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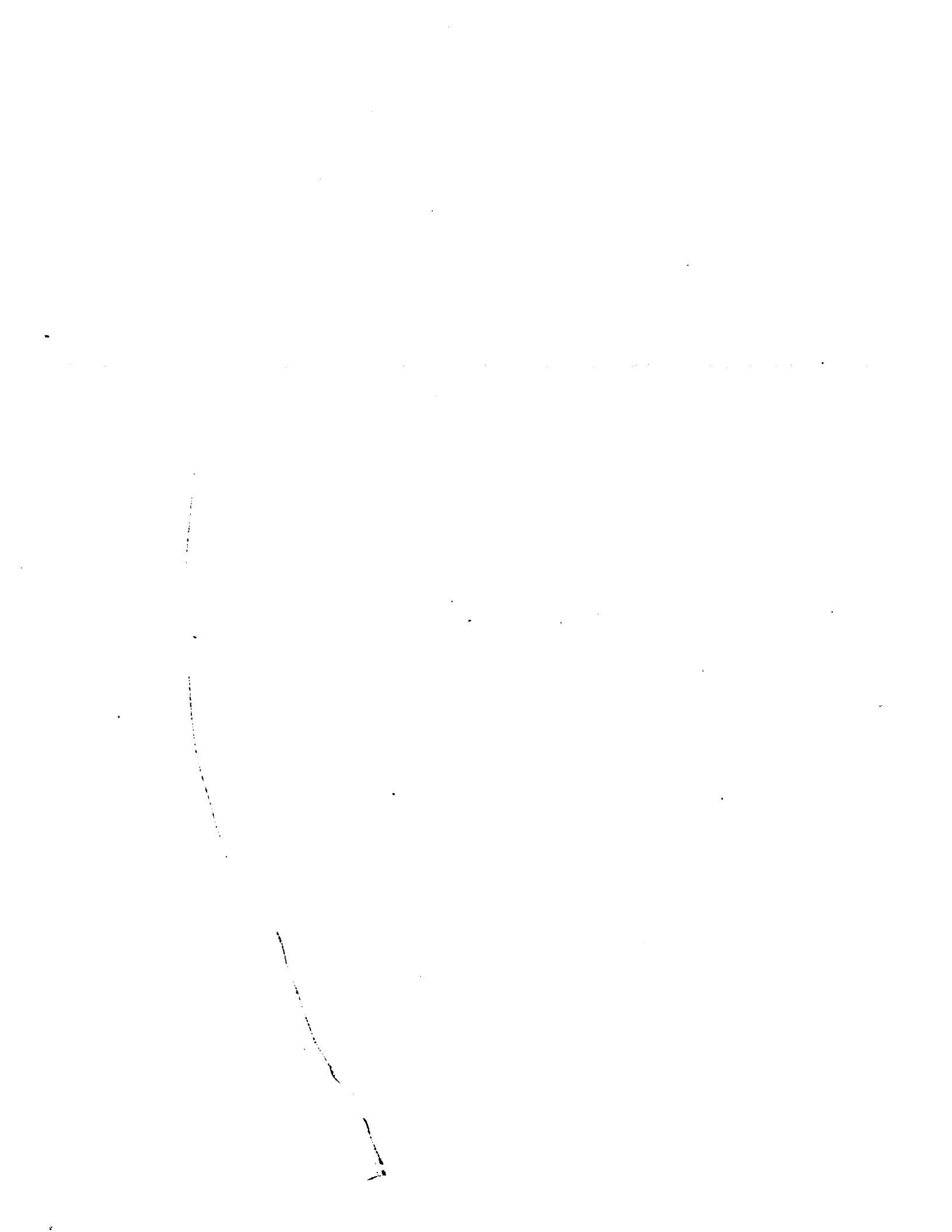
DEVELOPMENT OF ENDORPHINERGIC CONTROL OF FOOD INTAKE IN RATS

City University of New York PH.D. 1982



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DEVELOPMENT OF ENDORPHINERGIC CONTROL OF FOOD INTAKE IN RATS

by

Oladipupo O. Aroyewun

A dissertation submitted to the Graduate Faculty
in Psychology in partial fulfillment of the
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Abstract

DEVELOPMENT OF ENDORPHINERGIC CONTROL OF FOOD INTAKE IN RATS

by

Oladipupo O. Aroyewun

Advisor: Professor Gordon A. Barr

The pure opiate antagonist, naloxone, has been found to reduce deprivation-induced feeding in adult animals. This effect presumably reflects the blockade of opiate receptors by naloxone and the attendant pertubation of activity in endogenous opioid systems involved in food consumption. The aims of these experiments were to determine the developmental age of inception as well as the pharmacological and behavioural mechanisms of naloxone's anorexigenic action. Towards this end, altricial infant rats were used as subjects. The experimental approach involved specifying a series of behavioural and pharmacological elements that would characterise naloxone's anoretic action independently of toxic or narcotic effects.

The major findings of this study are as follows:

(1) Naloxone hydrochloride (5-30 mg/kg), or its structural congener naltrexone (10-50 mg/kg) given intraperitoneally to rats had no effect on the milk consumed by 3, 10 and 12 day olds. Naloxone attenuated food intake beginning 14 days postpartum and in a dose-related manner.

(2) Naloxone had no effect on the latency of eating but hastened the early cessation of feeding in 14 day olds.

(3) The relationship between the ontogeny of opiate receptors and feeding behaviour was examined by exposing developing rats to antenatal morphine. While no shift in the time-course occurred, a change in naloxone dose-response curves for feeding modulation was apparent in the 14 day-olds.

(4) Morphine treatment during the first 5 days of postnatal life accelerated the functional maturation of the system supporting naloxone's effect. Rats were responsive to naloxone at 10, 12 and 14 days of age.

(5) Chronic pretreatment with naltrexone potentiated naloxone's anorexigenic effect only at 14 days postpartum.

These findings suggest that naloxone, and, by inference, the endogenous opioid system, may not participate in the feeding of the young before their second week of birth; support the contention that naloxone's effects are mediated by the opiate-receptor mechanisms; and extend observations in adults to preweanling rats that endogenous opioid system may mediate feeding and appetite. The findings also suggest a 'satiety' mode of food-intake modulation for naloxone, and by inference, an

'anti-satiety' mode of food intake regulatory control for the endogenous opioid systems.

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vision helped this study to rise, phoenix-like, from the ashes of another; *sine quo non*.

To Kelvin James Walsh, for being there, always.

To Mum, who rose to the occasion. E ku ife, e ku iroju o.

Most sincere thanks.

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GENERAL INTRODUCTION

In the past decade, a plethora of important developments occurred in opiate pharmacology. The harbinger of these advancements was the observation that electrical stimulation of the periaqueductal grey of the brain of the rat produced sufficient analgesia to allow surgery (Reynolds, 1969), and that naloxone (N-allyl-noroxymorphine), an opiate antagonist, reversed this stimulation-produced analgesia (e.g. Akil, Mayer & Liebeskind, 1976). Such findings suggest that the periaqueductal gray region is part of an endogenous pain-inhibitory system that may mediate its function utilizing a neurochemical native to the brain with a potential analgesic property. Heretofore, it had been difficult to separate opiate alkaloid's addictive properties from its analgesic potential, but Reynolds' (1969) report raised the possibilities of inducing the body to produce its own analgesic or of developing a drug which has morphine-like analgesic effects without its addictive properties (see Martin, 1981a for review).

Since most drugs exert their actions as a result of combining with a specific receptor (Clark, 1933), initial efforts in the molecular biological approach to the question of analgesia and addiction were directed at isolating and characterising the receptors in the brain for opiates. As an obligatory step for pharmacological action, the receptor recognises the structure and steric features of the opiate molecule. Most opiates have a rigid T-shaped structure with two broad water-repelling surfaces

at right angles to each other, a hydroxyl (OH) and a positively charged nitrogen atom. Only the levorotatory isomer is active and substitution of the methyl group on the nitrogen by a larger alkyl group produces a series of antagonists (Jaffe & Martin, 1980).

Following the methodological concepts enunciated by Goldstein and coworkers (Goldstein, Lowney & Pal, 1971), studies employing a saturation displacement technique (i.e. displacement of radio-labeled opiate antagonist naloxone, or opiate agonist etorphine or morphine by unlabeled compounds) utilizing the steric property of opiates reported that synaptic membranes from different areas of the rat brain showed selective opioid recognition properties (Pert & Synder, 1973; Simon, Hiller & Edelman, 1973; Terenius, 1973). The opiates bound with high affinity and binding was saturable, suggesting a finite number of receptors (Flier, 1981; Weiland & Molinoff, 1981), while their inert mirror-image enantiomers, such as dextrorphan, the D-isomer of levorphanol, showed very little binding affinity (Simon et al., 1973).

Binding was found to be localized almost exclusively to neuronal elements and to exhibit, contrary to expectation, a heterogenous distribution that extended beyond the brain areas that are involved with pain i.e. laminae I and II of the spinal cord, spinal trigeminal nucleus, periaqueductal grey, periventricular grey, and medullary raphe nucleus (e.g. Yaksh & Rudy, 1978). Binding concentration was highest in striatum, low

in brainstem, absent in cerebellum, and intermediate elsewhere (Pert & Snyder, 1973). In addition, the ligand-receptor interactions were reported to show considerable heterogeneity. The various ligands did not show equal potencies or cause the same physiological effects, and neither did opiate antagonists show similar degrees of effectiveness against the various opioids (Hutchinson, Kosterlitz, Leslie, Waterfield & Terenius, 1975), suggesting the existence of multiple receptors for opiates (Martin, Eades, Thompson, Huppler & Gilbert, 1976).

Morphine and related compounds were later divided into at least three different classes on the basis of subjective effects in humans (Jasinski, 1977), different sensitivities towards naloxone (Jacquet, Klee, Rice, Iijima & Minamikawa, 1977) and dissimilar pharmacological profiles both in vitro (Hutchinson et al., 1975; Lord, Waterfield, Hughes & Kosterlitz, 1977) and in vivo (Cowan, 1981; Cowan, Geller & Adler, 1979; Gilbert & Martin, 1976; Martin et al., 1976). The subdivision was concomitant with the conceptualisation of three types of opiate receptors: mu- (morphine), kappa- (ketocyclazocine) and sigma- (N-allyl-normetazocine) receptors, with the action of each prototypic drug mediating its effects through its respective receptor (Martin et al., 1976).

Opiate receptors, however, have not evolved simply to interact with opiate alkaloids derived from the opiate poppy. Their high and unique affinity for opiates suggested the presence in the brain of an endogenous ligand with opioid properties

(Collier, 1972). This speculation was supported by the findings that extraction of biologic material from porcine brain inhibited the contraction of the mouse vas deferens (the de rigueur bioassay "screen" for opioid activity) and this inhibition was readily reversed by naloxone (Hughes, 1975). Also, this substance was able to compete with labeled opiates for receptor binding (Terenius & Wahlstrom, 1974). The endogenous morphinomimetics were later found to be three major but structurally distinct pentapeptides termed respectively, methionine-enkephalin (Met-enkephalin), leucine-enkephalin (Leu-enkephalin) (Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975), and beta-endorphin (B-endorphin) (Cox, Goldstein, & Li, 1976). Other minor but related pentapeptides, i.e. alpha- and gamma-endorphin, were found (Guillemin, Ling & Burgus, 1976); and major ones such as dynorphin have since been reported (Goldstein, Tachibana, Lowney, Hunkapiller & Hood, 1979).

The exact distribution of the endogenous opioid peptides was examined in the rat by immunofluorescent (Elde, Hokfelt, Johansson & Terenius, 1976) and unlabeled peroxidase-antiperoxidase techniques using antisera directed against Met-enkephalin (Simantov & Synder, 1976) or against B-endorphin (LaBella, Queen, Senyshin, Lis & Chrestien, 1977). The peptides were localized in both nerve-terminals and cell-bodies and showed a distribution generally consistent with that previously determined for the opiate receptors (Hokfelt, Elde, Johannson, Terenius & Stein, 1977; Simantov, Kuhar, Uhl & Synder, 1977; Sar, Stumpf, Miller, Chang & Cuatrecasas, 1978).

Met- and Leu-enkephalins were found in the same neuroanatomical network (Watson, Akil, Sullivan & Barchas, 1977), being predominant in the stria terminalis, globus pallidus, locus coerulus and the median eminence (DiGiulio, Majane & Yang, 1979; Sarne, Keren, Dalith & Weissman, 1980) in addition to their presence in the pain pathways (Uhl, Goodman, Kuhar, Childers & Synder, 1979). B-endorphin-containing fibers were found to constitute separate systems from enkephalin network (Bloom, Ling & Guillemin, 1978; Rossier, Vargo, Minick, Ling, Bloom & Guillemin, 1977) with highest concentration in the hypothalamic arcuate nucleus (Bugnon, Bloch, Lenys, Gouget & Fellman, 1979; Rossier et al., 1977; Sofroniew, 1979; Watson, Akil, Richard & Barchas, 1978) and with little or no significant amount in the globus pallidus or spinal cord (Watson et al., 1978). In addition to its presence in the brain, B-endorphin was also found in the pituitary gland, predominantly in the pars intermedia (Duka, Hollt, Przewlocki & Wesche, 1978). However, pituitary concentrations appear to be unrelated to the brain B-endorphin since hypophysectomy failed to alter brain content of opioid peptides (Cheung & Goldstein, 1976; Rossier et al., 1977; Wesche, Hollt & Herz, 1977) or modify the opioid activity of the brain (Brown, Blank & Holtzman, 1980), suggesting that these peptides may serve as neurotransmitters (Kosterlitz & Hughes, 1975).

Consistent with their distinctive distribution in the brain, the various endogenous opioids were found to differ in their sites of action. A receptor-type, delta receptor, was proposed

for enkephalins, based on enkephalin's ability to compete with opiate alkaloids in inhibiting electrically-induced contractions of mouse vas deferens in vitro and in situ (Lord et al., 1977), and on its selective protection of (3)H-enkephalin sites rather than (3)H-dihydromorphine sites from the irreversible inhibitor phenoxybenzamine (Robson & Kosterlitz, 1979). B-endorphin, on the other hand, was discovered to bind equally well to both mu-receptors and delta-receptors (Hazum, Chang & Cuatrecasas, 1979; Goodman, Synder, Kuhar & Young, 1980; Wuster, Schulz & Herz, 1980a), since it was effective in preparations enriched with both delta-receptors (mouse vas deferens, where morphine was less effective) and mu-receptors (guinea pig ileum, where enkephalin was less effective). Thus B-endorphin behaves like an alkaloid when displacing (3)H-naloxone (i.e. labeled alkaloid) and like enkephalin when displacing (3)H-enkephalin (Hazum, Chang & Cuatrecasas, 1978), a capacity that may reside in the length of its peptide chain (Wuster et al., 1980).

While appreciable controversy exists concerning the exact number of receptor-types (e.g. Amir & Amit, 1979; Hiller & Simon, 1980; Zhang & Pasternak, 1980; see Adler, 1980; Zukin & Zukin, 1981 for reviews) and their appropriate classification or nomenclature (Martin, 1981b), it is noteworthy that, like their alkaloid congeners, endogenous opioids exhibit different pharmacological profiles (Kastin, Olson, Schally & Coy, 1979; Olson, Kastin, Olson & Coy, 1979) and differential response to environmental stimuli (e.g. Opmeer, Loeber & van Ree, 1980).

These varied features and distinct properties of the endogenous opioid systems would seem to support the hypothesis that they serve a diversity of functions aside from simply pain modulation. Indeed, studies on the circadian variations in endogenous opioid levels revealed that the highest levels occurred during the nocturnal feeding phase with lowest level during the light phase of the cycle (Frederickson, Wesche, Edwards, Harrell & Burgis, 1978; McGivern & Bernston, 1980), increases that apparently are independent of painful physical stimuli. The latter observation in part prompted the suggestion that opioid peptides may be involved in the control of feeding and appetite (Margules, 1979). Further basis for this suggestion stems from the fact that opioid peptides and opioid receptors are widely distributed in the neuroaxis and that this ubiquity best serves behaviours that increase the likelihood of survival of species.

