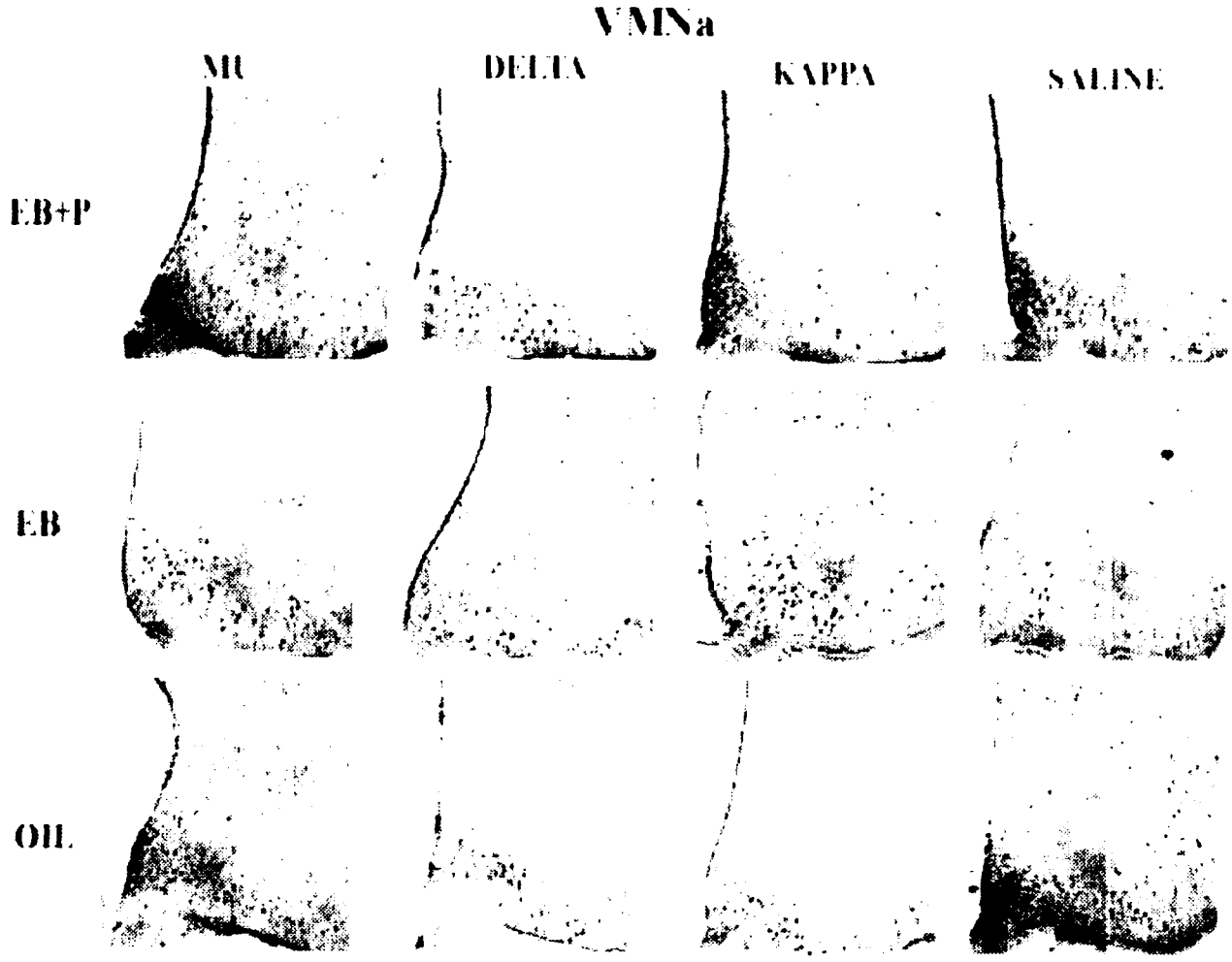

Figure 4a Representative digitized image of the VMNa taken at 40 X

There was also a significant main effect of drug treatment, $F(2, 18) = 48.64$, $p < 0.0001$, with significantly higher numbers of Fos cells in drug-treated rats compared to saline-treated rats across all hormone groups. There was also a significant interaction between drug and hormone treatment, $F(2, 18) = 28.17$, $p < 0.0001$. Post hoc comparisons of the interaction means revealed that rats treated with EB+P had a significantly greater number of Fos cells then the EB-alone and OIL groups. The OIL group also had a significantly greater amount of Fos induction compared to the EB-alone group. The EB + P and OIL alone groups which received drug treatment had significantly more Fos than their respective saline groups.

Effect of DPDPE. The EB + P group had moderate levels of Fos concentrated in the ventromedial region. In the EB-alone group there were low to moderate amounts of Fos scattered throughout the VMN. In the OIL group there were low amounts of Fos scattered throughout the VMN (Figure 4 and 4a).

The ANOVA detected a significant main effect of hormone treatment, $F(2, 18) = 14.59$, $p < 0.001$. Post hoc comparisons revealed that the EB + P and EB-alone group had a significantly greater amount of Fos than the OIL group. There was also a significant main effect of drug treatment, $F(1, 18) = 36.10$, $p < 0.0001$. Post hoc comparisons revealed that there was significantly more Fos induction in drug-treated rats compared to saline-treated rats across all hormone conditions. There was also a significant interaction between drug and hormone treatment, $F(2, 18) = 8.40$, $p < 0.01$. Post hoc comparisons of the interaction means showed that there was significantly more Fos induction in EB-alone treated rats compared to OIL-treated rats. There was also significantly more Fos induction in EB + P treated rats compared to OIL-treated rats. Both EB-alone and EB + P

treated rats which received drug treatment had significantly more Fos cells than the saline groups.

Effect of U50-488. The EB + P group had moderate levels of Fos scattered throughout the VMN. Both the EB-alone and OIL groups had lower levels of Fos that were scattered throughout the VMN. The EB alone group had a small concentration in the dorsomedial region of the VMN (Figure 4 and 4a).

The ANOVA detected a significant main effect of hormone treatment, $F(2, 18) = 14.00$, $p < 0.001$. Post hoc comparisons revealed that EB + P treated rats had significantly more Fos induction then EB-alone and OIL-treated rats across all drug groups. There was also a significant main effect of drug treatment, $F(1, 18) = 41.86$, $p < 0.0001$, with significantly more Fos induction in drug-treated rats compared to saline-treated rats across all hormone conditions. There was also a significant interaction between hormone and drug treatment, $F(2, 18) = 7.16$, $p < 0.01$. Post hoc comparisons revealed that there was significantly more Fos induction in drug groups given EB + P compared to EB and OIL drug-treated rats as well as EB + P drug-treated rats compared to rats given saline infusions.

Fos Induction in the VMNp

Effect of DAMGO. In the EB + P group there were fairly high levels of Fos in the VMNp with clustering in the ventrolateral region. In the EB-alone group there were low amounts of Fos throughout the entire region. In the OIL group there were low to moderate amounts of Fos that were slightly more concentrated in the ventrolateral region. (Figure 5 and 5a).