OPIOID PEPTIDES AND THE CONTROL OF FOOD INTAKE

There is substantial support for the notion that opioids are involved in the regulation of feeding. Low doses of B-endorphin administered intrahypothalamically (Grandison & Guidotti, 1977; Leibowitz & Hor, 1980; Tseng & Cheng, 1980) or intracerebroventricularly (Kenny, McKay, Woods & Williams, 1978) stimulated food intake in satiated or mildly food-deprived rats and reversed the satiety effect of a number of putative satiety factors such as cholescystokinin, bombesin and thyrotropin-releasing hormone (Morley & Levine, 1980; 1981). In addition, genetically obese mice and rats were reported to have greater amounts of B-endorphin than normal mice and rats (Margules, Moisset, Lewis, Shibuya & Pert, 1978; Rossier, Rogers, Shibasaki, Guillemin & Bloom, 1979). This finding has also been reported in the plasma of obese women (Givens, Wiedemann, Anderson & Ketabchi, 1980). Moreover, morphine, an exogenous opiate, caused an increase in food intake when injected into the paraventricular nucleus (McLean & Hoebel, 1980) or the ventromedial hypothalamus (Tepperman, Hirst & Gowdey, 1980) or when infused into the cerebral ventricles (Belluzzi & Stein, 1978) or when given peripherally (Ayahan & Randrup, 1973; Jalowiec, Panksepp, Zolovick, Najam & Herman, 1981; Kumar, Mitchell & Stolerman, 1971; Maickel, Braude & Zabik, 1977) to freely-feeding or mildly food-deprived rats.

Further impetus for this hypothesis came from research examining the effects of peripheral administration of opiate

antagonists (e.g. naloxone or naltrexone) on eating and drinking. Naloxone, an opiate antagonist, had been demonstrated to have little or no agonist action (Blumberg & Dayton, 1972; Jaffe & Martin, 1980) and to be free from pharmacological effects when given to subjects who had not been pretreated with narcotics (Foldes, 1973). However, as naloxone antagonised the effects of endogenous opioid peptides (Hughes, 1975; Watson et al., 1977, 1978) it would be expected to have behavioural effects in situations that elicited the release of endogenous opioids.

Acute systemic administration of naloxone to adult rats (Holtzman, 1974; Frenk & Rogers, 1979), mice (Brown, & Holtzman, 1979), guinea pigs (Schulz, Wuster & Herz, 1980), rabbits (McCarthy, Dettmar, Lynn & Sanger, 1981), sheep (Baile, Keim, Della-Fera & McLaughlin, 1981), and cats (Foster, Morrison, Dean, Hill & Frenk, 1981) caused decreased food intake under varying levels of food-deprivation and under different conditions of testing. Such effects have been demonstrated in operant responding for food in rats (Gellert & Sparber, 1977) and monkeys (Kelleher & Golberg, 1978). Naloxone was also shown to inhibit feeding induced by endogenous opioid dynorphin (Morley & Levine, 1981), and by lesions of the ventromedial nucleus of the hypothalamus (King, Castellanos, Kastin, Berzas, Mauk, Olson & Olson, 1979), and to suppress feeding in genetically obese animals (Margules et al., 1978; Rossier et al., 1979). In humans, naloxone was found to reduce food consumption in patients manifesting the Prader-Willis syndrome, which is characterised by

overeating and obesity (Kyriakides, Silverstone, Jeffcoate & Laurance, 1980). Chronic administration of naloxone induced long-term suppression of ad lib food consumption (Brands, Thornhill, Hirst & Gowdey, 1979, Jalowiec et al., 1981) and decreased weight gain (Brands et al., 1979). This evidence suggests that opioid agonists may play an important role in controlling food intake.

Naloxone's effect on food intake appears to be specific to its opioid antagonist activity. Morley and Levine (1981) have demonstrated that naloxone administration (systemic or intracerebral) suppressed feeding induced by intracerebroventricular injection of opioid peptide dynorphin. Grandison & Guidotti (1977) have reported that while systemic administration of the opiate antagonist failed to block feeding induced by intrahypothalamic norepinephrine or muscimol, even in large doses, it did block B-endorphin-induced feeding in small doses. The effect is stereospecific since reduction in food intake follows after the L-isomer of naloxone, but not after its D-isomer, even in high doses (Brown & Holtzman, 1980; Sanger, McCarthy & Metcalf, 1981). Furthermore, other opiate antagonists also produce these effects. For example, naltrexone, another opiate antagonist (Gritz, Shiffman, Jarvik, Schlesinger & Charuvastra, 1976), suppressed feeeding induced by B-endorphin injections (Grandison & Guidotti, 1977) or palatable diet (Apfelbaum & Mandenoff, 1981) in rats; and reduced weight gain in genetically obese mice (Recant, Voyles, Luciano & Pert, 1981).

The potency of the various opiate antagonists, however, differs considerably (Brown & Holtzman, 1981a; Maickel et al., 1977).

Brown and Holtzman (1980) have examined the relative potency of a number of narcotic antagonists regarding their ability to suppress consummatory behaviour following subcutaneous injection. The rank ordering of the potency of the various compounds ranging from naltrexone (most potent) through nalorphine (least potent) resembled the ordering determined for these compounds in displacing (3)H-naloxone from guinea pig ileal and rat brain binding sites and for the precipitation of morphine withdrawal jumping in dependent mice, suggesting that the food-suppressant effect of naloxone is similarly mediated through an interaction with opiate receptors and also that the naloxone effect produced by this route of administration is no less specific than that evoked by intracerebral injection.

More compelling proof that the different routes of administration poduce food reduction by the same mechanisms has been presented in a recent work by Tepperman et al. (1981). Using cannulae implanted into the ventromedial hypothalamus (VMH) of rats, they have observed that naloxone injected into the VMH attenuated food intake while morphine enhanced it, and that when naloxone was given subcutaneously to the rats it suppressed the intrahypothalamic morphine-induced feeding. When opioid peptide dynorphin was substituted for morphine similar results were obtained (Morley & Levine, 1981). These results suggest then that the effects produced by systemic injection of naloxone yield

changes in ingestive behaviour which are a reflection of action of the receptor populations, similar, if not identical to those acted upon by intracranially or intracerebrally administered naloxone.

The convergence of behavioural evidence in animals indicates that the naloxone effect is specific to food-intake reduction and not a manifestation of toxicity. Measures of drug-induced toxicity (see Goude, 1979 for review) correlate poorly with the naloxone effect. Acute administration of naloxone did not produce a conditioned taste aversion in low (LeBlanc & Cappell, 1975; Rogers, Frenk, Taylor & Liebeskind, 1978) or high doses (Jalowiec et al., 1981), suggesting that naloxone does not change food intake due to illness or malaise. Furthermore, naloxone attenuates food intake in a dose-related manner (Holtzman, 1974), with threshold doses for significant inhibition of food intake being as low as 0.5 mg/kg (Cooper, 1980). And doses of naloxone (Cooper, 1980) or naltrexone (Lowy & Kim, 1981) that inhibited solid food intake did not inhibit water intake, indicating the absence of illness covariance and also suggesting a selective effect since feeding and drinking control systems can interact (Toates, 1979).

The site of action of naloxone regarding food intake has been shown to be central (Grandison & Guidotti, 1977; King et al., 1979), even though opiate receptors are known to exist peripherally (e.g., Phillis & Kirkpatrick, 1979). Jones and Richter (1981) found that bilateral infusion of naloxone into the

lateral ventricles of food-deprived rats resulted in decreased food intake. Because the dose required was smaller than than the threshold dose of peripherally administered naloxone, they concluded that the inhibition was a central effect. Similar results have been reported with quaternary derivatives of naloxone or naltrexone, methly-naloxone or methyl-naltrexone, to which the blood-brain barrier is impermeable. They failed to affect consummatory behaviour when injected peripherally, but attenuated it at a very low dose upon intracerebroventricular administration (Brown & Holtzman, 1981c), suggesting that the anorexic effects of opiate antagonists are mediated primarily at sites within the central nervous system. Furthermore, the fact that hypophysectomy did not attenuate naloxone suppressant effect (Brown et al., 1980) indicates a lack of involvement of the pituitary endorphins in consummatory control.

Although the actions of naloxone on food intake in the adult animal are well established, there are ontogenetic limitations in these studies which restrict the extent to which the endogenous opioid system action vis-a-vis feeding behaviour can be generalised within the life span of a species. There is no evidence of naloxone's action on feeding in the rat infant. This neglect prevents access to the functional development of the endogenous opioid system, as well as knowledge about its specific role in infant feeding behaviour and broad role in the species survival, and to information that might be relevant to cases of early corrective clinical intervention during dysfunction.

Since critical ages exist across and within neurochemical systems such as the adrenergic, serotonergic, dopaminergic (e.g. Coyle & Campochiaro, 1976; Coyle & Yamamura, 1976; Porcher & Heller, 1972) that also monitor regulatory processes (see Morley, 1980 for review), it would seem likely that similar age-related changes also exist regarding the endogenous opioid system. Indeed, current evidence indicates that the rat is not born with a fully developed complement of opiate receptors (Kent, Pert & Herkenham, 1982; Simon & Hiller, 1978) or opioid peptides (Patey, de LaBaume, Gros & Schwartz, 1980). The opiate receptors make their appearance at about 15 days of gestation (Coyle & Pert, 1976), and undergo rapid proliferation from birth to weaning (Clendeninn, Petraitis & Simon, 1976; Patey et al., 1980), but do not reach adult density level until 20 weeks postpartum (Clendeninn et al., 1976). In addition, the various receptor types exhibit dissimilar patterns of development: some mimic adult density level perinatally (Koch, Sakly & Lutz-Bucher, 1980; Zhang & Pasternak, 1981), suggesting a predominant prenatal development, while others only start to increase postnatally and reach 62% of adult level on day 14 postpartum (Pasternak, Zhang & Tecott, 1980). Correspondingly, the developmental pattern of the endogenous opioid peptides has a time-course and a variability between peptides which is similar to the opiate receptors'. Endorphin levels are higher than enkephalin levels on embryonic day 16, when they are first detected in the rat brain, and remain so throughout the prenatal period (Bayon, Shoemaker, Bloom, Mauss

& Guillemin, 1979), while the opposite is true for the adult rat brain. The enkephalin levels increase markedly perinatally (Bayon et al., 1979; Patey et al., 1980). Furthermore, the opioid peptides and opiate receptors show regional variations during development (Chang, Cooper, Hazum & Cuatrecasas, 1979; Goodman, Synder, Kuhar & Young, 1980).

In view of the likely role of the endorphinergic system on adult feeding, these age-related changes in the population, distribution and characteristics of opiate receptors/peptides may correlate with the emergence and modification of feeding-related behavioural patterns. To the extent that changes in feeding patterns can be related to developmental changes of the opiate receptors/peptides, they might provide clues as to the specific mechanism mediating various aspects of feeding behaviour. This possibility adumbrates the concern of this research.

AIM OF STUDY

This study was undertaken to provide information on the functional ontogeny of the endogenous opioid system *vis-a-vis* feeding behaviour. Towards this end, pharmacological agents known to intervene precisely in the endogenous opioid system neurophysiological processes and behavioural functions were used to delineate development. Since such intervention affects ingestive behaviour of adult rodents, this research examined in infant rodents the developmental age of inception and the characteristic pattern of this relationship.

Specifically, the objectives were:

(1) To examine the responsivity of the neonate to naloxone which is thought to suppress food intake via alterations in the endogenous system.

(2) To ascertain that naloxone effect was pharmacologically specific, the ability of naltrexone to suppress food intake was also examined.

(3) To demonstrate the specific nature of the behavioural effects of naloxone, preweanling infants were observed under conditions of both active and passive feeding following naloxone treatment.

(4) To detect the detailed changes in feeding behaviour following naloxone intervention, a microanalytical observational technique was employed to examine the way in which naloxone inhibits food consumption and the way in which it may regulate patterns of feeding.

As the endogenous opioid system's mediation of food intake is thought to arise from the interaction between opioid peptides and opioid receptors and as naloxone-induced suppression of feeding is due to reduced ligand/receptor binding (Frenk & Rogers, 1979), chronic alteration of the interaction should result in a long-term modification of feeding behaviour. Therefore, the final objective was to assess the potential relationship between opiate receptor development and feeding behaviour, thusly:

(5) To examine the responsivity of preweanling offspring of females given chronic morphine treatment during the prenatal period to naloxone in the feeding situation, as a way of determining the role of the prenatally-developed receptors in feeding behaviour.

(6) To examine the responsivity of preweanlings, pretreated during early postnatal life with either chronic morphine or chronic naltrexone injections, to the naloxone effect, as a way of inferring the role of postnatal-receptors in feeding mediation.

PART 1: DEVELOPMENT OF OPIATE ANTAGONIST INHIBITION OF FEEDING IN RATS.

Prima facie, altricial mammalian infants depend upon their mother as the prime source of nutrition which they receive from the nipple via the integrated act of suckling. Post-weaning, the mammal is a free-foraging adult. Behavioural (e.g. Blass, Hall & Teicher, 1979) and neurological (e.g. Almli, 1978) studies have revealed that within the period from birth to weaning, feeding behaviour undergoes dramatic transformation shifting from being determined largely by sensorimotor reactions i.e. clinging, suckling, rooting (Kennedy, 1967) to having the multifactor control characteristic of the adult ingestive behaviour (Adolph, 1957; see Booth, 1978 for review).

The specifics of these behavioural transitions have been determined by the constructs used to explain and analyse adult feeding characteristics. In adults, investigators have elucidated the natural operations of the regulatory processes by identifying critical signals in the peripheral physiological systems which are monitored by the brain and ultimately reflected in behaviour (see Toates, 1981, for review). Since food intake in a freely feeding animal is a periodic series of discrete events (Wiepkema, 1971), the critical signals from the internal environment that provide a base for the execution, modulation, and termination of a feeding response have been assessed by using metabolic manipulations such as deprivation (Bare, & Cicala, 1960; Bellinger & Mendel, 1975; Burton, Rolls & Mora, 1975),

diabetes (Friedman, 1972; 1978), hyperinsulinaemia (Berthoud & Jeanrenaud, 1979; Hustvedt & Lovo, 1972) and modulation of the chemistry of the internal environment (Booth & Stribling, 1978; Blundell & Latham, 1978; Hoebel, 1977). Although these techniques have been criticized (e.g. Hinde, 1970), they have nonetheless been heuristic in the characterisation of two principal aspects of the ontogeny of rat ingestive behaviour.

First, infants of all ages are sensitive to food-deprivation. Houpt and Epstein (1973) found that one-day-old rats deprived for 4 hours gained 100% more than nondeprived controls when allowed to suckle their mother. Since the feeding response following food deprivation is a basic phenomenon providing evidence for the regulation, through food intake, of body energy deficits (LeMagnen, Devos & Larue-Achagiotis, 1980), it seems that sensitivity to energy deficits matures in utero. However, food-intake per se does not constitute the whole spectrum of feeding response following food-deprivation in the adult; the deprived adult animal also initiates or perform acts that are instrumental to obtaining an ingesta. In neonates, latency of attachment to the nipple and rate of nipple-shifting following milk delivery (Cramer, Blass & Hall; 1980; Hall, Cramer & Blass, 1975, 1977) have been used as measures of appetitive behaviour. Dollinger, Holloway and Denenberg (1980) found that 2-day-old rat infants did not respond to 11 hours of food-deprivation with a decrease in attachment latency, but that 9-day olds did decrease their attachment latencies significantly. Thus

the authors concluded that sensitivity to deprivational cues matures with age.

Second, intake controls that operate in the adult mammals mature sequentially. Although rats are prepared to eat in response to food-deprivation by 1 day of age, they did not eat in response to decreases in intracellular glucose utilization induced by 2-deoxyglucose until well after weaning (Drewett & Cordall, 1976). At the level of intake modulation and/or termination, Hall and Blass (1977) reported that the volumes of diet consumed by food deprived 9-13 day olds when milk was made freely available was not responsive to gastric fill or the nutritive quality of the load. Glucose administered by stomach tube did not inhibit feeding more than the same volume of water in 9-11 day-olds (Drewett & Cordall, 1976). At 15 days postpartum, intake was terminated by stomach distention (Hall & Blass, 1977) and nutritive preloads (milk) depressed intake to a greater extent than bulk (saline) loads (Hall & Rosenblatt, 1977, 1978). Moreover, cholescystokinin, a hormonal factor with potent inhibitory action on adult rat feeding (see Smith & Gibbs, 1981 for review), has been shown to be ineffective in suppressing milk intake in 10 day-olds while reducing milk intake in 15 day-olds in a dose related manner (Blass, Beardsley & Hall, 1979). Apparently there is a silhouette of adult ingestive behavioural controls in rats older than 15 days of age.

It seems from these studies that many physiological regulatory processes of adult feeding behaviour are notably weak

or absent in rats 13 days or younger. Some of these deficiencies may result from the immaturity of peripheral organ systems such as the kidney (Adolph, 1957), but others may be the result of the functional immaturity of central neurochemical systems which monitor the signals from the peripheral physiological systems. In the adult mammal, the endorphinergic system mediates in the translation of the physiological signals from the internal environment into goal-directed behaviour (van Ree, Smyth & Colpaert, 1979). However, nothing is known about the role of this system in mediating ingestion in neonates. Since the pure opiate antagonist naloxone has been suggested as a useful pharmacological tool for elucidating the possible physiological roles of endogenous opioid peptides (Goldstein, 1978), the present research examined the functional ontogeny of the endophinergic system vis-a-vis ingestive behaviour using this opiate antagonist.

GENERAL METHOD

Subjects

Offspring of male and female Long-Evans hooded rats (Rattus norvegicus), bred and reared in Hunter College colony, were used as subjects. Pregnant rats were checked for birth twice daily, at 8:00 a.m. and 6:00 p.m. Pups found at either of these times were declared born on that date, considered day 0. Dams and their litters were housed in 40 X 20 X 24 cm plastic terraria in a temperature- (21° C) and humidity-controlled room. The

light/dark regimen was 12:12 hours, with light onset at 7:00 a.m. Food (Purina pellets 5012) and water were always available. Three days after birth, each litter was reduced to 6-8 pups and otherwise left undisturbed until the time of testing.

Subjects were 3-, 10-, 14-, or 19-day olds of both sexes. The selection of these age groups was based on behavioural (Hall & Bryan, 1981), neurological (e.g. Almli, 1978) and biochemical (Pasternak et al., 1980) research indicating that rapid changes in development occur between 10 and 17 days postpartum. The inclusion of 3-day olds was based on the findings from autoradiographic techniques which indicates that receptor elimination occurs during development i.e. opiate receptors may be present in very young rats in brain areas where they are absent in adults (Kent, Pert, & Herkenham, 1982; Lewis, Palacios, Unnerstall, Niehoff, Molliver & Kuhar, 1981).

A litter was tested at one age only and each pup received a single dose of the test drug.

Deprivation Schedule

For the induction of food deprivation, all pups in a litter were removed from their mother 8 hour before testing and housed with their littermates in a plastic cage containing fresh wood-chip bedding. The cage was placed on a warm water incubator (32-34^{o}C). The choice of 8-hr deprivation was based on the finding that such a regimen is not too severe for infant rats (Lytle, Moorcroft & Campbell, 1971).

Infant Treatment

One hour before the end of the deprivation period, pups were removed from the incubator and labeled as to drug treatment with a Magic Marker. The subjects' bladders were then voided by stroking their anogenital area with a moist camel air brush. Individual rat was weighed to the nearest 0.01g on a Triple Beam balance (Ohaus Corporation, Florham, N.J.) and injected intraperitoneally with either naloxone hydrochloride (5, 10, 30 mg/kg), naltrexone naltrexone hydrochloride (10, 30, 50 mg/kg salt weight) or an equivalent volume (1 ml/100 g) of the 0.9% saline vehicle.

Immediately after injection of naloxone, or 15 minutes after injection of naltrexone, the subjects were tested in one of the three experimental techniques described below. This difference in the interval between drug administration and behavioural testing reflects the different rate of clearance in the body, which is twice as long for naltrexone as for naloxone in the adult (Gray & Robinson, 1974). However, the half-life of naloxone in neonates after intravenous administration is two to three times longer (Ngai, Berkowitz, Young, Hempstead & Spector, 1979) than that reported for adults (t 1/2 = 20 min, Tallarida, Harakal, Maslow, Geller & Adler, 1978), enough to permit a 1-hr test period.

To reduce experimental bias, all the drugs used in these studies were assigned individual codes. The experimenter was 'blind' as to the identity of the test drugs.

Testing Procedure

Milk intake was assessed under three conditions of milk availability. The subjects were allowed to suckle their natural, unanaesthetised mother, in the first condition, to swallow orally-infused milk in the second condition, and to lick milk off the floor in the third condition. In addition to milk-intake, the structural elements of ingestive behaviour were scored for the licking paradigm, using a rating system devised by Hall (1979).

Measurement of Consumption

At the end of the 1 hour period, each rat was weighed again. Percent weight gain during the test period was calculated as:

$$100 \times \frac{\text{body weight after experiment} - \text{body weight before experiment}}{\text{body weight before experiment}}$$

This formula compensated for body weight, since pups were not matched by weight. The absolute weight change (i.e. the difference between body weight before and after feeding) was also used as a measure of milk ingested. Houpt and Epstein (1973) have provided evidence for absolute weight gain as a valid measure of intake in preweanling rats.

Data are presented as mean $\pm$ standard error and analysed by analysis of variance and Dunnett's test. In order to facilitate comparison among different experimental protocols and drug groups, the data were further normalised to a percentage of the saline control values for each subject. The transformed data

were evaluated by Student t-tests. A P value of less than 0.05 was selected as the lower limit for statistical significance.

Experiment 1

Naloxone produces a food-intake reduction effect in deprived animals (Holtzman, 1974), presumably through its blockade of opiate receptors and the attendant pertubation of activity in endogenous opioid systems (Goldstein, 1978; Terenius, 1978). At present, these food-suppressant effects have been described only in adult rodents, but in view of the well-known age-related changes in the development of opiate receptors and the age-dependency of many of the opiate effects, it was of interest to examine the development of the food-suppressant effects of naloxone. Accordingly, the primary objective of this study was to examine the development of neonatal responsivity to naloxone action. Since opiate receptor system has been implicated in the mediation of a number of naloxone's physiological and behavioural effects, another aim of this study was to extend this observation to its effect on feeding behaviour. Towards this end, naloxone's structural congener, naltrexone, was evaluated for its ability to replicate naloxone's effect on the milk consumption of food-deprived preweanling rats.

As preweanling rats normally derive their nutrients from their mother via the suckling act (Blass et al., 1979) and thus interact with her during feeding, an undertanding of the ontogeny of feeding control must be related to the natural context of the

feeding act. As such, this study utilized the infants' home environment and was performed using 10 day-old or older animals, since current evidence indicates that it is only after 10 days postpartum that the infant can integrate elements of suckling (i.e. nipple search, nipple attachment and the consummatory act itself) into a feeding sequence, and thus contribute actively to the nursing interaction (Bolles & Woods, 1964; Drewett, Statham & Wakerley, 1974).

Method

Subjects and procedure

The subjects were 200 male and female offspring of rats, derived from the mating of 31 female rats. The animals were tested at 10-, 12-, 14-, or 19 days of age. At each age, 4 pups were removed from their mother at about 8:00 a.m. and put into the incubator for the deprivation regimen. The remaining pups from the litter were also removed at this time, as pilot study had revealed that the presence of littermates in the cage during the course of food-deprivation of test animals substantially reduced nursing by the dam. After drug injection, the animals were returned to their mother for ad lib feeding. At the end of the 1-hr test period, the subjects were removed from their mother and reweighed.

Results

Figure 1 shows milk intake during the 1-hour feeding period as a function of age and drug dose. Naloxone consistently reduced both absolute intake and intake as a function of body weight in 14 and 19 day old pups but had no effect on younger animals. ($F = 0.95, 0.24, 13.33$, and 15.42 for 10, 12, 14, and 19 day olds respectively for intake in grams; $F = 0.66, 0.73, 13.18$, and 16.71 for intake as a percent of body weight). Posthoc Dunnett's tests revealed that each dose of the drug at both 14 and 19 days of age was significantly different from the vehicle control

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Figure 1 about here

The results for naltrexone were similar to those for naloxone. Naltrexone decreased milk intake only after 14 days of age having no effect on 10 and 12 day old pups (Table 1). ($F = 1.05, 0.19, 7.28, 13.12$ for 10, 12, 14, and 19 day olds respectively for intake in grams; $F = 1.40, 0.19, 9.38$ and 16.11 as a percent of body weight). However, naltrexone was not as potent as naloxone in suppressing milk intake. Milk intake was reduced to 73.7% and 88.7% of 14 day olds saline control values by 10 mg/kg of naloxone and naltrexone respectively (Fig.2). Thirty mg/kg of naloxone and naltrexone decreased food intake to 39.0% and 72.5% respectively, $t(8) = 2.34$, $p < 0.05$. For 19 day

olds, it was 51.0% and 79.3% for 10 mg/kg naloxone and naltrexone respectively, $t(6)=3.11$, $p < 0.05$; and 45.0% and 55.0% for 30 mg/kg.

Table 1

EFFECTS OF NALTREXONE ON MILK INTAKE

	Age		
	10	14	19
Dose			
Saline	1.4 ± 0.2	2.3 ± 0.5	2.9 ± 0.2
10	1.2 ± 0.2	1.9 ± 0.3	2.4 ± 0.2
30	1.2 ± 0.2	1.6 ± 0.2	1.7 ± 0.3 *
50	1.1 ± 0.2	0.5 ± 0.2 *	1.1 ± 0.3 *
Saline	6.4 ± 0.9	6.7 ± 1.3	6.9 ± 1.2
10	5.7 ± 0.9	5.6 ± 0.8	5.3 ± 0.7 *
30	5.5 ± 0.7	4.3 ± 0.6	3.8 ± 0.9 *
50	4.9 ± 0.8	1.4 ± 0.5 *	3.0 ± 1.1 *

Entries in the top panel are grams of weight gain and in the bottom panel percent of body weight. Asterisks denote differences from appropriate controls ($p < 0.05$).

Figure 2 about here

Discussion

These results show that opiate antagonist naloxone fails to affect the control of food and appetite before 12 days of age.

Naloxone, however, decreased intake in 14 day olds. The effects of naloxone seen in 14 day-olds does not appear to represent a transient developmental phenomenon since the responses of 19 day-olds were virtually identical with those seen at 14 and with those reported for adult animals. At both ages, naloxone reduced milk intake; even the older animals showed more depression from their saline controls than did 14 day-olds ($p < 0.01$), suggesting a functional developmental trend in the system mediating such effect. That naltrexone produced effects similar to naloxone in the animals studied suggests a common link between the two drugs which may be the opiate receptor system. However, this study did not observe the rank order of potency (naltrexone > naloxone) that has been determined for the two drugs in in vivo and in vitro preparations (Creese & Synder, 1975; Pircio & Glys, 1975). The absence of such hierarchy, although somewhat puzzling, is consistent with previous reports on the effects of naltrexone on food intake in adult rats (Maickel et al., 1977). Perhaps such results should not be unexpected where species differences in naltrexone kinetics are concerned and particularly where multidetermined interacting behaviours are concerned. Species differences in naltrexone kinetics have been consistently reported (Inturrisi, 1976). The reversed rank order of potency observed here could be due to the procedural differences in the treatment groups or to higher entry of naloxone into the brain.

Experiment 2

The infant rat's suckling for food may initiate nursing (Dollinger et al., 1980) and its behaviour may influence maternal responses (Grosvenor & Mena, 1974). Nonetheless, the dam is the rate-limiting determinant of her young's intake (Friedman, 1975), indirectly in the amount of time she spends with her young, and directly in the amount of milk she provides to the pups at any point in time. Thus, responses of the mother may confound naloxone action on food-intake. In addition, dam's milk ejections have been reported as intermittent and brief, in the order of 5-10 secs (Lincoln, Hill & Wakerley, 1973) and pups detect the arrival of milk presumably by a change in nipple tugor (Drewett et al., 1974) and withdraw it by extensor response (Lincoln et al., 1973). Hence, naloxone may reduce milk-intake by affecting the "detection" of milk via the induction of 'drowsiness' (Kyriakides et al., 1980) or the extensor response via sedation. Furthermore, the sightless infants depend upon olfaction to locate their mother and the source of the food (Blass, Teicher, Cramer, Bruno & Hall, 1977; Cheal, 1975), and opiate receptors are known to be densely positioned at the anterior olfactory nuclei early in ontogenesis (Kent, Pert & Herkenham, 1982). Naloxone administration could compromise this olfactory guidance by blocking the relevant receptors and thus affect feeding in the suckling situation.

To obviate the maternal contribution and clarify the mechanism of naloxone action, an artificial feeding technique designed by Hall (1979b) was used in this study. This technique allowed for milk to be delivered slowly through a cannula imbedded in the pups mouth. The pup could either mouth and swallow the food or reject it by letting it pour out of its mouth. In this way, ingestion could be studied following drug administration without the confounding effects of suckling. Moreover, the technique permitted the study of younger pups than did the suckling model. The aim of this experiment was to assess naloxone's effect on the consummatory component of feeding behaviour and to determine whether or not it was mediated by behavioural sedation.

Method

Subjects and procedure

Four age groups of pups, 3, 10, 12, and 14 days were studied (n=20 per group). The technique of cannula implantation for this study was similar to that reported previously by Hall (1979). Essentially, a polyethene tube (PE-10, Clay Adams, Parsippany, N.J.) was flanged at one end by a flame and its other free end was passed through the unanaesthetized subject's mouth, just behind the root of the lower incissors, with the aid of a piece of stainless steel wire, angled at 45 degrees. The free end of the cannula was subsequently attached through a larger polyethene tube (PE-50, Clay Adams, Parsippany, N.J.) to a 5 cc syringe.

The syringe was mounted on a Harvard Apparatus infusion pump (Model 600-910) which delivered warm half-and-half milk at rates of 1.2, 2.3, 4.5 and 4.5 cc per hr for 3, 10, 12, 14 day-olds respectively. The oral cannula was implanted 4-hours before testing. The rats were individually housed in a small cage containing fresh beddings placed on top of a 32°C incubator, since ambient temperature has been shown to affect intake volume in developing rats (Johanson & Hall, 1980). At the end of the 1-hr infusion, subjects were gently cleaned and reweighed.

Results

Naloxone reduced the amount of orally infused milk ingested when measured by intake or by a percent of body weight in 14 day-olds but had no discernible effect on 3, 10, or 12 day-olds (Figure 3) ($F = 0.54$, .64, 0.52, and 9.10 for intake in 3, 10, 12, and 14 day olds respectively; $F = 0.05$, 1.78, 0.54, and 6.47 for intake as a percent of body weight). All doses were effective in the 14 day olds as measured by Dunnett's test.

Figure 3 about here

Comparison of the control intakes (percentage body weight) in the two protocols (suckling versus infusion) revealed no significant differences: Mean for saline-suckling = 8.8 $\pm$ 1.7; mean for saline-infusion = 6.5 $\pm$ 0.3, $t(4) = 1.316$.

Discussion

These findings extend the earlier report of the suppressant effects of naloxone on the milk intake of 14 day olds, but not on the younger ones. The extent of the suppression of milk intake in 14 day olds by naloxone in this study was more than that observed in the 'suckling' paradigm, in which the consumption of food was reduced by approximately 28% by 5.0-10.0 mg/kg of naloxone, and more than 55% by 30 mg/kg. In the current study, reduction was 50% and 66% for 5.0-10.0 mg/kg and 30 mg/kg respectively.

This discrepancy may be the consequence of major procedural differences between the two studies. In the earlier study, the infants were tested in their home cages and with their natural milk. Infant rats may have a natural preference for their mother's milk rather than the artificial milk used in the current experiment. And since a minor surgery i.e. cannula implantation was involved in the latter experiment, this may also account for the difference. However, the control level of milk intake is comparable to the control value in the previous experiment, which suggests that neither the artificial milk nor the surgery may account for the discrepancy. Rather, it would seem that the mother may act as a substitute for the immature physiological control mechanism of the young, and, in this instance, might have attenuated the the full effect of naloxone.

The experiment also yielded two other main results. Firstly, since the olfactory system has been implicated both in

the feeding of the young (Tobach, 1977) and as the site of early appearing opiate receptors (Kent et al., 1982), the lack of naloxone effect in the young suggests that the intake reduction in 14 day olds may not be mediated through the receptors in the olfactory system. If the opiate receptor system is necessary for the behavioural effect of naloxone, then the latter suggestion finds support in the notion of anatomic heterogeneity. Secondly, the results also suggest that the suppressive effect of naloxone in 14 day olds is not secondary to a reduction in activity level, which is an important point to establish, since it has been demonstrated that feeding and arousal systems can interact in infants (Blass et al., 1979).

Experiment 3

Demonstration of naloxone's capacity to reduce the total amount of food consumed by adult rats has been used to suggest the participation of the endogenous opioid system in feeding behaviour. However, there is reason to believe this technique may not be optimal for producing precise information about naloxone action or for providing a glimpse into the mode of endogenous opioid mechanism regulatory control.

Firstly, a single measure of the weight of food consumed in a given time may conceal information about the manner in which naloxone inhibits food consumption. For example, an animal may fail to eat because of the non-specific disruption of any controlled sequential behaviour by competing or interfering

activities. Indeed, there are suggestions that alteration in general arousal may be a covariate of naloxone effect since decreases in spontaneous motor activity of adult rats have been found to follow naloxone administration (Green, Isaacson, Dunn Lanthorn, 1979; Roger & Deacon, 1979); whereas, general activity may contribute to intake volume in young pups (Blass et al., 1979). Moreover, the single measure of weight of food consumed fails to indicate whether naloxone is acting to inhibit the onset of eating, to slow the progress of eating or hinder its execution, or to terminate prematurely an eating episode. Secondly, following a period of deprivation, adult animals vary the rate of their food consumption over time; since naloxone, like most drugs, has a time course of action, measurement of the bulk of food consumed may disguise the temporal profile of naloxone action and consequent changes in feeding profiles.