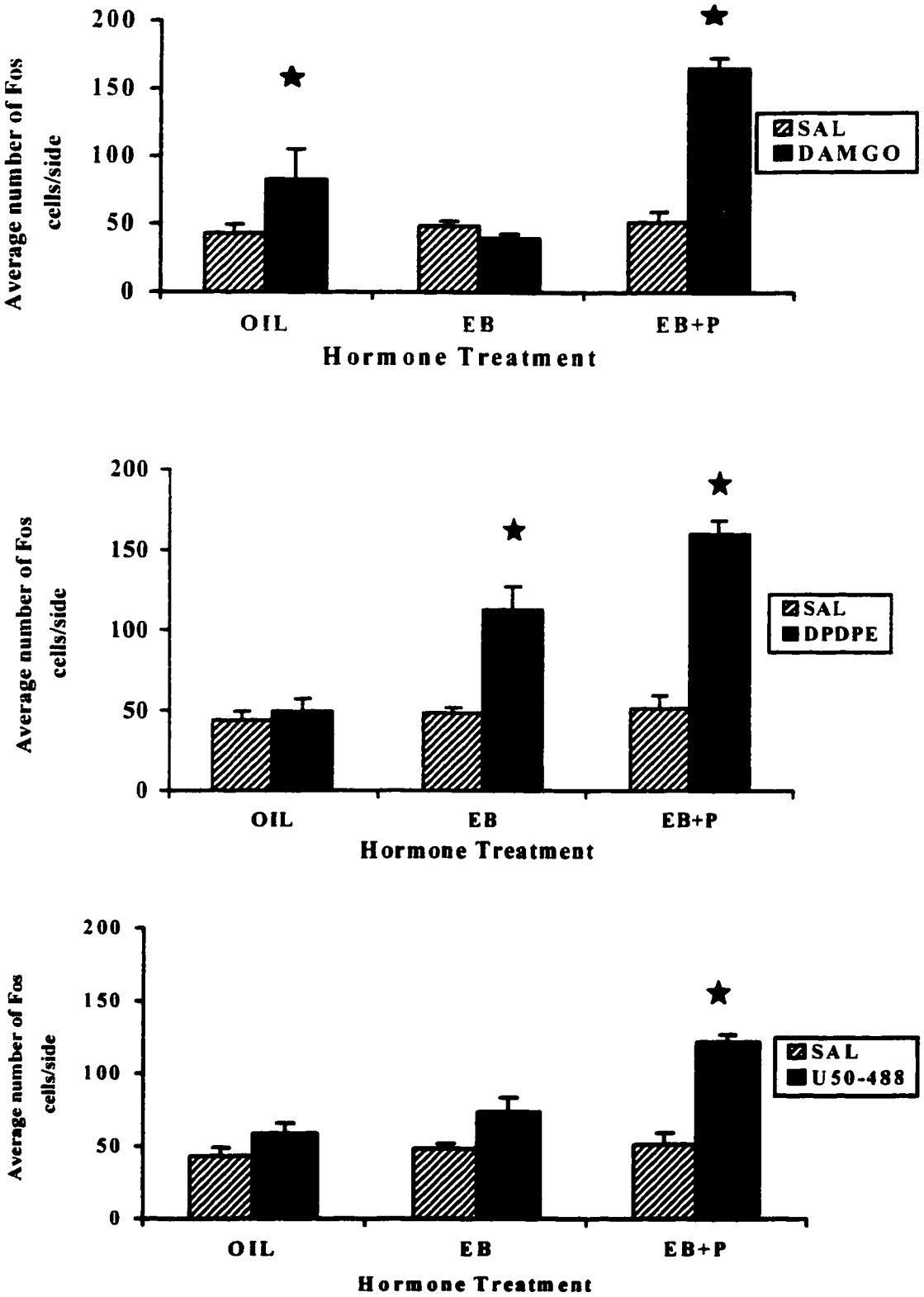

Figure 5. Average number of Fos cells/side in the VMNp. Solid bars represent means from OVX rats given hormone and drug treatment (n = 4/condition); hatched bars represent means from OVX rats given hormone and saline treatment (n = 4/condition). Vertical lines denote standard errors.

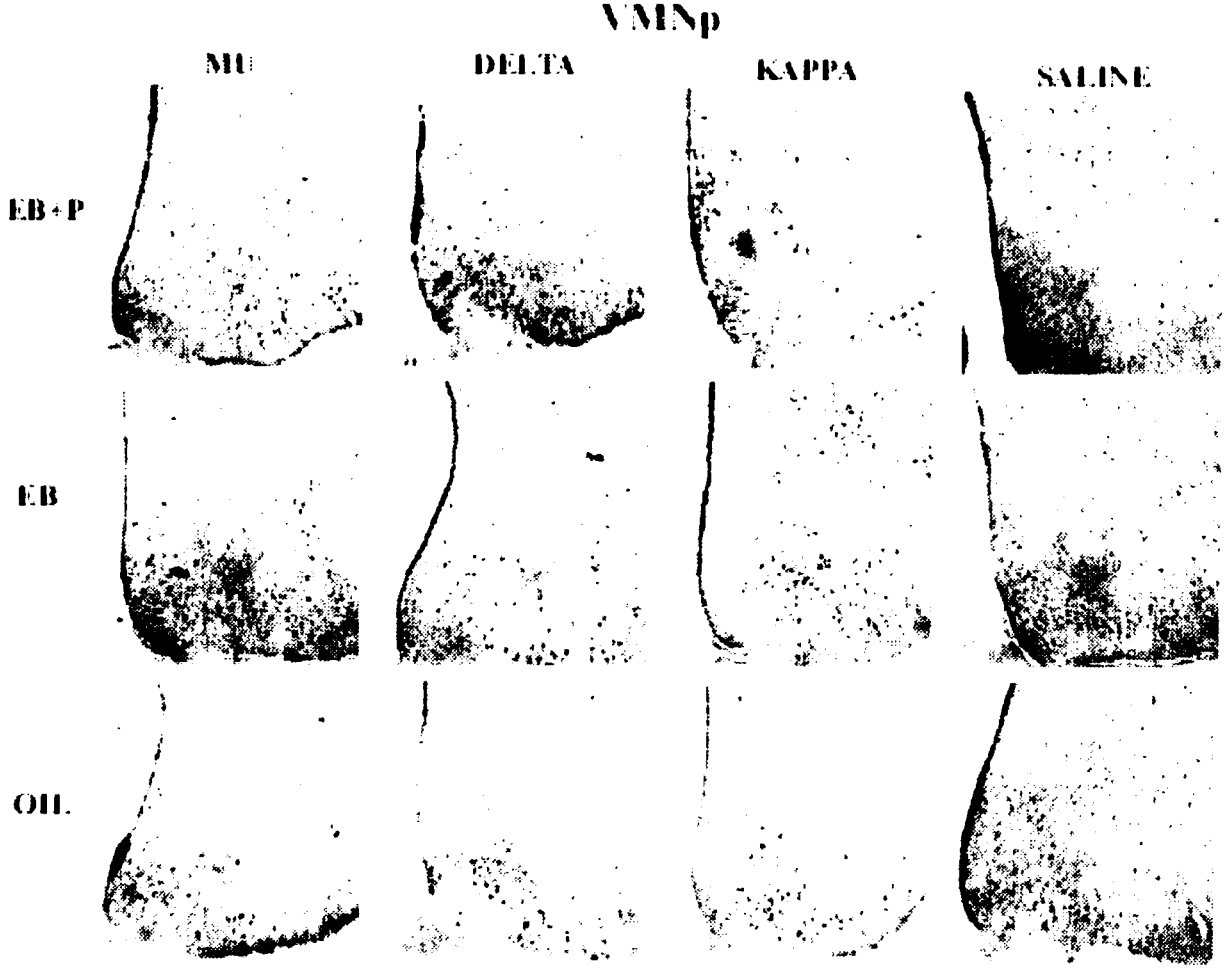

Figure 5a Representative digitized image of the VMNp taken at 40 X

The ANOVA detected a significant main effect of hormone treatment, $F(2, 18) = 19.24$, $p < 0.0001$. Post hoc comparisons indicated that the EB + P treated rats had significantly more Fos induction than EB-alone and OIL-treated rats across all drug conditions. There was also a significant main effect of drug treatment, $F(1, 18) = 30.48$, $p < 0.0001$, with significantly more Fos cells in drug-treated rats compared to saline-treated rats across hormone conditions. Finally, a significant interaction was found between the hormone and drug treatment, $F(2, 18) = 16.85$, $p < 0.0001$. Post hoc comparisons of the interaction means showed that drug-treated rats given EB+P had significantly more Fos than EB and OIL drug-treated rats. Drug-treated EB + P rats also had more Fos than those saline-treated EB + P rats.

Effect of DPDPE. In the EB+P group there were moderate to high levels of Fos that were slightly more concentrated in the ventrolateral area. In the EB-alone and OIL groups there were low amounts of Fos induction scattered throughout the VMNp. (Figure 5 and 5a).

The ANOVA detected a significant main effect of hormone treatment, $F(2, 18) = 23.34$, $p < 0.0001$. Post hoc comparisons revealed that the EB + P treated rats had significantly higher Fos induction than the EB-alone and OIL-treated rats across drug conditions. EB-alone treated rats also had significantly more Fos than OIL-treated rats across all drug conditions. There was also a significant main effect of drug treatment, $F(1, 18) = 70.20$, $p < 0.0001$, with significantly more Fos cells in drug-treated rats compared to saline-treated rats across all hormone treatments. Finally, there was a significant interaction between the drug and hormone treatment, $F(2, 18) = 17.47$, $p < 0.0001$. Post hoc comparisons of the interaction means revealed that rats treated with

drug and EB + P had significantly more Fos induction than rats treated with drug and EB-alone and OIL. EB alone drug-treated rats also had significantly more Fos than OIL drug-treated rats. Also, EB + P and EB rats drug-treated rats had significantly more Fos than EB + P and EB saline-treated rats.

Effect of U50-488. Low levels of Fos were observed in all three hormone groups. In the EB+P group the Fos was scattered throughout the VMNp. In the EB-alone group the Fos was slightly concentrated in the dorsomedial area. In the OIL group the Fos was scattered throughout the regions. (Figure 5 and 5a).

The ANOVA detected a significant main effect of hormone treatment, $F(2, 18) = 13.51$, $p < 0.001$, with significantly more Fos induction in EB + P treated rats compared to EB-alone or OIL-treated rats across all drug conditions. There was also a significant main effect of drug treatment, $F(1, 18) = 41.39$, $p < 0.0001$. Post hoc comparisons revealed that drug-treated rats had significantly more Fos compared to saline-treated rats, across all hormone treatments. Finally there was a significant interaction between the hormone treatment and drug treatment, $F(2, 18) = 8.70$, $p < 0.01$. Post hoc comparisons of the interaction means revealed that drug-treated EB + P rats had significantly more Fos than drug-treated EB-alone and OIL rats. Also, drug-treated EB + P rats had significantly more Fos than saline-treated EB + P rats.

Fos Induction in the MEApd

Effect of DAMGO. In both the EB + P and EB-alone group there were moderate amounts of Fos induction throughout the MEApd, with a slightly higher concentration in the ventromedial region. In the OIL group there were low to moderate levels of Fos scattered throughout this region. (Figure 6 and 6a).