The aim of these investigations was to detect possibly subtle changes in the action of naloxone not revealed by a simple measure of the amount of milk taken. In addition, a second purpose was to throw light on the mode of opioid peptide's food-intake control. Accordingly, feeding latency, feeding bouts and other behavioural components of feeding were observed in discrete time intervals during 1-hr tests. An examination of these qualitative aspects of feeding permits the possibile determination of whether naloxone-induced changes in feeding behaviour have been brought about by alterations in hunger i.e. suppression of onset of eating, or in satiety i.e. early termination of the initial bout of eating.

Method

Subjects and procedure.

Three age groups, 10, 12, and 14 days old were studied (n=24 per group). At the appropriate age, the pups were removed from their mother and put into the incubator. After the deprivation schedule and drug treatment previously described, they were tested. The feeding test was conducted in a small clear plastic cage (10 X 12.2 X 12.7 cm) placed in a a warm (32-34^{o}C) chamber. The cage floor was covered with a white towel onto which 30 ml, 35 ml or 40 ml of warm half-and-half milk was spread for 10, 12 or 14 day-olds respectively. The plastic cages were placed in the test chamber 1-hr prior to experimentation to attain the ambient temperature equivalent to the normal nesting body temperatures (36^{o}C) of the infant rats.

Latency to start feeding and the occurrences of mouthing were recorded. Mouthing was observed every 7.5 secs and was recorded as present when the subject dipped its snout/lip into the light pool of milk. Latency to start feeding was defined as the time before the occurrence of the first two successive instances of eating. For data analysis, an eating bout was defined as two successive instances of mouthing, since Kissileff (1970) has shown that, for normal rats with free access to food, meal distribution is independent of the criteria used to separate meals from each other.

Six other categories of behaviour were also recorded at 7.5 second intervals: resting, twitching or grooming, fore-limbs-assisted probing (the posterior end of the pup remains stationary as the pup scrabbles with its front paw), locomoting, cage-wall climbing, and jumping or tumbling. Each behavioural category was rated in the order indicated above on a 6-point scale (0 through 5) according to the system developed for studying ingestion in young rats (Hall, 1979). For data analysis, the 1-hr test was divided into six 10-min segments.

Results

Intake

The effect of naloxone on licking is presented in Figure 4. Only after 14 days of age did naloxone inhibit feeding. ($F = 0.386$, 1.41, and 16.43 for 10, 12, and 14 day-olds respectively; $F = 0.23$, 1.57, and 15.33 for the same ages for intake as a percent of body weight). Again, posthoc Dunnett's test showed that, at 14 days of age, all doses significantly decreased intake using both measures.

Figure 4 about here

Comparison of the control intakes (percentage body weight) under suckling and licking paradigms revealed no significant difference: Mean for saline-suckling = 8.8 ± 1.7; mean for

saline-licking = 11.0 ± 1.0, t(7) = 1.05. Analysis of variance performed on the control intakes in the three paradigms also revealed no difference: $F(2,7) = 2.9$, $p < 0.12$.

Latency to feed

Mean latencies for rats 10, 12, and 14 days of age injected with either saline or various doses of naloxone are presented in Table 2. Naloxone had no consistent effect on the latency with which feeding occurred at any age or at any drug dose. Analysis of variance revealed no significant effect of naloxone at which feeding started: $F(3,15) = 0.59$, 0.84, and 1.91 for 10, 12 and 14 day-olds respectively.

Table 2

MEAN LATENCY (IN SEC) TO FIRST FEEDING BOUT

Dose	Age 10	12	14
Saline	48.1	54.5	125.0
5.0	3.8	24.8	60.0
10.0	34.8	16.7	75.0
30.0	48.1	47.0	288.8

Mouthings/Feeding bouts

Analysis of variance, performed on the total number of mouthings with drug dose and time as factors, revealed a significant dose effect only at 14 days of age, ($F = 2.35$, 0.10, and 10.61 for the dose effect at 10, 12, and 14 days). Results are presented in Table 3. Dunnett's tests demonstrated that at

14 days of age all doses significantly reduced mouthings ($p < 0.01$ in all instances).

Table 3

EFFECT OF NALOXONE ON MOUTHING

	Age		
	10	12	14
Saline	5.0 ± 0.8	7.1 ± 0.9	9.5 ± 1.0
5.0	4.5 ± 0.9	6.2 ± 0.9	3.5 ± 0.7 *
10.0	4.4 ± 0.7	7.1 ± 0.8	5.5 ± 0.9 *
30.0	4.6 ± 0.8	6.4 ± 0.8	3.4 ± 0.5 *

*Significantly different from saline control values $p < 0.05$. Entries are the mean ± SEM per 10 minute period.

The two successive mouthing criteria for separating bouts of eating resulted in a clearly defined feeding profile for 14 day olds. The data of number of feeding bouts/5 min across the 1 hour feeding period conformed to a 4 by 12 factorial design, having repeated measures, with 4 levels of drug treatment and 12 factorial levels of time. An analysis of variance of these data yielded a reliable effect due to doses, $F(3,9) = 12.05$, $p < 0.002$; a reliable effect across the 12 periods, $F(11,33) = 2.13$, $p < 0.05$; but no reliable interaction, $F(33,99) = 0.87$. Figure 5 shows the temporal profile of naloxone effects. Naloxone's suppressive effect is initialed early and maintained throughout the 1-hr observation, with major changes occurring approximately 15 minutes post-injection. For the three doses of naloxone, feeding is initiated normally and then curtailed with the initial bout of eating approximately proportional to the dose injected.

Figure 5 about here

Activity.

Activity was measured as a composite of the six behaviours (see General Methods) and analysed by a two-way analysis of variance for each age (Dose X Block of time). There was a significant dose effect only at 14 days of age ($F = 1.17$, 0.82 and 4.35 for 10, 12, and 14 days respectively) and in no case a dose by time interaction (Figure 6). Dunnett's tests demonstrated that, at 14 days of age, all doses significantly enhanced activity ($p < 0.01$ in all instances). Table 4 presents the data.

Table 4

EFFECT OF NALOXONE ON ACTIVITY

	Age		
	10	12	14
Saline	5.3 ± 1.1	19.5 ± 3.7	22.0 ± 3.0
5.0	6.1 ± 1.2	20.3 ± 3.0	34.0 ± 4.0 *
10.0	8.6 ± 1.3	24.1 ± 3.4	31.8 ± 3.0 *
30.0	9.2 ± 1.3	25.5 ± 3.7	45.6 ± 3.8 *

*Significantly different from saline control values $p < 0.05$.
Entries are the mean ± SEM per 10 minute period.

Figure 6 about here

Discussion

The results of the last experiment confirm and extend the findings of the two previous ones that naloxone's food suppressant effect is evident only two weeks postpartum. Milk intake of the 14 day old test subjects was reliably decreased in a dose-dependent manner. The suppression appears to be a robust effect one that is relatively independent of variations in experimental protocol. The extent of intake reduction in this study was equal to that obtained under 'infusion' paradigm. In the current experiment, 5.0-10.0 mg/kg of naloxone reduced intake by 50%, while 30 mg/kg reduced it by 65%.

The experiment also revealed that the naloxone has no effect on latency and thus did not postpone the onset of feeding, but appeared to modulate the initial bouts of feeding and further reduce feeding bouts with time. It seemed possible that this delayed effect of naloxone was simply due to the rate of uptake. The use of intraperitoneal route of administration in the present experiment may have inadvertently reduced the bioavailability of the drug. The fact that naloxone is lipophilic in nature and is rapidly diffused upon administration (Kaufman, Semon & Koski, 1975) suggests that the latter cannot account for the lack of naloxone's effect on feeding latency. Since there was no appreciable difference between the feeding bout profiles for the three doses of naloxone, and no difference in the latency of eating, it would appear that a certain amount of food must be consumed in order for the inhibitory action of naloxone to take

effect. This suggestion raises the possibility that naloxone exerts its inhibition over feeding by enhancing feedback signals from food consumption. This possibility would be consistent with the finding that intraventricularly administered endogenous opioid takes 22 minutes and intrahypothalamic administered morphine takes 30-120 minutes to initiate feeding in free-feeding or satiated adult rats. Since the phenomenon in which feeding is terminated or modulated by the consequences of food ingestion is commonly termed satiation, naloxone may act to promote satiety. Secondly, all three doses of naloxone increased activity in 14 day olds, but had no effect on younger animals, which suggests that naloxone may activate non-appetitive behaviour.

GENERAL DISCUSSION

The above results reveal that opiate antagonist naloxone does not affect food intake early in ontogenesis, but commences its food-suppressant effect two weeks postpartum.

Under three models of milk availability, it was discovered that intraperitoneal administration of naloxone had no effect on deprivation-induced milk intake of 3, 10, or 12 day old rats. The infants responded to deprivation by suckling their natural mother until satiated or until the mother ceased lactating (Experiment 1), by swallowing milk delivered freely to their mouths (Experiment 2) or by licking milk off the floor when it was made freely available (Experiment 3). Under similar conditions, naloxone inhibited the food intake of 14 and 19 day old rats.

The intake reduction following naloxone administration in 14 and 19 day olds is not likely to be due to the nauseant or toxic action of the drug. This is apparent because the drug did not incapacitate the rats sufficiently to compromise their search for and location of ingesta, since latencies to begin eating were never longer in the drug treated animals than in the controls (Experiment 3). Furthermore, the drug did not reduce intake in 3-, 10-, and 12-day-old rats, which are much more vulnerable to a variety of stresses than the older animals studied here. It is not likely that special receptors mediating malaise develop in parallel with those mediating intake.

These data suggest that the naloxone effect may be specific to food reduction and that the absence of such an effect in 12 day-old or younger rats indicates age-specific changes in the mechanism which mediates this effect.

It has been suggested that the existence of a receptor system is a perquisite for drug action. Indeed, receptors for narcotics have been marked by naloxone (Coyle & Pert, 1977) or naltrexone (Clendeninn, et al., 1976), and have revealed developmental changes with anatomical (Kent et al., 1982; Tsang & Ng, 1980) and functional (Pasternak Zhang & Tecott, 1980) peaks occurring two weeks postpartum.

Consistent with the view that this naloxone food suppressant effect is mediated by antagonist action at the opiate receptors, it was found that naltrexone produces effects similar to naloxone. While it had no effect on the milk intake of 10 and 12

day olds, it had a dose-related food-suppressant effect in 14 day-old and older rats, in accord with other studies of adult rats (Maickel et al., 1977). Although the existence of a relationship between dose and food-reduction over the dose range used is not unequivocal evidence of a specific receptor interaction, it is an expected consequence of such interaction. Moreover, the comparable ED50 doses of naloxone obtained suggest the existence of a common link between the varied feeding conditions, which may be opiate receptor based.

These observations support the contention that naloxone attenuates food consumption by interacting with normal control of feeding. Since opiate antagonist blocks B-endorphin induced feeding but not feeding induced by intrahypothalamic norepinephrine or muscimol (Grandison & Guidotti, 1977), it has been suggested that the effects of opiate antagonist on feeding behaviour may be a function of the action of this drug on endogenous opioid systems. To assess the pattern of its mode of control, measurement of food intake parameters has revealed that naloxone may induce an early cessation of the feeding act, since it did not have any effect on the onset of eating, and may thus function to block an endorphin-mediated mechanism which inhibits satiety.

Consistent with the view that the opioid system may serve as an anti-satiety control, it has been found that direct administration of B-endorphin into the ventromedial hypothalamus stimulated food intake in satiated animals (Grandison & Guidotti,

1977). B-endorphin induced feeding was blocked by the administration of the opioid antagonist naltrexone. The medial hypothalamus is one of several areas of the CNS with a high concentration of opiate receptor sites (Kuhar, Pert & Synder, 1973) and the ventromedial hypothalamus is viewed as a satiety center, since destruction of this area of the CNS leads to profound hyperphagia and obesity in a variety of animals (e.g. Hetherington & Ranson, 1942; Powley, 1977). It is possible that the function of the VMH may be properly ascribed to the endogenous opioid system and that immaturity of the system in the infant may thereby account for the lack of naloxone effect in 12 day old or younger animals. Indeed, opiate receptor development has been found to follow a caudal-rostral pattern of development. At one week postpartum, dense naloxone binding occurs only in the spinal cord, medulla pons, and midbrain, while increases become apparent in the midbrain, hypothalamus, and striatum from 14-21 days (Bardo et al., 1981; Tsang & Ng, 1980).

The absence of a functional 'satiety' system may at first appear to contravene teleological reasoning. However, there is ample evidence that the lactating rat plays a significant role in the feeding of the young and is the rate-limiting factor in the amount of milk consumed by the young. This evidence, combined with the fact that infants spend a good part of their time sleeping, ensures that only a finite period is devoted to eating. Indeed it has been reported that any manipulation which increased arousal in the young also increased intake (see Friedman, 1975

for review); and 9-13 day old pups allowed unrestricted access to food consumed a large and inappropriate volume of diet irresponsive of pathological stomach distention (Blass et al., 1979). Thus it appears that the absence of endogenous opioid role in the feeding of the young may not signify its irrelevance to species survival, but may rather ensue from the evolution of the species that has designated the opioid system's early role in lactating rats.

While the data of this study are compatible with the hypothesis that naloxone suppresses feeding behaviour in the rat by interfering with the activity of endogenous opioid system, definitive evidence in support of this theory is lacking. A study of structure-function relationship may therefore help to unveil the strength of this theory, and this task was the aim of the second part of this research.

PART 2: ON THE POTENTIAL RELATIONSHIP BETWEEN ENDORPHINERGIC SYSTEM'S DEVELOPMENT AND FOOD INTAKE.

GENERAL INTRODUCTION.

Endogenous opioids are thought to produce their biologic effects by first binding to receptors on the target cell (Simon & Hiller, 1978), and one consequent effect of this interaction is the regulation of feeding behaviour (Grandison & Guidotti, 1977). Indeed, naloxone's food suppressant effect is attributed to reduced ligand/receptor interaction. Thus, chronic environmental intervention in opioid/receptor interaction may produce long-term alterations in behaviour. Intervention restricted to the developing organism may be more heuristic in evaluating this relationship, since it is generally known that alterations occur in structure in the process of normal growth of the brain which may be correlated with the emergence and modification of behavioural function. Studying the functional maturational processes of altered development may permit a proper evaluation of the opioid system/food intake relationship.

Brain development in the rat encompasses prenatal and postnatal periods (Zagon, 1975), and studies on the ontogenetic development of the endogenous opioid systems have revealed similarly two developmental epochs. Clendeninn, Petraitis & Simon (1976) using binding technique and naltrexone as a ligand, found that receptors could be detected in the brain of the 14 day rat fetus, but that the receptor population was 6-8 fold lower than that of the adult rat, when expressed per mg protein. Coyle

& Pert (1977) did similar work using naloxone as ligand, and reported that the (3H)naloxone binding increased 200 fold between the 15th day of gestation and birth, so that the density of the whole brain receptors at birth was 40% of adult level. Postnatal increases are small during the first week, but show a 16 fold increase from day 14 through postnatal day 21, decreasing thereafter until adulthood (Clendeninn et al, 1976; Coyle & Pert, 1977). Opiate receptors marked by enkephalin have been reported to appear at embryonic day 20 and to follow a gradual time-course of appearance (Kent et al., 1982). Tsang & Ng (1980), in their studies of postnatal development of the met-enkephalin receptors in the forebrain, cerebellum and brainstem of the rat, noticed a slight increase in the brainstem during the first week, a 5-fold increase at day 14 postpartum, and a decline to adult level at day 20. The forebrain did not begin to show an increase until day 7, reaching adult level at day 17 postpartum. The cerebellum showed its greatest level on day 4, but declined thereafter to adult level.

While most investigators did not observe any changes in the affinity of the receptors with increasing age, Pasternak et al (1980) reported postnatal differentiation of high and low affinity receptors that correlated respectively to morphine analgesic and respiratory depression properties. They found that the high affinity receptors increased by 3 fold between days 2 and 14 while in the same period low affinity receptors did not change, having reached a high level perinatally.

Investigators studying the development of endogenous opioid peptides have also reported prenatal and postnatal changes in the neurochemicals. Bayon et al. (1979), using radioimmunoassay, reported that endorphin immunoreactivity could be detected as early as embryonic day 13 and revealed marked increases thereafter, such that by embryonic day 16 it assumed a regional distribution resembling the adult pattern. From day 17 to postnatal day 5, it showed only modest increases, but thereafter it revealed several fold increases in all brain regions. In contrast, enkephalin is not detectable until embryonic day 16, with a regional distribution unlike that of the adult. However, the absolute rates of increase of enkephalin immunoreactivity which are much lower than those of endorphin before birth, increase several fold for the perinatal period. Patey et al (1980) examined the postnatal development of enkephalin in the rat using liquid scintillation spectrometry and found that met- and leu-enkephalin concentration exhibit an 11 fold increase between birth and 21 days, when they reach the adult level. Similar postnatal increases were reported for the 'enkephalinase', enkephalin-degrading enzyme, with the same time-course paralleling the changes in the enkephalin levels and receptor population. Of particular interest in these studies is the fact that the caudal-to-rostral pattern of development decribed for neuroanatomy, neurophysiology and neurochemistry (Jacobsen, 1978) also applies to endorphinergic system's development.

Therefore the foetal and early postnatal periods in rats may be critical stages in the functional maturation of endogenous opioid system. Exposure to opiates during either of these periods may induce alterations in development which may correlate with later changes in feeding behaviour. In the studies reported, developing rats were exposed to opiates indirectly during gestation (Experiment 1) and directly during the early suckling period (Experiment 2) and tested for response to naloxone-suppressant effect at the appropriate age.

Since addiction to drug following indirect (Kandall, 1977) or direct (Blasig, Herz, Reinhold & Zieglgansberger, 1973) exposures is a property which is also common to narcotics, this study also observed the occurrences or absences of narcotic-withdrawal signs (e.g. tremors, cyanosis) resulting from termination of drug-pretreatment or precipitated by naloxone test drug in order to isolate naloxone's food-related effects from narcotic effects.

GENERAL METHOD

Animals:

Female Long-Evans rats (Hunter College Colony, New York) weighing 250 to 275 g were housed individually in cages in a temperature-controlled colony room with an automatically timed cycle of 12 hours of light and 12 hours of darkness. Purina Laboratory Chow (Ralston Purina Company, St. Louis, Mo.) and tap water were continuously available. Mating was accomplished by

pairing a female with a male of known fertility from the same stock for a 5- to 7-day period. The finding of sperm in vaginal smears was taken as a sign of copulation. At this time (designated as the first day of pregnancy), the males were removed and the females were either randomly assigned to control and treatment groups (Experiment 1) or left undisturbed for breeding purposes (Experiment 2). Maternity cages were inspected twice daily during the light hours and the day on which a litter was first observed was designated day 0.

Evaluation of Feeding Behaviour:

Infant rats were tested for their responsivity to naloxone (1.0 mg/kg, 5.0 mg/kg) food-suppressant effect using the 'licking' paradigm previously described. The dose range employed in this study was chosen as a tool for examining any changes which might have occurred in the endorphinergic system, since previous studies in adults have found that opiate-pretreatment may alter the receptor population (Davies, Akera & Brody, 1975) efficacy of naloxone for these receptors (Tulunay, & Takemori, 1974). To reduce experimental bias, all the drugs used in these studies were assigned individual codes. The experimenter was "blind" as to the identity of the treatment drugs and the test drugs.

The test groups of offspring used in Experiment 1 were derived from an initial maternal pool of 16 pregnant rats and 160 pups. The test groups of infants used in Experiment 2 were derived from 40 pregnant rats and 242 pups.

Evaluation of Withdrawal Signs

Withdrawal sign or gross behaviour, as defined by Villarreal and Karbowski (1973), was considered to be present when rats exhibited continuous tremors, muscular rigidity, irritability, clutching or biting persisting for at least 1 minute together with inhibition of normal feeding activities (licking, grooming, probing). No attempt was made to grade the intensity of this behaviour, the latter having been found to be subjected to great observational errors (Wei, 1973; Wei, Loh & Way, 1973). The presence or absence of any of the signs was simply noted.

Drugs.

The following drugs were employed in this study: morphine sulphate (Eli Lilly & Company, Indianapolis, Ind.), naltrexone hydrochloride and naloxone hydrochloride (Endo Laboratories, Garden City, N.Y.). All drugs were were dissolved in sterile saline (0.9%) and diluted to the appropriate concentrations. Drug doses were expressed in terms of salts. Injections were made subcutaneously for the pregnant rats, 1 ml/kg (Experiment 1) and for the infant rats (Experiment 2) in a volume of 1 ml/100g body weight. Drug solutions were replaced at approximate 14 day intervals as necessary.

Analysis of Data

Milk intake, expressed as grams of weight gain or as percentage of body weight, was analysed at each age using a two-way analysis of variance, with Pretreament-drug as between group

variable and Naloxone-doses (test drug) as within group variable. Subsequent tests between controls and experimental groups were made using the Newman-Keuls test. Body weights were also analysed at each age using a two-way analysis of variance with Pretreatment drug as between group variable. The chi-square test was used to analyse frequency data such as mortality rates (Tables 5 and 7). All other comparisons were made using Student's t test for paired observations. Drug effects were considered significant if the P value was less than 0.05.

Experiment 1

It is well established that the developing embryo is most susceptible to environmental agents during the period of major organogenesis (Murphy, 1965). Thus, in a given period, the structure at its maximal differentiation and growth is most susceptible relative to other structures. Since some opiate receptors and opioid peptides are known to appear earlier than others, prenatal exposure to morphine may induce alterations in their development with concomitant changes in their postnatal functions.

Historically, morphine alkaloid has been used to induce changes in the endophinergic system (e.g. Friedler, 1974; see Kornetsky, 1970 for review), because of the similarities in the pharmacodynamic, pharmacokinetic properties and biologic effects of morphine and endogenous opioid peptides. When morphine is administered (intravenously or subcutaneously) to the intact rat, it concentrates on microscopic sites in the brain which contain

opiate receptors (Hollt & Herz, 1978), and is eliminated slowly with a half-life equivalent to the half-life of receptor-dissociation, while being rapidly depleted from the brain tissues not containing significant amounts of opiate receptors such as cerebellum and serum (Perry, Mullis, Oie & Sadee, 1980). Moreover, cross-tolerance between morphine and Met-enkephalin occurs with respect to dependence maintenance (Bhargava, 1980; Tortella & Moreton, 1980), and between morphine and B-endorphin with respect to analgesia (Blassig & Herz, 1976; van Ree, de Wied, Bradbury, Hume, Smyth & Snell, 1976). This suggests that the same mechanisms are responsible for the effects of endogenous and exogenous opioids on some behaviours.

When injected into the pregnant rat, morphine crosses the placenta readily (Blane & Dobbs, 1967; Sanner & Woods, 1965; Villee, 1965), enters foetal circulation (Kirby, 1979a), is retained by the foetal brain cells following maternal administration (Steele & Johannesson, 1975), and may function as a potent behavioural/biochemical teratogen specific to the endophinergic system.

Developmental observations have shown that chronic prenatal morphine administration has little effect on morphological development (Kirby, 1979b), skeletal structures (Johannesson & Becker, 1972) somatic growth (Kirby, 1980) or reproductive success of the offspring at low doses (O'Callaghan & Holtzman, 1976), but compromises infant viability or survival rate at high doses (Davis & Lin, 1971; Zagon & McLaughlin, 1977a, b).

Studies of the behavioural consequences of prenatal exposure to morphine have focussed mainly upon the alterations in the post-weanling animal's behaviour, especially morphine-induced analgesia and emotionality. Mature offspring of rats treated before or during gestation exhibit attenuated responsiveness and long-term tolerance to the analgesic effect of a single dose of morphine in a hot-plate test (Friedler, 1974; Johannesson & Becker, 1972). In addition, measurements of the dose-response and time-effect relationships of the analgesic characteristics of morphine in 21-, 35- and 77-day-old offspring of morphine-treated dams have revealed age-related differences. Analgesia decreases faster in younger rats (21 days) than in older animals, and greater doses of morphine are required in adults to produce a degree of analgesia comparable to that obtained in 21-day-olds (O'Callaghan & Holtzman, 1976).

Hyperactivity, as measured by the open-field technique, has also been reported to result from early exposure to morphine. Davis and Lin (1972) have demonstrated that offspring of rats treated with increasing doses of morphine reaching 45 mg/kg/day during gestation exhibit high motility scores at 30 and 70 days after birth. Sobrian (1977) replicated this finding and extended the observation to include preweanling offspring. He found that rat pups of morphine treated mothers showed normal spontaneous motor activity between birth and 10 days of age, but that after this time they became increasingly more active than the controls, with significant increases in activity level occurring during the third and fourth postnatal weeks.

Studies investigating the subtle changes in the nervous system that relate to these behavioural modifications have reported alterations in areas with a dense opiate receptor population. Tsang and Ng (1980) demonstrated that treatment of rats with morphine hydrochloride (10 mg/kg i.p.) before and during pregnancy induced an increase in (3H)Met-enkephalin receptors in the cerebellum, brainstem and whole forebrain of the offspring when assessed in the first week postpartum. Kirby (1980) found a reduction in the volume of the foetal rat spinal cord following prenatal morphine treatment. The spinal cord of the adult rat is known to have a dense opiate receptor population (Atweh & Kuhar, 1977).

If endogenous opioid receptors have a role in nociception and a role in feeding behaviour as reported, then feeding behaviour may be susceptible to alterations similar in scope to that reported for analgesic response following antenatal morphine treatment. That there exists a commonality in the mechanisms underlying analgesia and feeding has been demonstrated. Twenty four hours food deprivation increases the analgesic threshold (Bodnar, Kelly, Spiaggia & Glusman, 1978), and naloxone tends to normalize the threshold (McGivern, Berka, Berntson, Walter & Sandman, 1978) and reduce compensatory overeating in rats (Cooper, 1980). In addition, stress induces an increase in B-endorphin activity (Margules, 1979) and feeding (Rowland & Antelman, 1976) and naloxone inhibits this feeding (Lowy et al., 1980; Morley & Levine, 1980). An anorexic agent, such as

amphetamine, is ineffective in this regard (Antelman, Caggiula, Black & Edwards, 1978).

In this present experiment, preweanling offspring of rats treated during gestation with morphine were given a single injection of naloxone at different age periods and subsequently assessed for milk-intake in order to examine the potential relationship between opiate receptor development and feeding behaviour. Since high motility has been reported to result from prenatal morphine exposure, another aim was to examine if changes in activity covaried with naloxone-food suppressant effect.

Subjects.

Groups of gravid rats with body weight of about 250g were injected subcutaneously into the dorsal side of the neck twice daily at 10-hr intervals with morphine sulphate 7.5 mg/kg or like volumes of saline from day 5 of gestation until parturition. This schedule was chosen in order to provide for an *in utero* exposure of the embryo to morphine during the period of rapid organogenesis. (Murphy, 1965). The dosage represented the mean of the increasing dose regimen which had previously been shown to cause changes in postnatal reaction to pain and analgesic agents (O'Callaghan & Holtzman, 1976). Body weights were recorded every morning prior to injection. The control groups were treated identically except that saline was administered instead of morphine.

The number of offspring in each litter was recorded within 12 hours of delivery and pups found dead were recorded as

stillbirths. At this time, 9 randomly-selected pups were removed from their mother, weighed, and assigned to a surrogate mother that had been timed to deliver within the preceeding 24 hours. The fostering technique was implemented to insure that any observed behavioural changes in the pups resulted from prenatal exposure to morphine and not from morphine-induced changes in postnatal maternal behaviour (Way & Adler, 1962).

Infant rats at 10-, 12-, 14-days of age, 3 pups per litter, were assigned to developmental evaluations using naloxone and testing for milk consumption over a 1 hour session using the licking paradigm. The offspring of the saline- and morphine-treated females will hereafter be designated as saline- and morphine-offspring.

Results

Gross Behavioural Measures

Initially, the pregnant rats administered morphine exhibited some loss of weight as well as gross behavioural excitation, including extensive grooming, rearing, sniffing, eating and stretching. However, some of these characteristic effects of morphine (e.g sniffing, stretching) disappeared by the 10 day of drug administration. At term, no difference was observed in body weight between morphine and saline treated animals. There were no prenatal or postnatal maternal deaths. Eight of the 9 (88.9%) morphine treated females and 6 of the 7 (85.7%) saline-treated females who were sperm positive gave birth to viable litters. No significant incidence of stillbirths was found.

The perinatal effects of prenatal morphine administration are listed in Table 5. No differences in litter size or perinatal mortality were found between morphine and saline groups, but significantly greater neonatal mortality (expressed as a percent reduction in offspring) occurred during the first 10 days. Prior to testing, no further deaths were recorded, and no obvious or significant abnormalities were observed in either group.

Table 5

PERINATAL EFFECTS OF PRENATAL MORPHINE ADMINISTRATION TO THE RAT

	MORPHINE	SALINE
Percent Females delivering Offspring	88.9	85.7
Mean Number of Offspring per litter	11.25 ± 0.5	12.33 ± 1.2
Total # of live births	86.00	75.00
Mean Birth weight in grams	5.65 ± 0.1	6.45 ± 0.3
Percent infant mortality		
Stillborn	1.44	1.35
Day 2-10	8.14*	0.50
Day 10-14	0.00	0.00

* Significantly different from offspring of saline-treated females, $p < 0.001$

The possibility that the cause of high mortality in morphine-offspring may have involved two of the foster mothers

was considered and tested by fostering extra pups from saline-groups with these dams, three pups to a dam. All six pups were not viable when observed at day 1 post-fostering and were completely moribund on day 2. The two dams were considered "rejecting" mothers and excluded.

The body weights of morphine-offspring and their saline-control did not differ significantly at birth or at postnatal days 10, 12 and 14, when they were determined (Table 6).

Table 6

NEONATAL BODY WEIGHT (IN GRAMS) OF MORPHINE- AND SALINE-OFFSPRING VALUES LISTED ARE MEANS ± SEM FOR MALE AND FEMALE PUPS COMBINED.

Age (days)	Morphine	Saline
10	22.40 ± 1.13	23.12 ± 0.68
12	27.97 ± 1.52	28.48 ± 1.20
14	35.45 ± 1.75	35.02 ± 1.73

Intake

The grams of weight gain were submitted to a 2 X 3 analysis of variance for each age with a factor for pretreatment drugs and a factor for two doses of naloxone plus saline. There was no effect due to morphine-saline pretreatments: $F(1,9) = 0.07$, $p > 0.05$; various doses of naloxone: $F(2,18) = 0.0378$, $p > 0.05$; or morphine-saline by naloxone interaction: $F(2,18) = 0.48$, $p > 0.05$ at 10 days of age.

At 12 days, there were also no significant effects of morphine-saline pretreatments: $F(1,9) = 3.49$, $p > 0.05$; naloxone

doses: $F(2,18) = 0.31$, $p > 0.05$; or morphine-saline by naloxone interaction: $F(2,18) = 1.19$, $p > 0.05$.

At 14 days of age, this ANOVA yielded no significance for the factor associated with morphine-saline pretreatments: $F(1,10) = 0.88$, $p > 0.05$. There was, however, a reliable dose-effect with subject taking less with increasing dose: Mean for control = 3.742 g; mean for the low dose = 2.39 g; mean for the high dose = 2.02 g; $F(2,20) = 49.92$, $p < 0.001$. Employing the Newman-Keuls test of significant ordered pairs with probability level of 0.05 a clear dose relationship was demonstrated with a consistent decrease in the slope across the doses (Fig. 7).

There was a reliable pretreatment by test drug interaction: $F(2,20) = 5.55$, $p < 0.01$. Further analyses of the scores of the interaction indicate that naloxone reduced consumption compared to vehicle controls in both morphine- and saline-offspring: $F(2,30) = 51.5$, $p < 0.001$ for the morphine-offspring, $F(2,30) = 13.51$, $p < 0.001$ for the saline-offspring. However, naloxone-induced food reduction was greater in morphine offspring than saline offspring. Food intake in morphine offspring was reduced to 41% and 21% of saline control values by 1.0 mg/kg and 5.0 mg/kg respectively for morphine offspring, and for saline offspring it was 71% and 57%. Thus the dose of naloxone that reduced food intake to 50% of the control intake (ED50) was 1.0 mg/kg for morphine offspring and more than 5.0 mg/kg for saline offspring. Test of simple main effects revealed that at the high dose, gram intake in morphine-offspring was significantly less

than for saline-offspring ($p < 0.025$). No differences were observed between the morphine- and saline-group control intake.

When scores were transformed into grams consumed/grams of body weight before feeding and analysed, the same factors emerged as reliable source of variance.