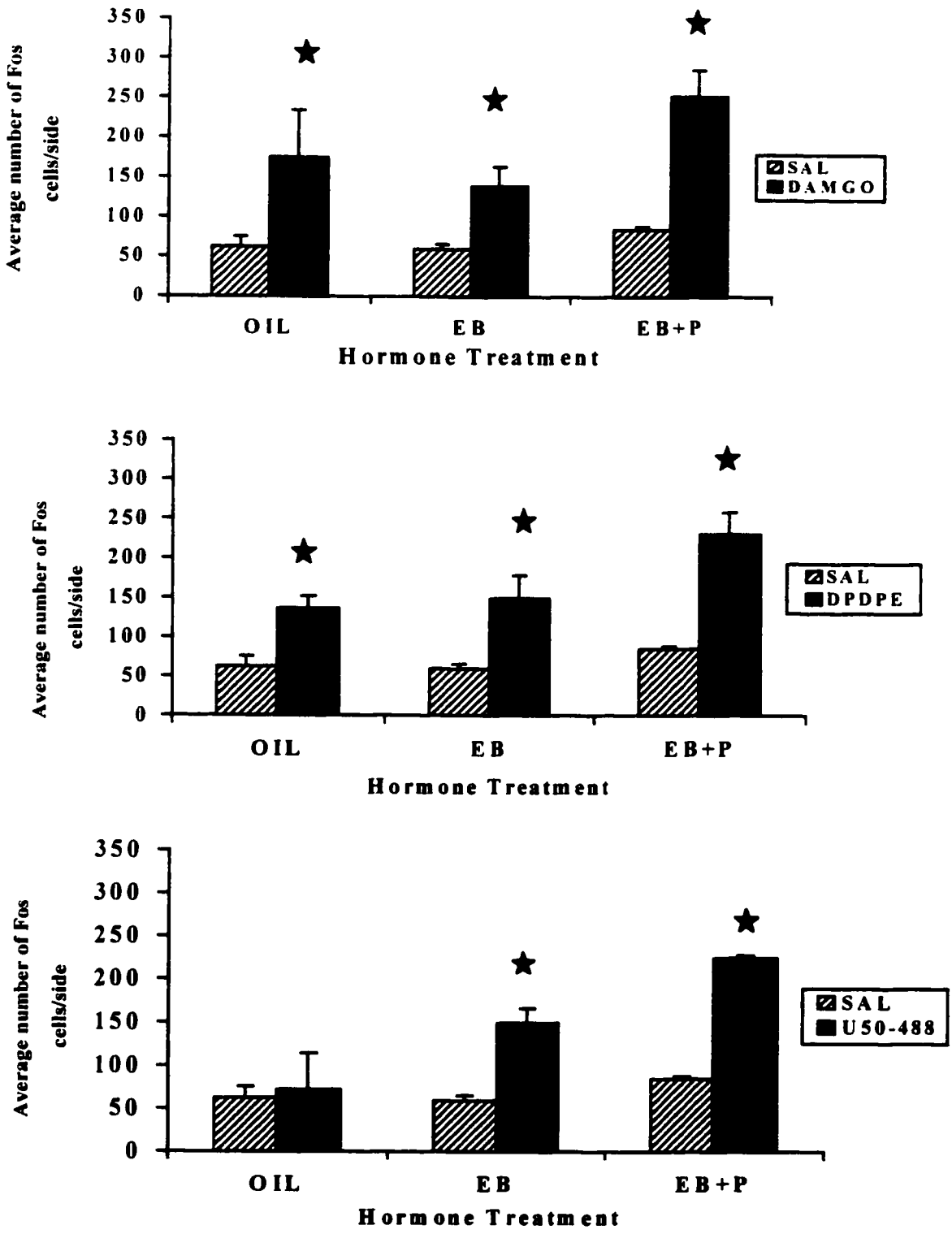

Figure 6. Average number of Fos cells/side in the MEApd. Solid bars represent means from OVX rats given drug and hormone treatment (n = 4/ condition); hatched bars represent means from OVX rats given hormone treatment and saline (n = 4/ condition). Vertical lines denote standard errors.

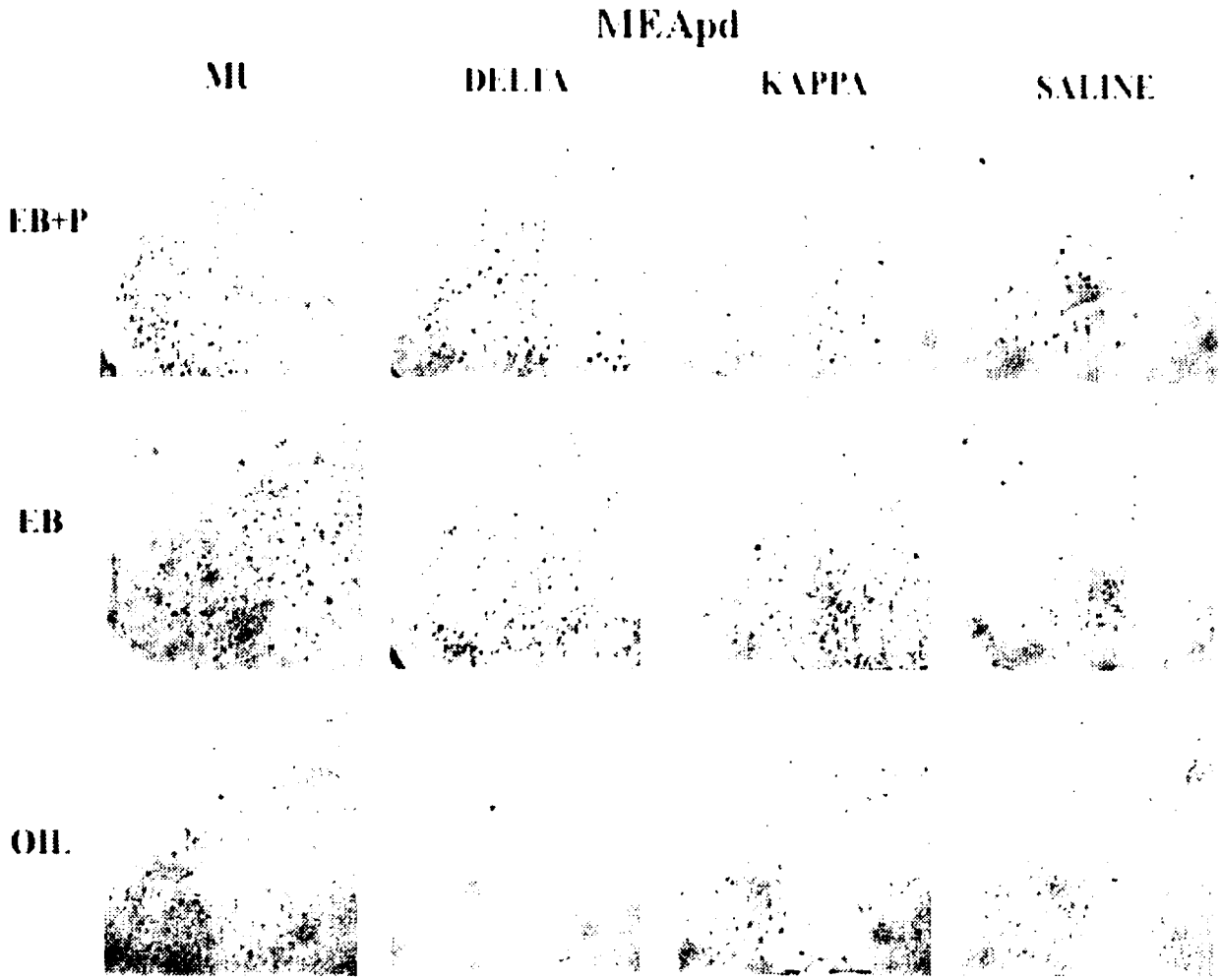

Figure 6a Representative digitized image of the ME Apd taken at 40 X

The ANOVA detected a significant main effect of drug treatment, $F(1, 18) = 23.62$, $p < 0.001$. Post hoc comparisons revealed more Fos induction in drug-treated rats compared to saline-treated rats across all hormone conditions. There was no significant main effect of hormone treatment or significant interaction between drug and hormone treatment.

Effect of DPDPE. Both the EB + P and EB-alone groups had moderate-to-high levels of Fos induction in the ventromedial region. In the OIL group there were low-to-moderate levels which were seen close to the optic tract (Figure 6 and 6a).

The ANOVA indicated a significant main effect of hormone treatment, $F(2, 18) = 6.04$, $p < 0.01$. Post hoc comparisons revealed that EB + P treated rats had a significantly higher amount of Fos induction than the EB-alone or OIL-treated rats across all drug conditions. There was also a significant main effect of drug treatment, $F(1, 18) = 45.8$, $p < 0.0001$. Post hoc comparisons revealed that significantly more Fos cells were found in drug-treated rats compared to saline-treated rats across hormone conditions. There was no significant interaction between hormone and drug treatment.

Effect of U50-488. In the EB+P group there were moderate to high amounts of Fos cells scattered throughout the region. In the EB-alone group there were moderate amounts of Fos induction which were slightly more concentrated in the ventromedial region. In the OIL condition there were low levels of scattered Fos cells throughout the region. (Figure 6 and 6a).

The ANOVA detected a significant main effect of hormone treatment, $F(2, 18) = 10.07$, $p < 0.01$, with significantly more Fos cells found in EB + P treated rats across all drug conditions compared with EB-alone or OIL-treated rats. There was also a significant

main effect of drug treatment, $F(1, 18) = 25.09$, $p < 0.0001$, with significantly more Fos cells found in drug-treated rats compared to saline-treated rats across all hormone conditions. Finally, there was a significant interaction between drug and hormone treatment, $F(2, 18) = 5.68$, $p < 0.02$. Post hoc comparisons of the interaction means revealed significantly more Fos cells in drug-treated EB+P rats compared to drug-treated OIL rats. Also, drug-treated EB + P and EB-alone rats had more Fos cells than EB + P and EB-alone saline-treated rats.

Overall Fos Induction in the MCG

There were more Fos cells in the MCGv across all drug and hormone conditions and there was also a scattered amount of Fos found in all aspects of the MCG. (Figures 7, 8, 9 and 9a).

Effect of DAMGO. In the dorsolateral aspect, Fos was scattered throughout the area and was seen in fairly low to moderate amounts in all three hormone conditions. The ANOVA indicated that there was a significant main effect of drug treatment, $F(1, 18) = 7.95$, $p < 0.01$, with significantly more Fos induction found in drug-treated rats compared to saline-treated rats across all hormone conditions. There was no main effect of hormone or interaction between drug and hormone treatment.

In the dorsomedial aspect there were low to moderate amounts of Fos in all three conditions. The ANOVA detected a significant main effect of drug, $F(1, 18) = 14.07$, $p < 0.01$. Post hoc comparisons indicated that significantly more Fos cells were found in drug-treated rats compared to saline-treated rats across hormone conditions.

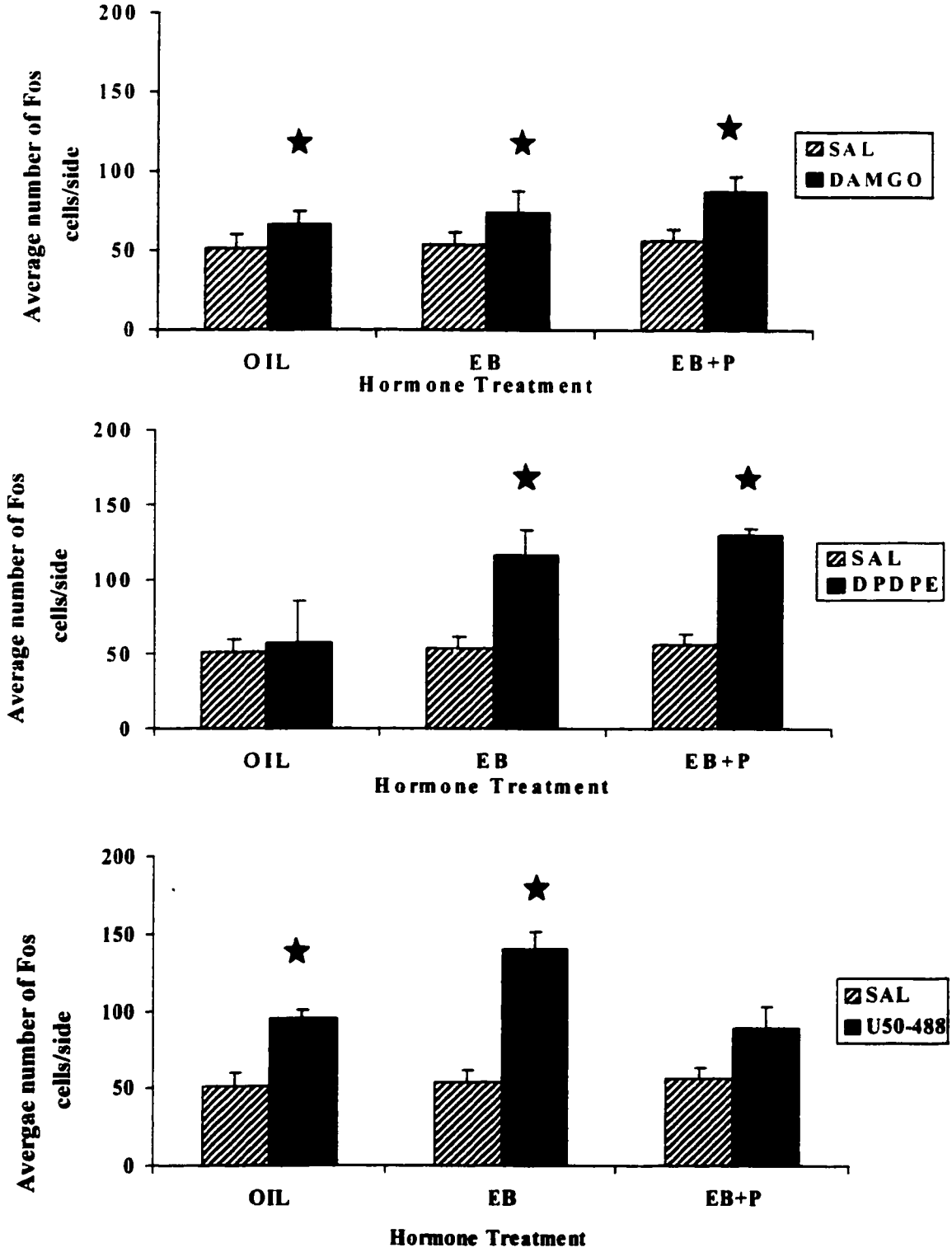

Figure 7. Average number of Fos cells/side in the MCGdl. Solid bars represent means from OVX rats given hormone and drug treatment (n = 4/condition); hatched bars represent means from OVX rats given hormone and saline treatment (n = 4/condition). Vertical lines denote standard errors.

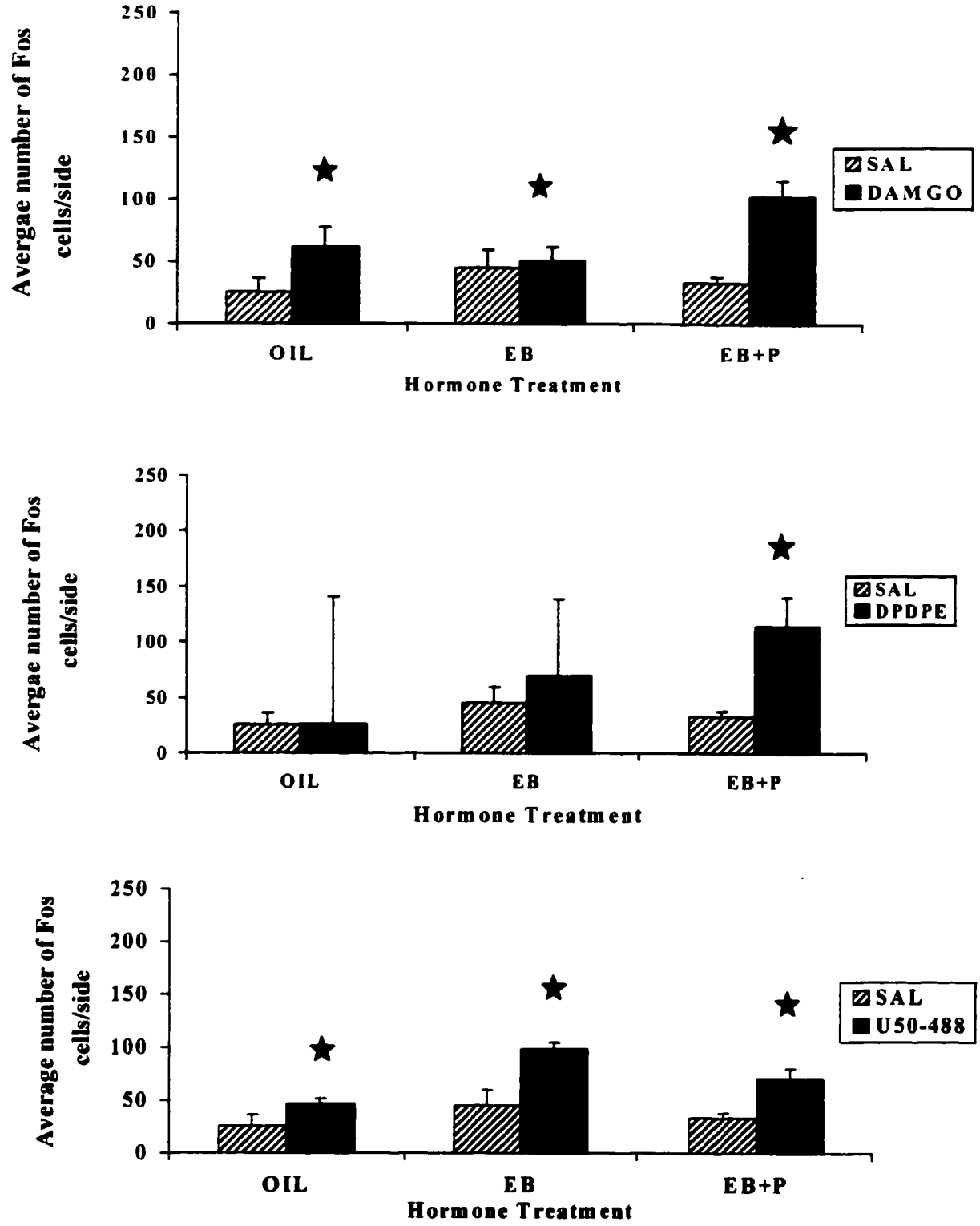

Figure 8. Average number of Fos cells/side in the MCGdm. Solid bars represent means from OVX rats given hormone and drug treatment (n = 4/condition); hatched bars represent means from OVX rats given hormone and saline treatment (n = 4/condition). Vertical lines denote standard errors