Figure 7 about here

Activity

Activity was measured as a composite of the six behaviours (see Methods) and analysed by a three-way analysis of variance for each age and treatment (Dose X Block of time X Treatment). At 10 days of age, the ANOVA revealed no effect due to drug-pretreatment, $F(1,10) = 0.95$; doses of naloxone, $F(2,20) = 2.35$; naloxone by drug-pretreatment interaction, $F(2,20) = 1.10$; time-interval, $F(5,50) = 1.72$; interval by group interaction, $F(5,50) = 0.44$; naloxone by interval interaction, $F(10,100) = 0.54$; and naloxone by interval by group interaction, $F(10,100) = 0.67$. A similar repeated measures ANOVA performed on the activity of 12 day-olds saline- and morphine-offspring resulted in the same nonsignificant main effects, but a reliable time-interval effects: $F(5,50) = 15.04$, $p < 0.001$ and a slight effect due to interval by group interaction, $F(5,50) = 2.29$, $p < 0.059$. Similar patterns emerged at 14 days: $F(1,8) = 0.97$ for effect due to drug-pretreatments; $F(2,16) = 2.54$ for effect due to naloxone

doses; $F(2,16) = 2.83$, for naloxone by pretreatment interaction; $F(5,40) = 24.39$, $p < 0.001$, for time-interval effects; $F(5,40) = 0.70$, for the effect due to interval by group interaction; $F(10,80) = 1.22$ for the effect due to naloxone by interval interaction; and $F(10,80) = 1.92$, $p = 0.054$ for naloxone by interval by group interaction.

Discussion

Administration of 7.5 mg/kg morphine twice daily during the last two trimesters of gestation had no demonstrable effect on the development of the neuronal substrate mediating naloxone effect. No food intake reduction followed naloxone administration to 10 and 12 day old offspring of morphine or saline treated mothers. Naloxone reduced food intake only at 14 days postpartum, and in a dose-dependent manner. The dose-response relationship for the suppression of food intake by naloxone is very similar to the one previously established under the same experimental paradigm (Part 1, and Aroyewun & Barr, 1982), and indicates that the dose-dependent suppressant effects of naloxone on the 14 day olds' milk intake is a replicable phenomenon.

The apparent immutability in the ontogeny of the neuronal substrate mediating naloxone anorexigenic action may be due to the competitive antagonism (Goldstein et al., 1974) of residual morphine or its metabolites which are still bound to the tissues of the 10 and 12 day olds. This possibility is unlikely, however, considering the low drug regimen used in this study,

that the animals were tested 10 or 12 days after the termination of drug administration, the fact that the half-life of morphine is less than 6 hours, at least in adults (Jaffe & Martin, 1980). Moreover, morphine pretreatment did not alter the ontogeny of naloxone-associated activity effect, as activity was unaffected in 14 day old morphine offspring compared to saline-offspring. This would suggest differential effects of morphine on the substrates mediating food intake and activity.

Morphine pretreatment, however, altered naloxone dose-response curves for anorexia in the 14 day-olds, and thus the efficacy of naloxone. Food-intake responses thus appeared to be a sensitive index of the antagonist effects of naloxone in morphine-pretreated animals, since the lowest dose produced more than a 50% reduction in food consumed. Although teratogenic effects of morphine have been reported (Arcuri & Gautieri, 1973; Harpel & Gautieri, 1968), the behavioural changes in the responsivity of morphine offpsring to naloxone occurred in the absence of gross malformations. The effect is apparently of prenatal or early postnatal origin as morphine-treated offspring were fostered at birth to non-drugged mothers.

Since naloxone food-suppressant effect is stereoselective (Sanger et al., 1981) and therefore involves receptor mechanisms, and since the resultant effect of receptor occupancy may be proportional to the number of receptors occupied (Flier, 1981), it is possible that the change in naloxone efficacy may reflect either a structural or a quantitative change in the receptors for

the opiate antagonist. The Tsang and Ng (1980) study is suggestive. They found that prenatal morphine administration resulted in an initial increase in the Met-enkephalin receptors one week after termination of treatment i.e. after parturition, followed by a rapid decline from day 10 to day 30 postpartum, with the lowest binding at approximately 14-15 days of age. This period of decline parallels the time in which the saline treated groups registered their highest receptor growth.

However, this interpretation raises problem for the ontogenetic alteration observed in naloxone-associated activity effect. By the latter account, naloxone should increase activity significantly in 14 day old morphine offspring, since the receptor population has now been reduced. That the converse was observed may be indicative of the receptor heterogeneity that has been postulated for opiates. Indeed, the receptors in the hypothalamus, one of the areas in the brain that may function to control feeding behaviour, are known to exhibit mild increases prenatally (Coyle & Pert, 1977). However, the spinal cord, an area with possible role in activity which is also known to contain opiate receptors in its substantia gelatinosa (Atweh & Kuhar, 1977), develops more precociously than the brain. Thus it is possible that both receptor subpopulations may have come under the influence of morphine at different time in ontogeny, and thus may proliferate at different rates or in different quantities as a function of early stimulation by morphine.

Another possibility for the lack of alteration in the ontogeny of endorphinergic system mediating food intake may be that normal receptor development is contigent upon the exposure of developing receptor cells to endogenous opioids, which would be consistent with the idea that the morphine receptor (mu-receptor) may be a postsynaptic receptor (Pert, 1981). Since morphine can both activate the receptors as well as decrease the level of endogenous opioid, it may restrict the access of the endogenous opioids to their receptors and thus delay their development.

Whatever the mechanism for this change, it can, in light of these results, be concluded with confidence that the effects of feeding are due to engagement of opioids at opioid receptors, i.e. there is pharmacological specificity. With somewhat less confidence, it can be concluded that the effect is due to receptors that show marked increases in number and/or affinity during the postnatal period.

EXPERIMENT 2

The maturation of brain opiate receptors is a major determinant of some opiate effects. The ontological development of opiate receptors has been assessed in the brain of rat for both the total increases in receptor population (Coyle & Pert, 1977) and in terms of specific changes in the receptor-type (Pasternak et al., 1980). Studies concerned with receptor-type have reported that the rat is not born with a full complement of opiate receptors (Simon & Hiller, 1978). Naltrexone (Clendeninn

et al., 1976) or morphine binding (Pasternak et al., 1980), which is an indication of ontological development of opioid receptors, increases rapidly during the postnatal preweanling period, with marked peaks occurring the first two weeks postpartum (Pasternak et al., 1980; Tsang & Ng, 1980). These increases correlate with pharmacological and behavioural efficacy of narcotics for these receptors (Auguy-Valette, Cros, Goudarderes, Gout & Pontonnier, 1978), which suggests that treatments during the preweanling period which may alter opiate receptor ontogeny and concomitantly, endophinergic food intake mediation.

In adult animals it has been reported that chronic administration of morphine decreases receptor binding (Davis, Akera & Brody, 1975; 1979) and increases the efficacy of naloxone (Tulunay & Takemore, 1974) whereas chronic administration of opiate antagonists increases the receptor binding (Lathi & Collins, 1978; Schulz, Wuster & Herz, 1979) and decreases the efficacy of naloxone (Pilcher, 1980). Furthermore, long-term morphine treatment decreases the level of B-endorphin in the pituitary and discrete brain areas of the rat (Gianoulakis, Woo, Drouin, Seidah, Kalant & Chrestien, 1981; Hollt, Przewlocki & Herz, 1978; Przewlocki, Hollt, Duka, Kleber, Gramsch, Haarman & Herz, 1979), and increases the level of 'enkephalinase' (Malfroy et al., 1978), while long-term naloxone treatment increases the endogenous opioid levels (Wuster, Schulz & Herz, 1980b). Thus, endophinergic systems which are fully mature can adapt to opiate administration, and that this adaptation has pharmacological and behavioural consequences.

Sandman et al. (1979) reported that infants treated with B-endorphin during their first week of life displayed a long-lasting change in pain threshold as adults. Sonderegger et al. (1977) have achieved similar results using subcutaneous morphine pellet (75 mg of morphine base) implantation. They found reduced sensitivity to the analgesic action of a single injection of morphine when the rats were tested on day 48 postpartum using the hot-plate technique. Chronic exposure to morphine has also been shown to alter the hypoactive (Mallari & Kleem, 1978) effects of a single injection of morphine on activity levels. Bardo & Hughes (1981) found that rats treated perinatally with morphine were more responsive to a test drug of acute morphine (5.0 mg/kg) than the controls on day 27 postpartum. Similar results have been achieved when the interval between the last injection of treatment drug and test drug was only one day (Bardo et al., 1982). Conversely, early pretreatment with opiate antagonists such as naloxone (Sandman et al., 1979) sensitizes the adult rat to the effect of morphine. Paul et al. (1978) reported that rat pups given daily subcutaneous injections of naltrexone (10 mg/kg) throughout infancy showed shorter latency to morphine-induced analgesia as adults in a tail-flick antinociception procedure. Bardo et al. (1982) have demonstrated that chronic naloxone treatment throughout infancy (1-21 days) produced an increase in the number of mu-receptors. These studies indicate that opiate receptors which are undergoing postnatal development may be particularly susceptible to chronic drug intervention, and that

such intervention may alter the interaction between endogenous opioids and their binding sites.

The purpose of the present investigation was 2-fold. First, it was designed to examine if postnatal opiate treatment altered the ontogeny of the endogenous opioid system and if such alterations were accompanied by behavioural modification of naloxone's food-suppressant effect. Since morphine-antagonist activity of naloxone has been shown to be enhanced in animals previously treated with morphine or other narcotic analgesics, the second objective of this study was to determine whether or not the suppressant effect of naloxone on milk intake is similarly altered in 14 day old rats treated with morphine or naltrexone.

Method

Subjects

Animals were offspring of female rats mated in our laboratory. One day after parturition, each litter was culled to 9 pups.

Drug Pretreatment

One third of the animals from each litter was administered morphine sulphate (5 mg/kg), another third with naltrexone hydrochloride (10 mg/kg) and the last third with normal saline vehicle. All injections were made subcutaneously at the nape of the neck and were given once daily at noon from day one to day 5. Each rat from the split-litter (Martin & Moberg, 1981) was marked

for identification on the first treatment day (Geller & Geller, 1966). Each animal was weighed on a Digimetric (Sybron Corporation, New Haven, Ct.) to the nearest .01g prior to every injection. The pups were kept in huddles before and after injection and spent no more than 5 minutes away from their mother. Following treatment on Day 5, all animals were left undisturbed until testing. A litter was tested once at 10-, 12-, or 14-days of age. The pups were tested for milk-intake under the licking paradigm, as described in the general method.

Results

Mortality, and Body Weight

The data obtained from the measurements of the two parameters (i.e. mortality and body weight) during the postnatal period are presented in tables 7 and 8. Naltrexone (10 mg/kg) pretreatment did not alter either the mortality rate from the last day of drug administration and test days (Table 7), or the body weights determined on each test day (Table 8). While morphine pretreatment (5 mg/kg) did not significantly alter the mortality rate from day 5 to test days when compared to saline group, the difference was significantly more appreciable when compared to naltrexone group. Morphine reduced body weights in all age groups (Table 8). Saline pretreatment had no effect on body weight but did increase mortality rate when compared to naltrexone group: $X^2(2) = 7.14$, $p < 0.03$.

Table 7

EFFECT OF POSTNATAL OPIATE-TREATMENT ON MORTALITY

	Drug		
	Saline	Morphine	Naltrexone
Total Live	112	97	92
Total Dead	28 *	23 *	8

* significantly different from naltrexone group, $p < 0.03$.

Table 8

EFFECT OF POSTNATAL OPIATE-TREATMENT ON BODY WEIGHT

	Mean body weight + SEM, g		
Age (days)	Saline	Morphine	Naltrexone
10	22.27 ± 0.41	19.95 ± 0.36*	21.22 ± 0.88
12	23.88 ± 1.06	20.79 ± 1.54*	23.42 ± 1.12
14	28.52 ± 0.61	23.44 ± 0.55*	26.75 ± 0.81

* significantly different from saline group, $p < 0.05$.

Gross Behavioural Measure

No unusual behaviour was observed when naloxone was given to morphine- or naltrexone-treated animals. There were no peculiar postures, hyperactivity, observable involuntary somatic responses such as muscular rigidity and shivering, signs indicative of naloxone-precipitated drug-withdrawal in adults.

Intake

Figure 8 shows milk intake during the 1-hour feeding period as a function of drug-pretreatments and naloxone doses. Naloxone produced a dose-related decrease in food intake (g) of the 10 day olds. Analysis of variance confirmed the major pattern of

results depicted in Figure 8 and showed an effect of naloxone doses: $F(2,36) = 4.30$, $p < 0.02$, and drug-pretreatments by naloxone interaction: $F(4,36) = 3.98$, $p < 0.01$. Test of simple main effects revealed that naloxone reduced consumption only in morphine-pretreated animals, $F(2,36) = 11.50$, $p < 0.001$ without any effects on the other groups, and that the intake of naltrexone- and saline-pretreated animals did not differ from each other: $F(2,36) = 0.572$, $p > 0.05$.

At 12 days of age, there was an effect due to pretreatment drugs: $F(2,15) = 3.84$, $p < 0.04$; naloxone dose effect: $F(2,30) = 3.46$, $p < 0.04$; but no drug-pretreatments by naloxone interaction: $F(4,30) = 0.99$, $p > 0.05$. Significant differences between groups were evaluated by Newman-Keuls test of significant ordered pairs with probability level of 0.05. The test revealed that morphine-pretreated animals took in less milk than saline-pretreated animals. A significant dose response effect was indicated in morphine-pretreated animals in that the intake in grams at the high dose of 5.0mg/kg was smaller than gram intake at 1.0mg/kg ($p < 0.05$, Newman-Keuls test).

The effect of naloxone on milk intake of 14 day-old is depicted in Figure 8. The 3 by 3 analysis of variance confirmed the expectation that two weeks old animals receiving vehicle take in more milk than animals receiving acute naloxone: Mean for vehicle control = 3.14 g; mean for low dose of naloxone = 2.46 g; and mean for high dose of naloxone = 2.34 g; $F(2,30) = 36.25$, $p < 0.001$.

There was a reliable drug-pretreatments by naloxone interaction: $F(4,30) = 4.36$, $p < 0.01$. Further analyses of the scores of interaction indicate that naloxone reduced consumption compared to vehicle controls in all the three groups: $F(2,30) = 8.49$, $p < 0.01$ for saline-pretreated groups; $F(2,30) = 98.98$, $p < 0.001$ for morphine-pretreated groups; and $F(2,30) = 8.97$, $p < 0.01$ for naltrexone-pretreated groups. Dunnett's test revealed revealed that for morphine-subjects, all doses were different from vehicle control values ($p < 0.01$) and only the high dose was different from the control values ($p < 0.01$) in the saline- and naltrexone-pretreated animals.

When the scores were transformed into grams consumed/grams of body weight before feeding and analysed, the same factors emerged as reliable source of variance for all the groups except the 10 day olds (Figure 8).

Figure 8 about here

Discussion

The results of the present study indicate that postnatal exposure to morphine may induce alterations in the ontogeny of endogenous opioid system. Morphine pretreatment hastened the appearance of the anorexigenic effect of naloxone, as milk intake was reliably decreased in 10, 12, and 14 day olds rats exposed to morphine during the postnatal days 1-5.

The reduction of milk intake observed in morphine treated animals was not due to competition, either by residual morphine or its metabolites, for the following reasons : 1) Since morphine induces feeding (Tepperman, Hirst & Gowdey, 1981), any tissue-bound morphine present would nullify the anorexigenic action of naloxone by competitive antagonism, or 2) It would require a large dose of naloxone to produce a minimal effect and a low dose would be ineffective altogether. Such was not the case, since food reduction following a 1.0 mg/kg of naloxone in morphine treated animals was even greater than the size of reduction that followed the most effective dose (5.0 mg/kg) in the saline-pretreated group. 3) The changes in naloxone-induced food reduction were still apparent 5, 7, and 9 days after the last administration of morphine, whereas the amount of morphine in the brain after 1 day was likely to be negligible (Mullis, Perry, Finn, Stafford & Sadee, 1979). 4) The failure to observe any withdrawal symptoms in morphine-treated animals following naloxone administration strongly suggests that the suppression of ingestion was not mediated by some nonspecific mode of action. Since naloxone and morphine have access to the same receptor sites, therefore the changes observed here maybe structural change at the opiate receptor. Conceivably, chronic morphine exposure caused a decrease in the receptor population and thereby reduced the number of receptors occupied by the antagonist for effective action. This would result if the opioid chemicals function as neurotransmitters (Frederickson, 1977), in which case

the decreases in receptor system may serve as a feedback loop to modulate the effect of the continuous presence of morphine. Decreases in receptor binding following morphine-pretreatment have been demonstrated indirectly by a number of investigators (Way et al., 1969; Smits & Takemori, 1970) and directly by studies using brain slices instead of homogenates for the assays (Davies Akera, Brody, & Watson, 1975).