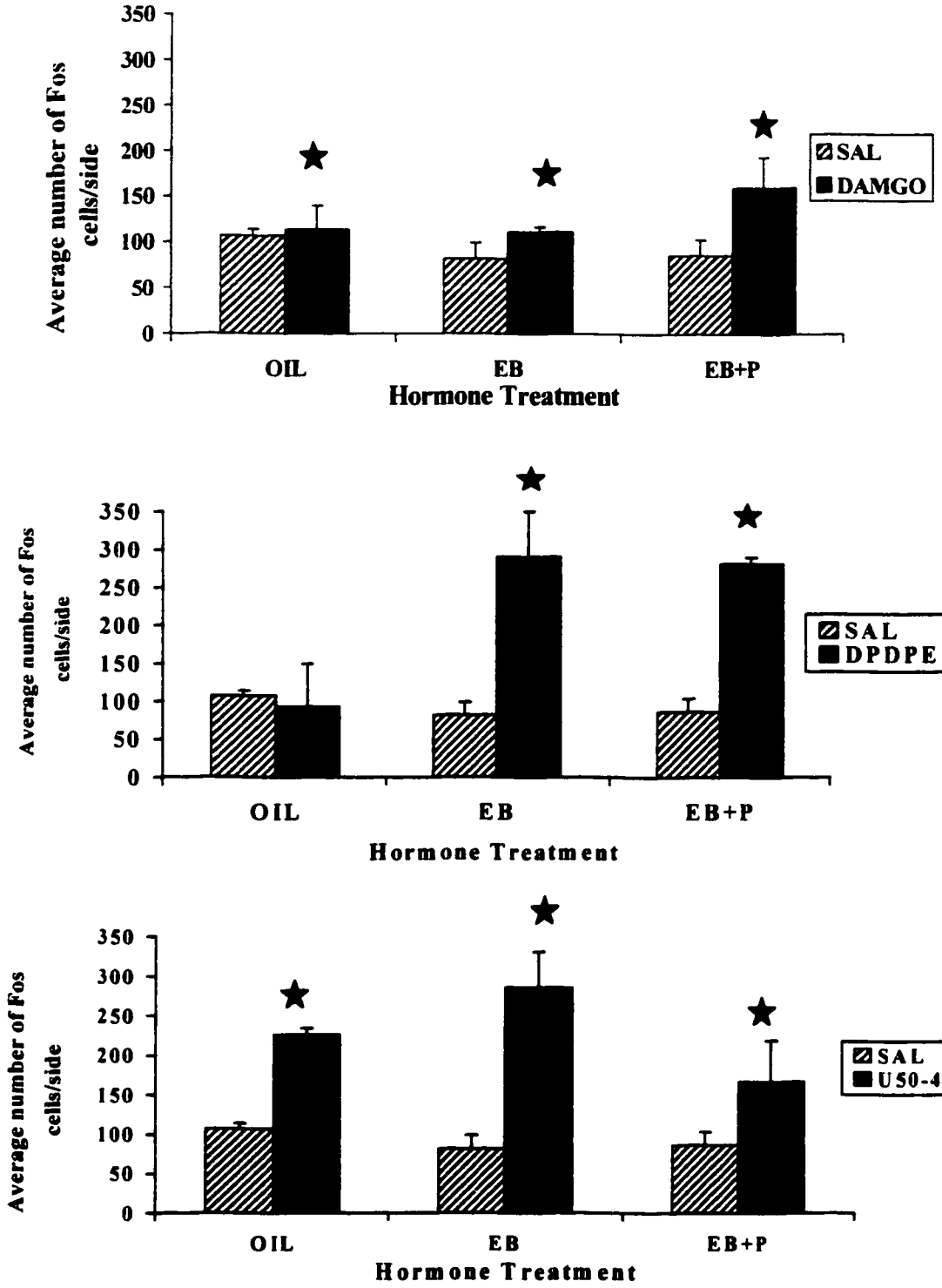

Figure 9. Average number of Fos cells/side in the MCGv. Solid bars represent means from OVX rats given hormone and drug treatment (n = 4/condition); hatched bars represent means from OVX rats given hormone and saline treatment (n = 4/condition). Vertical lines denote standard errors.

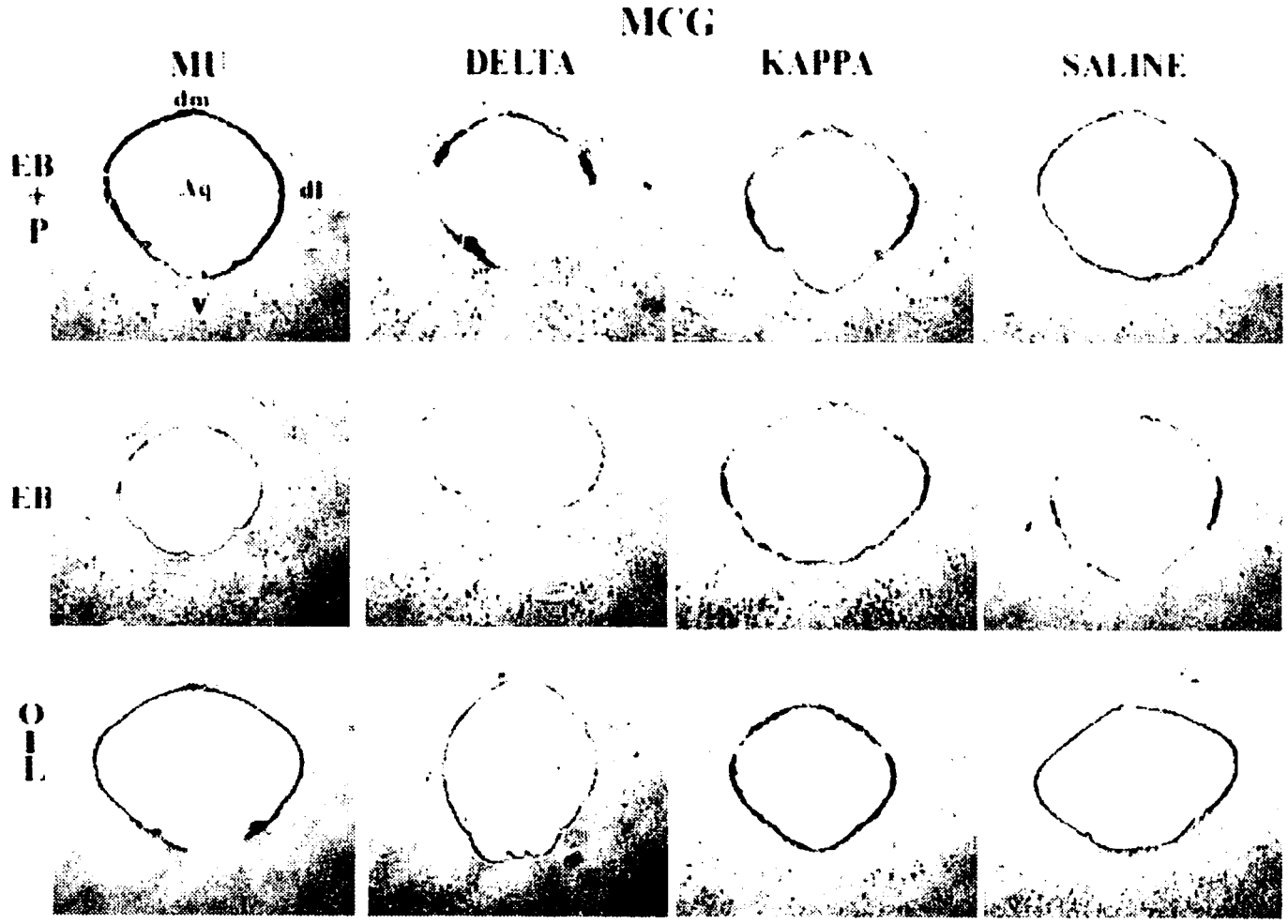

Figure 9a Representative digitized images of the MCG taken at 40 X

There was no main effect of hormone treatment or significant interaction between drug and hormone treatment.

In the ventral aspect, there were a moderate amount of Fos cells found in all three hormone conditions. However, in the OIL condition the amount of Fos induction appeared to be slightly lower. The ANOVA detected a significant main effect of drug treatment, $F(1, 18) = 4.80$, $p < 0.05$, with significantly more Fos cells in drug-treated rats compared to saline-treated rats across all drug conditions. However, there was no significant main effect of hormone treatment or significant interaction between drug and hormone treatment.

Effect of DPDPE. In the dorsolateral aspect, Fos cells were scattered throughout the region in all three hormone conditions. The EB + P condition appeared to have slightly more Fos cells than in the EB-alone condition. In the OIL condition there was less Fos induction than in the EB + P and EB-alone condition. The ANOVA detected a significant main effect of hormone treatment, $F(2, 18) = 3.88$, $p < 0.05$. Post hoc comparisons revealed that EB + P rats had a significantly higher amount of Fos induction compared to OIL-treated rats across all drug conditions. There was also a significant main effect of drug treatment, $F(1, 18) = 15.56$, $p < 0.001$. Post hoc comparisons revealed significantly more Fos cells found in drug-treated rats compared to saline-treated rats across hormone conditions. There was no significant interaction between hormone and drug treatment.

In the dorsomedial aspect, Fos cells were scattered throughout the region in all three hormone conditions. There appeared to be higher amounts of Fos induction in the EB + P groups compared to the EB-alone or OIL groups. The ANOVA detected a

significant main effect of hormone treatment, $F(2, 18) = 4.96$, $p < 0.05$, with significantly more Fos cells in EB + P hormone-treated rats compared to OIL-treated rats across all drug conditions. There was also a significant main effect of drug treatment, $F(1, 18) = 7.94$, $p < 0.05$, with significantly more Fos cells in drug-treated rats compared to saline-treated rats across all drug conditions. There was also a significant interaction of hormone and drug treatment, $F(2, 18) = 3.57$, $p < 0.05$. Post hoc comparisons of the interaction means indicated that there were significantly more Fos cells in drug-treated EB + P rats compared to drug-treated OIL rats and compared to saline treated rats.

In the ventral aspect, the EB+P and EB-alone groups had large amounts of Fos induction, compared to the OIL group in which there was a lower amount. The ANOVA a significant main effect of hormone treatment, $F(2, 18) = 3.91$, $p < 0.05$, however, post hoc comparisons revealed no significant differences between hormone groups across drug conditions. There was also a significant main effect of drug treatment, $F(1, 18) = 20.17$, $p < 0.001$. Post hoc comparisons revealed that drug-treated rats had significantly higher numbers of Fos cells than saline-treated rats across all hormone conditions. There was also a significant interaction between drug and hormone treatment, $F(2, 18) = 6.30$, $p < 0.01$. Post hoc comparisons of the interaction means indicated that EB + P and EB-alone rats treated with drug had significantly more Fos than OIL rats treated with drug. Drug-treated rats given EB + P and EB alone had significantly more Fos than saline-treated rats.

Effect of U50-488. In the dorsolateral aspect, all three hormone conditions, had scattered Fos induction throughout the region. In the EB-alone group there were moderate to high amounts of Fos induction whereas in the EB + P and OIL conditions

there were lower amounts. The ANOVA detected a significant main effect of hormone treatment, $F(2, 17) = 4.13$, $p < 0.05$, however, post hoc comparisons did not reveal significant differences between the hormone treatments across all drug conditions. There was also a significant main effect of drug treatment, $F(1, 17) = 50.69$, $p < 0.0001$, with significantly more Fos cells in drug-treated rats compared to saline-treated rats across all drug conditions. There was also a significant interaction of hormone and drug treatment, $F(2, 17) = 4.37$, $p < 0.05$. Post hoc comparisons of the interaction means revealed significantly more Fos cells in rats treated drug and EB alone compared to rats treated with drug and EB + P or OIL. Rats treated with drug and EB alone or OIL had significantly more Fos cells compared to rats treated with saline and EB alone or OIL.

In the dorsomedial aspect, the EB + P and EB-alone groups had moderate amounts of Fos whereas there were lower amounts of Fos induction in the OIL groups. The ANOVA detected a significant main effect of hormone treatment, $F(2, 17) = 6.82$, $p < 0.01$. Post hoc comparisons revealed significantly more Fos cells in EB alone-treated rats as compared to OIL-treated rats across all drug conditions. There was also a significant main effect of drug treatment, $F(1, 17) = 22.87$, $p < 0.001$, with significantly more Fos cells in drug-treated rats compared to saline-treated rats, across all drug conditions. There was no significant interaction of hormone and drug treatment.

In the ventral aspect, all hormone conditions had scattered Fos throughout the region. In both the EB-alone and OIL groups there were moderate amounts of Fos induction. The EB + P treatment appeared to induce slightly less Fos induction. The ANOVA detected a significant main effect of drug treatment, $F(1, 17) = 32.07$, $p < 0.0001$, with significantly more Fos cells in drug-treated rats compared to saline-treated

rats. There was no significant main effect of hormone treatment or significant interaction between drug and hormone treatment.

Discussion

The findings outlined in this thesis demonstrate that ovarian hormones alter the ability of opioid agonists to induce Fos in the female rat brain. Different regions of the brain respond differently, depending on the hormone regimens used and opioid receptor subtype stimulated. In all regions drug treatment induced a significant amount of Fos compared to saline. However, there were differences in Fos induction in all brain regions depending on the drug treatment and hormone regimen. There was a significant interaction between the effects of hormone treatment and ICV infusions of the μ-opioid receptor agonist, DAMGO, on Fos induction in the mPOA and VMN. Following ICV infusions of the δ-opioid receptor agonist, DPDPE, and hormone treatment there was significant Fos induction in the LS, mPOA, VMN, and MCG compared to OIL injections. ICV infusions of the κ-opioid receptor agonist, U50-488, interacted with hormone treatment to increase the level of Fos induction in the VMN, MEApd and the MCGdl. It is noteworthy that different patterns of Fos induction were observed in the LS, mPOA, VMN, MEApd and MCG following drug infusions and hormone treatments. These findings elucidate functional pathways in the brain where opioid activation is modulated by hormone treatment. Interestingly, in some instances, the opioids induced significant Fos expression in regions where the specific opioid receptor were not located, suggesting an indirect activation.

Under the conditions of the present experiment, hormone injections alone did not induce Fos in any of these sites. The reason for this lack of effect may be the time of

injections relative to the time that the animals were sacrificed. Auger and Blaustein (1997) found that EB + P treatment induced significantly more Fos than EB alone in the VMH if animals were injected with P and perfused one hour following this injection. It may be that in order to see the effects of hormone treatment on Fos induction, the hormone needs to be administered closer to the time that the animals are sacrificed. In the present study, P was administered four hours before the drug infusion and animals were sacrificed 75 min following the drug infusion. Consistent with these results, other studies have found that Fos is not expressed when animals are sacrificed four hours after the hormone injection (e.g. Pfaus et al., 1993; Pfaus et al., 1996).

Lesion data can provide information about how brain regions regulate different behaviours, such as female sexual behaviour. The behaviour that is impaired following the lesion of a brain region suggests that the opposite behaviour would be seen following activation of that region. The remainder of this discussion will focus on the induction of Fos observed in the LS, mPOA, VMN, MEApd and MCG and will interpret these findings in light of existing lesion and pharmacological data.

Lateral Septum

Lesions of the LS have been shown to facilitate lordosis behaviour in female rats (Yamanouchi & Arai, 1990). The effects following lesions indicate that the LS normally acts to inhibit lordosis. In the present study, it was found that infusions of both DAMGO and U50-488 significantly increased the induction of Fos in the LS, regardless of hormone treatment. Following ICV infusions of DPDPE, however, there was a significantly greater number of Fos labeled cells observed in female rats treated with EB + P than in those treated with EB alone or OIL (see Figure 1).

Role of μ-opioid receptors. This study found that DAMGO increased Fos induction in the LS significantly, albeit consistently, across all hormone treatments. However, there is no significant μ-opioid binding in the LS (Mansour et al., 1987) and it is unlikely that DAMGO binds non-selectively in the LS. Accordingly, DAMGO must have acted indirectly to induce Fos in this region, which could occur, for example, at another site which projects fibers to the LS. In line with this reasoning, connections between the mPOA and LS do exist, and research has shown μ-opioid receptor localization in the mPOA (Mansour et al., 1987). Conceivably, μ-opioid receptor activation in the mPOA by DAMGO could induce Fos in the LS via these projections. Because the present results suggest that μ-opioids have an effect in the LS, albeit indirectly, it is possible that μ-opioid actions on the LS regulate sexual behaviour of the female rat. These findings, however, do not allow for speculation as to how this effect may be regulated by hormones, because Fos induction was equivalent among the different hormone treatments.

Role of δ-opioid receptors. In the present study it was found that females infused with DPDPE and treated with EB + P had significantly higher amounts of Fos cells than females treated with EB alone or OIL. This hormone-dependent effect was only found following infusions of DPDPE, suggesting a unique modulation by hormones on δ-opioid transmission in this site as compared to the activity of the other opioid receptor subtypes.

Behavioural studies have shown that ICV DPDPE infusions facilitate proceptive behaviours in female rats primed with EB + P (Pfaus & Pfaff, 1992). In the present study, Fos induction was observed following ICV DPDPE infusions in female rats primed with EB + P. δ-opioid receptors have been previously demonstrated to exist in the LS

(Mansour et al.. 1987). Therefore. it is possible that. following these ICV infusions of DPDPE. δ-opioid agonists acted directly in the LS given the anatomical proximity of the LS to the lateral ventricle as well as the existence of δ-opioid receptors in the LS. Therefore. the present results suggest a hormone-dependent δ-opioid influence in the LS which, although speculative. may play a role in facilitating proceptive behaviours.

It has been shown that infusions of ß-endorphin into the third ventricle inhibit lordosis. whereas infusions into the lateral ventricle facilitate it (Gorzalka et al., 1997). Because ß-endorphin binds with equal affinity to both μ- and δ-opioid receptors. these results suggest that the activation of different populations of receptors account for these effects. As mentioned, in the present study, lateral ventricular infusions of DPDPE induced Fos in the LS. Activation of δ-opioid receptors, as other opioid receptors, hyperpolarize neurons (e.g. McFadzean, 1988). Therefore, δ-opioid receptors in the LS possibly act to disinhibit the role of the LS in sexual behaviour, thereby causing a facilitation of proceptive and receptive components of female sexual behaviour.

Role of κ-opioid receptors. This study found that U50-488 significantly increased Fos activation consistently across hormone treatments. Moderate κ-opioid binding exists in the LS (Mansour et al., 1987) and, therefore, the present study suggests that these ICV infusions of U50-488 may have produced κ-opioid activation directly in the LS. It may be that κ-opioid activity in the LS is also capable of disinhibiting female sexual behaviour. However, the present data do not allow for speculation as to how this effect may be regulated by hormones.