Other possibilities, such as altered disposition of naloxone in the brain during the challenge test (Shen & Way, 1975; Lange, Fujimoto, Roerig & Wang, 1977), may also account for heightened ability of naloxone to reduce food intake in the animals. This possibility is unlikely, however, since both latency to feed and duration of action of naloxone in morphine-pretreated animals did not differ from those of control animals (data not shown). Thus, the result of this study may be consistent with receptor-mediated naloxone anorexigenic effect. But the interesting paradox is that although the the receptors are presumably fewer, they are active earlier.

Chronic injection of naltrexone had no effect on the development of endorphinergic system. No food intake reduction followed naloxone administration to 10 and 12 day olds that had been pretreated with naltrexone. Chronic naltrexone, however, did not reverse the anorexigenic effect of naloxone as food intake was also reduced in 14 day olds. The absence of naltrexone reversal of naloxone's anorexigenic effect at 14 days raises the possibility that naltrexone action may not be specific

to the feeding-related opiate receptors, where naloxone has high affinity. Differences in the effects of these drugs have consistently been demonstrated. Inter alia, it was demonstrated that, in feeding, naloxone is a more potent anorexigenic agent than naltrexone (Part 1). Thus, chronic naltrexone pretreatment may have had no effect on the relevant receptors. Moreover, naltrexone is a highly lipid-soluble drug with a rapid onset of effect and a limited duration of action. It is possible, therefore, that a lipid-soluble drug, such as naltrexone, because of its high diffusibility (Kaufman et al., 1975), might prove to be less anatomically specific in terms of its ability to alter the neuronal system subsisting naloxone effect. Indeed, previous attempts to demonstrate increase in pharmacologically relevant receptors following acute (Pert & Synder, 1976) or chronic (Hitzemann, Hitzemann & Loh, 1974; Schulz et al., 1979) narcotic antagonist administration have proved unsuccessful. This failure has led Schulz et al. (1979) to emphasise that continuous exposure of the tissue to sufficiently high concentrations of the antagonist, such as provided by pellet implantation, appears to be an essential requirement for induction of these changes. They further suggest that peripheral injections of opiate antagonist do not allow this condition to be met. This then would be consistent with the result obtained with naltrexone in this study and with published evidence. Amir and Amit (1979) demonstrated that naltrexone administered subcutaneously once a day at 10 mg/kg for 21 days to adult rats did not affect nociceptive

reponding in non-stressed rats. Similar results were reported by Harry and Rosecrans (1979) who found that chronic oral naltrexone had no effect on the responsiveness to pain when administered perinatally.

Some investigators have, however, reported behavioural alterations that are attributable to prior chronic opiate antagonist exposure. Paradigmic differences may account for the discrepancy between such observations and those reported here. In those studies, changes in behaviour of naloxone- or naltrexone-pretreated rats were found to occur following acute opiate agonist challenge (Paul et al., 1978; Tang & Collins, 1978), but not after the antagonist challenge (Sandman et al., 1979). It is possible, therefore, that tolerance, as a dimunition in effectiveness after a repeated administration, may not be a property of opiate antagonist. The results of these experiments then suggest that the endorphinergic system responsible for food modulation maybe more susceptible to chronic receptor activation rather than chronic receptor blockade.

GENERAL DISCUSSION

The results of these experiments clearly indicate that naloxone's effects on the preweanling rats are both age-dependent and dose-related. Following systemic administration of naloxone, infant rats from age 3 through 12 unequivocally displayed no feeding or behavioural perturbation in three paradigms involving active, passive and natural feeding conditions. At two weeks postpartum, the spectrum of naloxone anorexigenic action observed in adults (Holtzman, 1974) began to emerge. Fourteen day old or older rats reduced their milk intake in a dose-dependent manner.

This dose-dependent relationship suggests that specific opiate receptors are involved (Synder, 1975). Additional evidence in this regard derives from the similarities in the age-dependency and dose-dependency of naltrexone and naloxone effects in the young; and in the fact that the effects of chronic morphine and acute naloxone were not additive. The most compelling evidence for receptor mechanisms' involvement came from the finding that chronic morphine modulated the time-course and dose-response of naloxone anorexia, a phenomenon that obtains when the same substrate mediates the action of two competitive drugs. These observations then are consistent with the hypothesis that endogenous opioid systems might participate in normal control of food intake.

The question which may now be asked is whether the evidence is sufficient to allow the formulation of any more detailed accounts of the precise physiological or behavioural mechanisms

involved. Morley (1980) has suggested that endorphinergic mechanisms in the lateral hypothalamus are responsible for mediating appetite for food and thus, presumably, food seeking and eating. A number of hypothalamic nuclei contain enkephalin terminals, and three days of food deprivation has been found to reduce the levels of B-endorphin in the hypothalamus (Gambert, Garthwaite, Pontzer & Hagen, 1980). However, the finding that naloxone also suppresses food intake in rats with ventromedial hypothalamic lesions (King, Castellanos, Kastin, Berzas, Mauk, Olson & Olson, 1979) makes it unlikely that the hypothalamus is the sole site of action of naloxone.

The presence in the peripheral systems of opioid peptides has also raised the suggestion that opioids may modulate consummatory behaviours via these channels. Met-enkephalin has been reported to increase intestinal activity, including blood flow, motor activity, and oxygen consumption (Pawlik, Walus & Fondacaro, 1980). However, naloxone has no high affinity for the delta-receptors (Met-enkephalin receptors) (Pert, 1981) and is also ineffective in in altering intestinal motility (Howd, Adamovics & Palekar, 1978). Recently, Jones and Richter (1981) have suggested that several components of the vagal system, including the dorsal motor nucleus and the nucleus tractus solitarius, may act as the likely sites for opioid/receptor interaction since they contain a large concentration of enkephalin terminals and opiate receptors. Vagotomy was reported to block naloxone's suppressant effects. What mechanisms maybe involved are unknown.

In the preceeding experiments behavioural analysis of feeding revealed that naloxone has no effect on the latency to feed but may hasten the early termination of the feeding act, and, by inference, endogenous opioid systems may mediate mechanism which inhibits a feeding-satiety state. However, the physiological mechanism involved may have a much wider role in the control of behaviour. One theory is that opiates and their antagonists may affect ingestion by modulating central reward processes which may be quite directly involved in appetitive behaviours. Belluzzi and Stein (1977) have implicated endogenous opioid systems in drive-reduction reward. Unfortunately, the most straightforward prediction of their hypothesis, that opiate agonists would decrease feeding, does not hold true. At low doses, which presumably best stimulate physiological conditions in the infants, morphine increases feeding (Aroyewun & Barr, in preparation). Another hypothesis suggests that the satiety effect of naloxone reflects a change in the emotional tone of animals as opposed to a shift in the bias of control systems for energy homeostasis (Panksepp, Herman, Vilberg, Bishop & DeEskinazi, 1980). However, this hypothesis is not supported by the data obtained in these experiments. Twelve day old or younger animals did not show the effect of such a bias.

It is probable that the role of endogenous opioid in mediating feeding may be to modulate activity in other, more primary neurochemical systems. A variety of neurochemical mechanisms, situated in the hypothalamus and elsewhere, are

involved in the control of food intake (Blundell & Latham, 1979). There is also evidence that endogenous opiates rather than act as classical neurotransmitters substances, may have an interactive or modulatory role in neurotransmission. In fact, it has been reported that peptides, including enkephalins, may co-exist with classical transmitters such as dopamine and noradrenaline in the same neurones or endocrine cells (Hokfelt, Johansson, Ljungdahl, Lundberg & Schultzberg, 1980). Relatively little is yet known about such interactive processes, particularly in relation to the role of peptides in the regulation of behaviour. It is in this regard that the results of these experiments hold potential promise. For, Rosengarten and Friedhoff (1979, 1980) reported biochemically, and Shalaby and Spear (1981) confirmed behaviourally, that perinatal treatment with antagonists such as haloperidol decrease dopamine receptor density, while adult treatment increases it. The results herein provide behavioural evidence in support of biochemical reports of unaltered receptor binding following chronic but intermittent administration of narcotic antagonist (Schulz et al., 1979). Thus the two systems differ in their response to chronic blockade, and such differences maybe useful in distinguishing them functionally vis-a-vis feeding behaviour. In addition to the behavioural model in these experiments, the animal model is also a rich source of information indispensable to the understanding of the endorphinergic system's role in natural behaviours. It has been found, for example, that naloxone reduces water intake as well as

food intake from rat to monkey (Brown & Holtzman, 1981), and the dose that decreases food intake has no effect on water intake (Jones & Richter, 1981; Lowy & Yim, 1981). suggesting the involvement of two endorphinergic subsystems in eating and drinking (Sanger, 1980). Since the rat infant lives mainly on its milk, which is part water and part nutrients, and based on the finding of this report that endogenous opioid may mediate milk intake only after two weeks postpartum, it is possible that studies employing infants may help dissociate the subsystems that mediate water and food intake in the adult. And, indeed may reveal different developmental trends. Such endeavour will be important in determining the physiological significance of endogenous opioid system.

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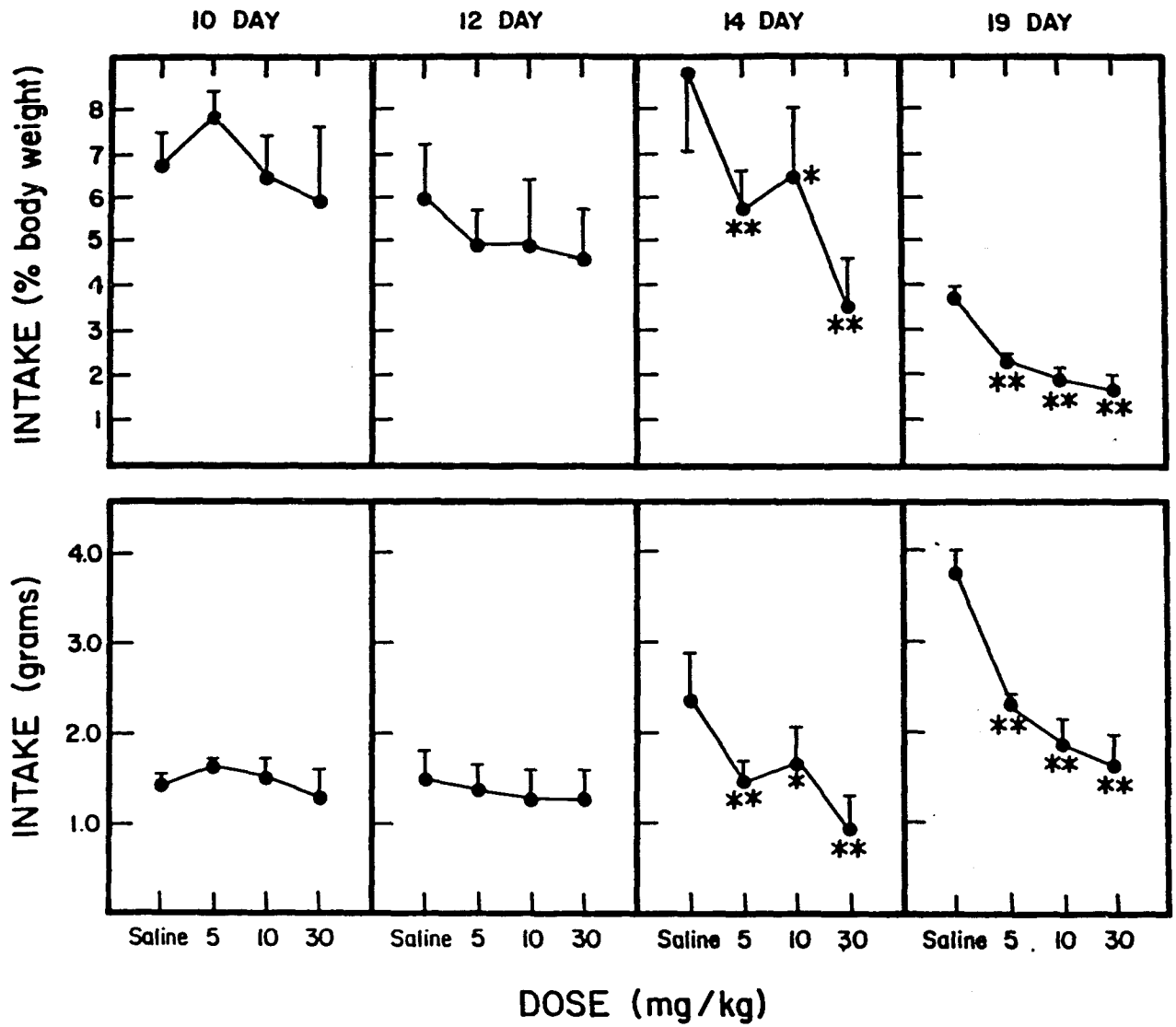

10 DAY
12 DAY
14 DAY
19 DAY
INTAKE (% body weight)
8
7
6
5
4
3
2
1
INTAKE (grams)
4.0
3.0
2.0
1.0
Saline 5 10 30
Saline 5 10 30
Saline 5 10 30
Saline 5 10 30
DOSE (mg/kg)