Medial Preoptic Area

Lesions of the mPOA facilitate lordosis and inhibit pacing and proceptive behaviours (Hoshina et al., 1994; Veney & Rissman, 1997). This suggests that the mPOA, following activation, inhibits lordosis and facilitates pacing and proceptive behaviours. The present findings demonstrated that infusions of DAMGO significantly increased the induction of Fos in the mPOA in rats treated with EB + P and EB alone as compared to OIL. Following DPDPE infusions, there was significantly more Fos induction observed in rats treated with EB alone than with EB + P and OIL in the mPOAa and with EB alone than with OIL in the mPOAp. The infusions of U50-488 increased the induction of Fos, although there were no significant differences across hormone treatments (see Figures 2 and 3).

Role of μ-opioid receptors. The present study found that in both the mPOAa and the mPOAp, rats treated with EB alone and EB + P in the drug group had a higher number of Fos cells compared to rats treated with OIL. Because μ-opioid binding has been observed in the mPOA (Desjardins et al., 1990), Fos induction probably occurred following direct activation by DAMGO.

Infusions of the selective μ-opioid antagonist, ß-funaltrexamine to the mPOA, facilitated lordosis in OVX female rats primed with EB (Hammer et al., 1989). Recently, Acosta-Martinez and Etgen (1999) found that infusions of DAMGO into the mPOA inhibited lordosis behaviour in female rats and naloxone facilitated it, suggesting that DAMGO activation of μ-opioid receptors in the mPOA might inhibit lordosis. Therefore, μ-opioid binding in the mPOA appears to induce an inhibition of lordosis. Because μ-opioids act on neurons through a process of hyperpolarization (e.g. McFadzean, 1988),

direct activation following a μ-agonist infusion should be inhibitory. Therefore, the present Fos activation following the infusions of DAMGO suggests that a population of inhibitory neurons is being activated.

As mentioned previously, infusions of ß-endorphin into the third ventricle inhibit lordosis whereas infusions into the lateral ventricle facilitate it (Gorzalka et al., 1997). In the present study, Fos activation occurred in the mPOA following lateral ventricular infusions of μ-opioid agonists. The lateral ventricular infusions most likely drained into the third ventricle and then activated μ-opioid receptors within the mPOA. Therefore, the previously observed ß-endorphin inhibition of lordosis following third ventricular infusions may have been mediated by μ-opioid receptors and, as discussed, μ-opioid inhibition of lordosis has been observed.

Role of δ-opioid receptors. In the present study, Fos induction was greater following DPDPE infusions in EB-treated females than those treated with EB + P or OIL in the mPOAa and in EB-treated females compared to those treated with OIL in the mPOAp. Pfaus and Pfaff (1992) found that DPDPE infusions reduced the number of rejection responses of OVX rats primed with EB alone. In both studies, results were observed following ICV infusions of DPDPE in female rats primed with EB alone. This suggests that the observed δ-opioid receptor activation responsible for this decrease in rejection responses may be mediated through activation in the mPOA. Alternatively, it is possible that the effects of EB + P reduce the frequency of rejection responses to the same extent as DPDPE infusions. Consequently, in a female rat that is primed with EB + P the rejection responses may already be maximally reduced such that the addition of DPDPE would not have an additional effect.

Role of k-opioid receptors. The present study found a significant effect of κ-opioid agonist treatment regardless of the hormonal state of the female rat. Because there is very dense κ receptor binding located in the mPOA (Mansour et al., 1987) it is possible that the κ-opioid agonist, U50-488 activated Fos induction directly in the mPOA. ICV infusions of κ-opioid agonists have been shown to facilitate lordosis behaviour in rats (Pfaus & Pfaff, 1992) and it is possible that the κ-opioid induced Fos activation may be indicative of a population of neurons in the mPOA involved in facilitating proceptive or receptive behaviours. However, these data do not allow for an understanding of how this effect may be regulated by hormones.

Ventromedial Hypothalamus

The VMN, when lesioned, decrease proceptive behaviours and lordosis (Pfaff & Sakuma, 1979). The behavioural effects observed following lesioning suggest that the VMN acts to facilitate both proceptive behaviours and lordosis in female rats. The present study found that both infusions of DAMGO and U50-488 induced a significant amount of Fos in females treated with EB + P, as compared to EB alone or OIL in the VMN. Following infusions of DPDPE, there was significant Fos induction observed in females treated with EB + P or EB alone, as compared to OIL in the VMNa, whereas, this induction was shown in females treated with EB + P, as compared to EB alone or OIL in the VMNp. (see Figures 4 and 5).

Role of μ-opioid receptors. The Fos activation observed following ICV infusions of a μ-opioid agonist in the VMN in the present study are consistent with the results of Chang and Harlan (1990), who found that ip injections of morphine induced significant amounts of Fos in the VMN. However, because the VMN does not contain detectable

populations of μ-opioid binding sites (Mansour, e al., 1987). DAMGO must be activating the VMN indirectly for this effect to have occurred. The VMN receives input from the mPOA (Simerly, 1995) and DAMGO binding sites exist in the mPOA (Mansour et al., 1987). Therefore, DAMGO may bind in the mPOA and act on the VMN indirectly to induce Fos expression.

Although, the present findings suggest that there is an indirect μ-opioid effect on the VMN which is regulated by hormone influence, researchers have injected μ-opioid agonists directly into the VMN and observed effects on female sexual behaviour. For example, infusions of morphine or DAMGO into the VMH inhibit lordosis (Vathy et al., 1991; Acosta-Martinez & Etgen, 1999). These results are difficult to reconcile given the lack of μ-opioid receptors in the VMN (e.g. Mansour et al., 1987). Therefore, the question of where DAMGO may bind remains unresolved. One possibility is that morphine or DAMGO infused into the VMN diffused into the third ventricle. If this is the case, DAMGO may have acted downstream from the VMN, possibly in the MCG, which, is another site that acts to facilitate lordosis.

Role of δ-opioid receptors. Following DPDPE infusions in the present study, Fos induction occurred in females treated with EB + P or EB alone to a greater extent than in OIL-treated females in the VMNa. However, in the VMNp, females treated with DPDPE and EB + P had greater Fos induction that those treated with DPDPE and EB alone or OIL. This suggests that subregions within the VMN may have different hormonal requirements under which δ-opioid action occurs. For example, the ventrolateral aspect of the caudal portion of the VMN has been identified as a region of the hypothalamus which is involved in the estrogen regulation of lordosis. Following application of

crystalline estradiol in the ventrolateral region of the caudal VMN, lordosis is potentiated following flank stimulation in OVX female rats (Pfaff, 1980). In addition, Pfaus and Pfaff (1992) found different behavioural effects, depending on the hormonal priming of the female rat, following ventricular infusions of DPDPE. In female rats that received DPDPE infusions and EB alone or EB + P, a facilitation of lordosis was observed. In contrast, a facilitation of proceptive behaviour was observed only in rats treated with EB and P. Therefore, it may be that Fos activation in the caudal portion of the VMN following DPDPE infusions in females primed with EB + P represents a mechanism involved in the regulation of lordosis, whereas the activation seen in the VMNa represents a mechanism involved in some other aspect of female reproduction.

Role of κ-opioid receptors. Following U50-488 infusions in the present study, Fos induction occurred in females treated with EB + P to a greater extent than in EB-alone or OIL-treated females. The possible sites in which κ agonists may act to affect female sexual behaviour have not yet been investigated. Pfaus and Pfaff (1992) found that ICV infusions of k-opioid agonists facilitated lordosis in female rats primed with EB alone as well as with EB + P. Therefore, the present results suggest that there may be κ-opioid action which contributes to the regulation of lordosis in the VMN. This speculation is also consistent with the existence of κ-opioid receptors in the VMN (Mansour et al., 1987), indicating that the present Fos induction is probably a result of direct κ-opioid activation in the VMN.

Posteriodorsal Medial Amygdala

It has been shown that lesions of the MEApd do not affect female sexual behaviour. (Mascó & Carrer, 1980). In the present experiment it was found that both

DAMGO and DPDPE increased the amount of Fos across the different hormone treatments in a consistently significant manner in the MEApd. However, infusions of U50-488 induced a significant amount of Fos in females treated with EB + P as compared to OIL (see Figure 6).

Role of μ-opioid receptors. As mentioned, the effect of DAMGO treatment on Fos induction in the MEApd did not vary significantly between hormone groups in the MEApd. The MEApd has a high density of μ-opioid receptor binding (Mansour et al., 1987) and, therefore, ICV infusions of DAMGO may have produced direct μ-opioid activation in the MEApd. Although there may be μ-opioid activity in the MEApd which modulates female sexual behaviour, these results do not allow for speculation as to the hormone mechanisms by which these μ-opioid actions are regulated.

Role of δ-opioid receptors. As observed following DAMGO infusions, ICV infusions of DPDPE induced a consistently significant amount of Fos across the hormone groups. δ-opioid receptor binding exists in the MEApd and, therefore, it is possible that ICV infusions of DPDPE produced δ-opioid activation in the MEApd (Mansour et al., 1987). Although there may be δ-opioid activity in the MEApd which modulates female sexual behaviour, it is again not possible to comment on what role hormones may play in regulating this activity.

Role of κ-opioid receptors. Infusions of U50-488 induced Fos that varied significantly depending on the hormonal state of the female rat. As mentioned, κ-opioid agonists have not yet been infused into specific brain regions in order to investigate effects on female sexual behaviour. Findings from Pfaus and Pfaff (1992) showed that

ICV infusions of U50-488 dramatically facilitated female sexual receptivity (as measured by lordosis quotients) in rats primed with EB alone. In females primed with EB + P, however, this facilitation was slight, albeit statistically significant. However, where this regulation might take place is still unknown (Pfaus & Pfaff, 1992). Because moderate amounts of κ-opioid receptors exist in the MEApd (Mansour et al., 1987), and because the MEApd is located near the lateral ventricle, these ICV infusions could have produced direct κ-opioid activation in the MEApd.

As mentioned, lesions of the MEApd do not affect female sexual behaviour (Mascó & Carrer, 1980). However, a dense Fos induction has been reported in the MEApd of female rats following copulation with intromission or vaginal cervical stimulation (VCS) (Erskine, 1993; Pfaus et al., 1993). It has been suggested that this Fos induction may not be related to female sexual behaviour, but may, instead, be related to other neuroendocrine functions which are activated by VCS and regulated in the MEApd (Pfaus et al., 1996). Interestingly, lidocaine infusions around regions of the MEApd of intact female rats before and after paced intromissions block pseudopregnancy (Coopersmith, Gans, Rowe, & Erskine, 1996). Therefore, the MEApd may affect neuroendocrine mechanisms (e.g. pseudeopregnancy or estrus termination) which are activated by VCS (Pfaus et al., 1996). The present finding of Fos activation following ICV infusions of U50-488 effects following hormone treatment suggest that κ-opioid action plays a role in the MEApd by mediating these neuroendocrine effects.

Mesencephalic Central Gray

Lesions of the MCG have been found to impair lordosis. This indicates that activation of this region facilitates lordosis (Sakuma & Pfaff, 1979). The present results

showed that DAMGO significantly increased Fos induction across all hormone treatments within the MCG. Following infusions of DPDPE, there was a greater amount of Fos induction in the dorsomedial aspect of the MCG in female rats that were primed with EB + P, as compared to EB-alone or OIL. However, in the ventral aspect of the MCG, Fos induction was significantly higher in female rats primed with EB-alone or EB + P, compared to OIL (see Figures 7, 8, and 9). Following infusions of U50-488, there was a greater amount of Fos induction in the dorsolateral aspect of the MCG in female rats primed with EB alone compared to rats treated with EB + P or OIL.

Role of μ-opioid receptors. As mentioned, there was a significant induction of Fos following infusions of DAMGO into the lateral ventricle, and this result was equivalent across hormone groups. Although there is μ-opioid receptor expression in the MCG (Tempel & Zukin, 1987), the MCG is located in a distal anatomical position relative to the lateral ventricle. Therefore, DAMGO may have acted in another site that projects to the MCG, as opposed to acting directly. Connections exist between the mPOA and the MCG (Morrell, Greenberger & Pfaff, 1981) and research has shown μ-opioid localization in the mPOA (Mansour et al., 1987). It is possible then, that μ-opioid receptor activation in the mPOA by DAMGO induces Fos in the MCG via these projections. The present study found no significant interaction between the effect of DAMGO and hormone treatment on Fos induction in the MCG. Since the MCG plays a facilitatory role on lordosis and there was a significant induction of Fos following DAMGO infusions, there may be μ-opioid action which contributes to the regulation of female sexual behaviour in the MCG. However, the present data do not allow for speculation as to the specific manner in which hormones may regulate this action in the

MCG.

Role of δ-opioid receptors. Following DPDPE infusions in dorsomedial aspect of the MCG in the present study. Fos induction occurred in females treated with EB + P to a greater extent than females treated with OIL. DPDPE infusions did, however, induce significant amounts of Fos in the ventrolateral aspect in female rats primed with EB or EB + P as compared to female rats treated with OIL. Because the present study found that the effects of ICV DPDPE infusions interact with hormones differently in distinct aspects of the MCG to induce Fos, it is important to consider the previous behavioural findings at greater lengths.

It has been reported that infusions of ß-endorphin into the dorsal MCG of female rats primed with EB + P or EB alone inhibited lordosis (Sirinathsinghji,1984). Although ß-endorphin may bind non-selectively to both μ- and δ-opioid receptors, there is a much larger population of μ-opioid receptors than δ-opioid receptors in the MCG (Mansour et al., 1987; Tempel & Zukin, 1987). Therefore, it may be possible that the inhibition of lordosis was due to μ-opioid receptor activation in that study. The present results suggest that DPDPE infusions induce Fos expression in different regions of the MCG depending on the hormonal priming of the female rats. ICV DPDPE infusions have been found to facilitate lordosis behaviour in female rats primed with both EB + P or EB alone. Because there are δ-opioid receptors in the MCG (Blackburn et al., 1988) it is possible that they may influence female sexual behaviour if activated selectively, with a selective δ-opioid receptor agonist or antagonist.

The MCG is too distant from the anatomical location of the lateral ventricle to suggest with any confidence that lateral ventricular infusions of DPDPE would be able to

diffuse into the MCG. Because connections between the VMH and the MCG exist (e.g. Hennessey et al., 1990), it may be possible that the infusions of DPDPE acted in the VMN and induced Fos expression in the MCG via these fiber projections. This also suggests the possibility that a δ-opioid effect, which originates in the VMN and acts on the MCG, plays a role in the facilitation of sexual receptivity. This hypothesis is consistent with the findings of Pfaus and Pfaff (1992) that ICV DPDPE infusions facilitated lordosis in female rats.

Role of κ-opioid receptors. There was a significant induction of Fos following infusions of U50-488 in the dorsolateral aspect of the MCG in female rats primed with EB alone compared to EB + P and OIL. As mentioned, κ-opioid agonists have not yet been infused into specific brain regions in order to investigate effects on female sexual behaviour. Although there is κ-opioid receptor expression in the MCG (Tempel & Zukin, 1987), the MCG is located in a distal anatomical location from the lateral ventricle. Therefore, κ-opioid action may be acting in another brain region that projects to the MCG. Connections exist between both the mPOA and VMH and the MCG (Morrell, Greenberger & Pfaff, 1981) and research has shown that κ-opioid expression exists in the mPOA and VMH (Manosur et al., 1987). Therefore, κ-opioid activation in the mPOA or VMH by U50-488 may induce Fos in the MCG via these connections. Although it is possible that the MCG may be a site in which κ-opioid activation affects female sexual behvaiour, the present results can only suggest that the observed Fos induction is likely to be an indirect effect, due to the anatomical location of the lateral ventricles.

Summary and Conclusions

In sum, the present findings of the experiment of this thesis have demonstrated

that estrogen and progesterone alter the ability of opioid agonists infused ICV to induce Fos protein within different regions of the female rat brain. Secondly, these findings elucidated areas of Fos induction within each of the brain regions discussed. These Fos clusters may be thought of as subregions of the brain that may serve as anatomical markers for future studies that attempt to activate functional pathways using direct opioid infusions. Thirdly, there was the unexpected finding of the opioid agonists inducing a significant amount of Fos protein in brain regions where the specific opioid receptor was not located. These results allowed for the speculation that there was activation of indirect as well as direct pathways where Fos expression was observed.

Several issues remain to be investigated. Among these is the effect that opioid agonists or antagonists have on female sexual behaviour when administered via cannulation into these brain regions directly. Only the mPOA ,VMN and MCG have been studied for direct opioid effects on female sexual behaviour (e.g. Sirinathsinghji et al., 1983; Hammer et al., 1989; Vathy et al., 1991; Acosta-Martinez & Etgen 1999). Furthermore, the full complement of appetitive and consummatory sexual behaviours displayed by female rats has not yet been examined for opioid effects. Therefore, a paucity of data exist on the role of opioids on female sexual behaviour. The present thesis suggests that opioids may act on other regions of the brain, such as the LS and MEApd, to influence female sexual behaviour, or may act in tandem in several brain regions.

Another important issue that remains to be investigated is the phenotype of the Fos positive cells that were identified in this study. In order to characterize the activated Fos cells, a double labeling study must be conducted. As mentioned, opioid agonists act by inhibiting the neurons that they bind to (e.g. McFadzean, 1988). However, in some

instances it has been found that an opioid agonist will facilitate female sexual behaviour (e.g. Pfaus & Pfaff, 1992). Therefore, the observed Fos induction in the present study would be an indication that the opioid agonist inhibited a population of inhibitory neurons, thus allowing that specific behaviour to be facilitated. For example, following ICV infusions of DPDPE, Fos activation was observed in the caudal VMN which is also a brain region that regulates the facilitation of lordosis. Interestingly, Kow, Harlan, Shivers and Pfaff (1985) found that infusions of glutamate into the VMN inhibited lordosis. It could be speculated that δ-opioid receptors, which when activated facilitate lordosis, act on these glutamate neurons by a process of disinhibition in the VMN, thereby allowing lordosis to be facilitated. Therefore, it would be of interest for future studies to investigate the nature of these Fos positive cells, perhaps in a double labeling study for glutamate and Fos protein.

In conclusion, the induction of Fos was found to be significantly different in various brain regions depending on the specific opioid receptor subtype and the hormone regimen that was administered to OVX female rats. By shedding light on brain regions where the cellular response depends on an interaction between opioid actions and hormonal priming, these results contribute to the understanding of opioid action in areas of the female rat brain involved in female reproductive behaviour.

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