FIG. 1

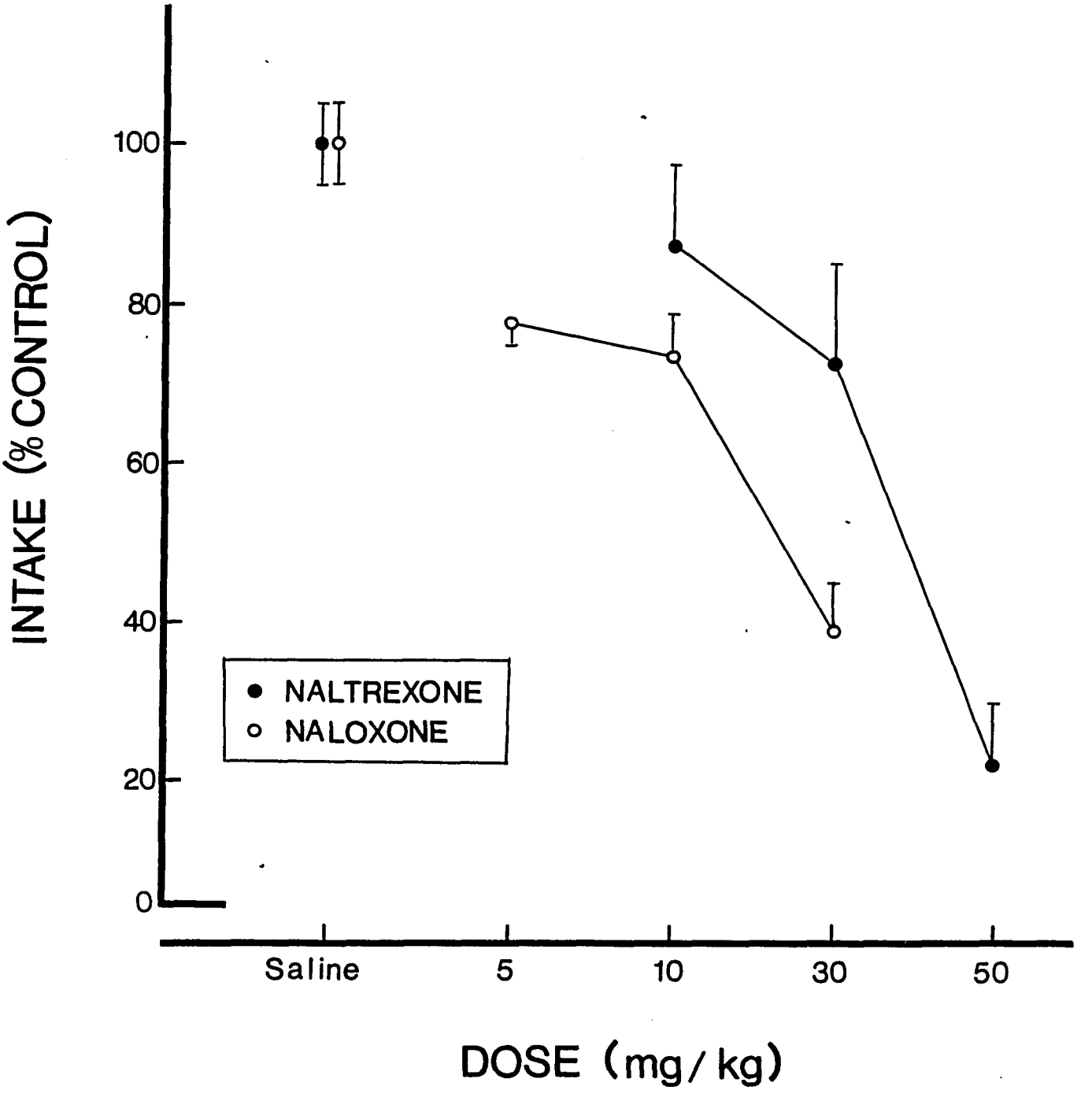
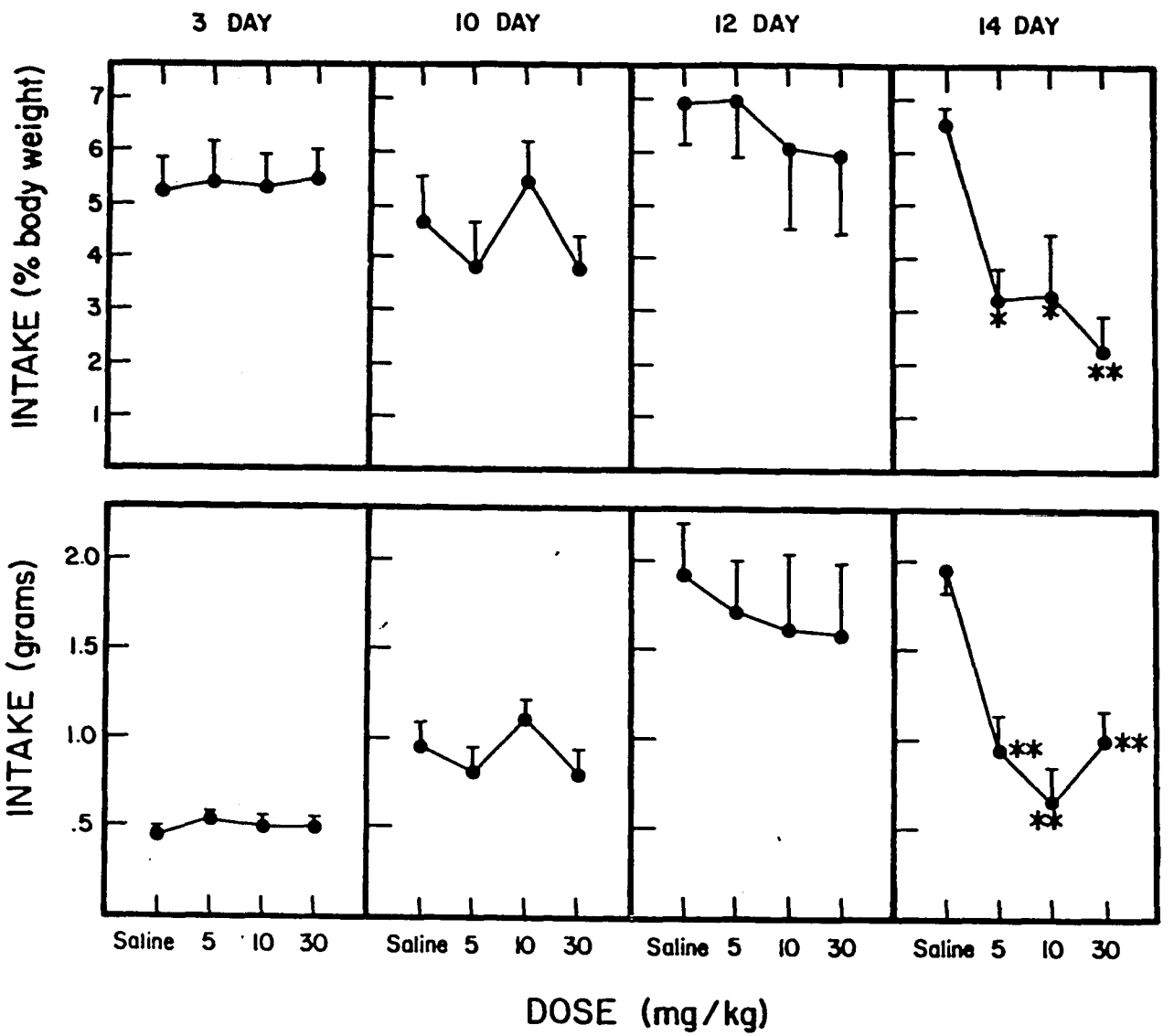

INTAKE (% CONTROL)
100
80
60
40
20
0
NALTREXONE
NALOXONE
Saline
5
10
30
50
DOSE (mg/kg)

FIG 2


3 DAY
10 DAY
12 DAY
14 DAY
INTAKE (% body weight)
7
6
5
4
3
2
1
*
*
**
INTAKE (grams)
2.0
1.5
1.0
.5
**
**
**
Saline 5 10 30
Saline 5 10 30
Saline 5 10 30
Saline 5 10 30
DOSE (mg/kg)

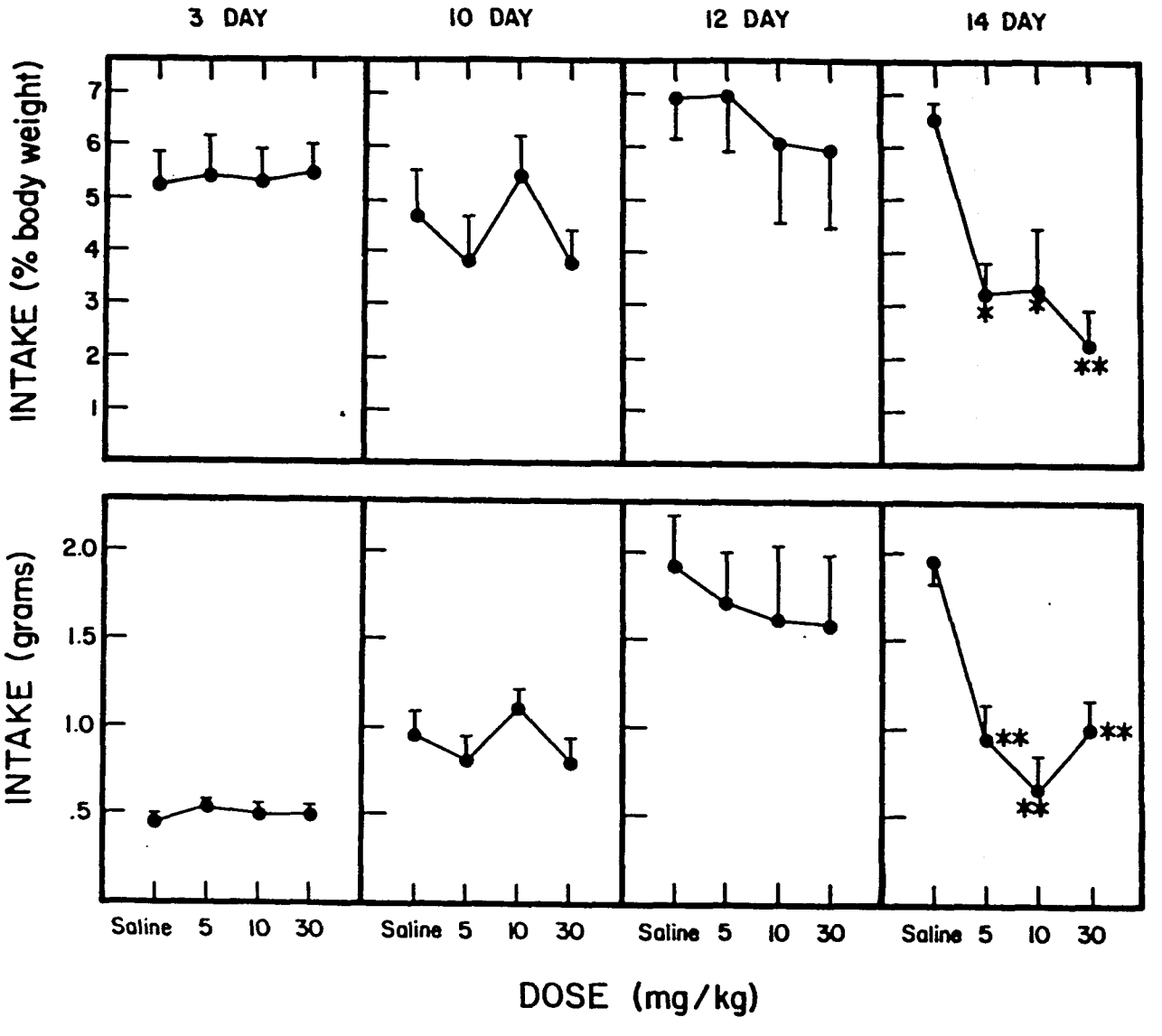

3 DAY
10 DAY
12 DAY
14 DAY
INTAKE (% body weight)
7
6
5
4
3
2
1
INTAKE (grams)
2.0
1.5
1.0
.5
Saline 5 10 30
Saline 5 10 30
Saline 5 10 30
Saline 5 10 30
DOSE (mg/kg)

FIG. 3

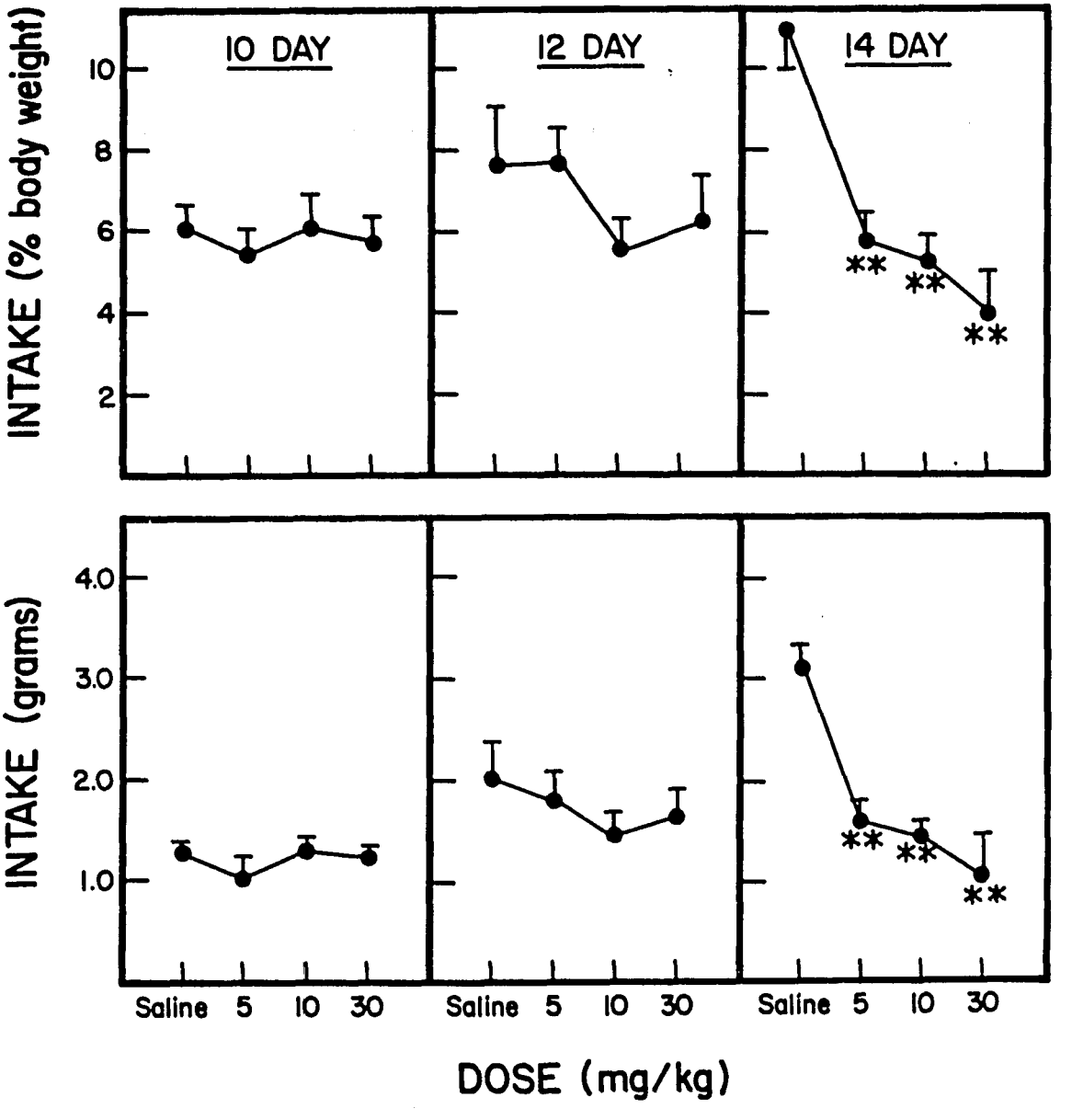

10 DAY
12 DAY
14 DAY
INTAKE (% body weight)
10
8
6
4
2
INTAKE (grams)
4.0
3.0
2.0
1.0
**
Saline
5
10
30
DOSE (mg/kg)

FIG. 4

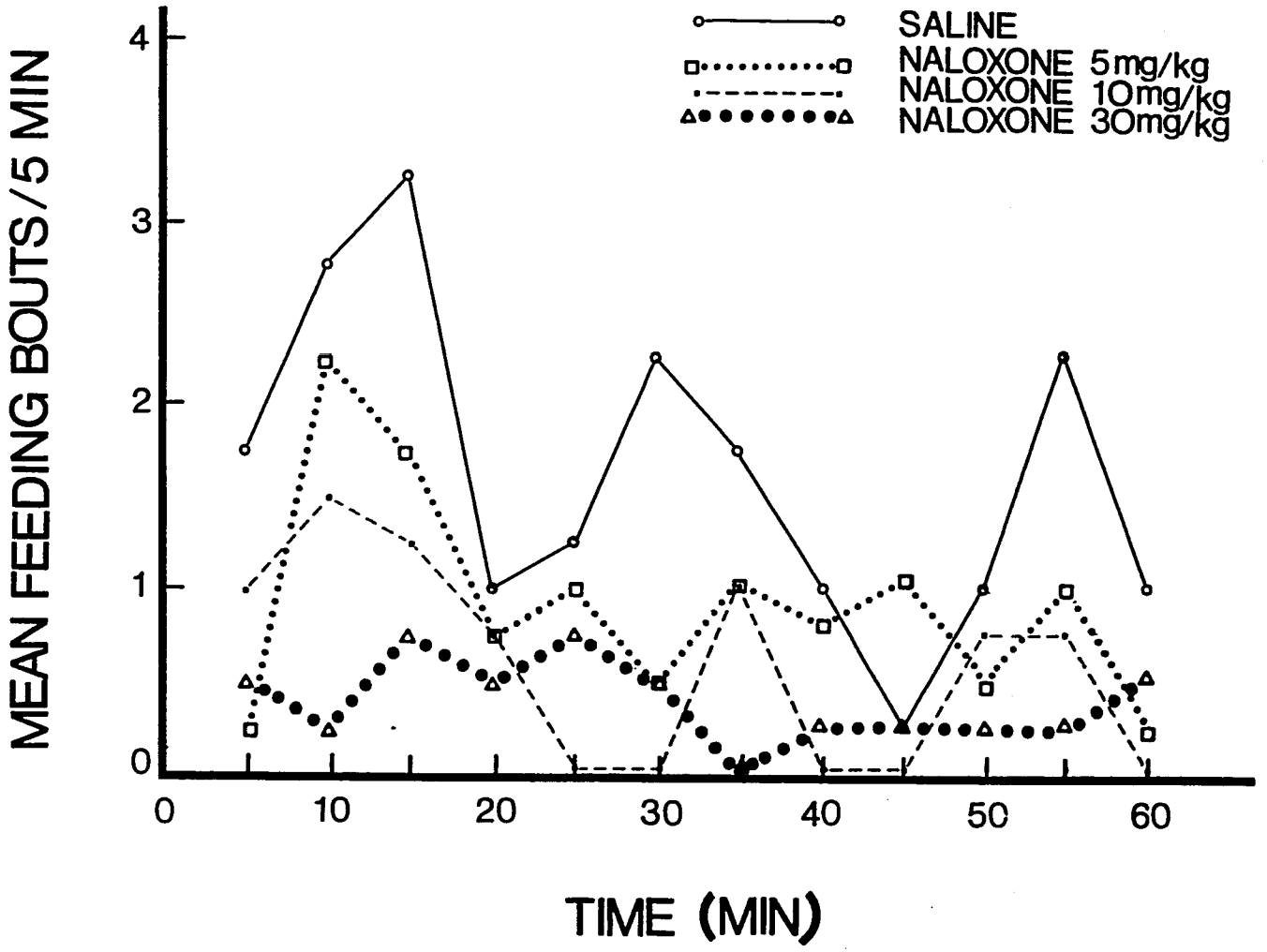

SALINE
NALOXONE 5mg/kg
NALOXONE 10mg/kg
NALOXONE 30mg/kg
MEAN FEEDING BOUTS/5 MIN
0
1
2
3
4
0
10
20
30
40
50
60
TIME (MIN)

FIG 5

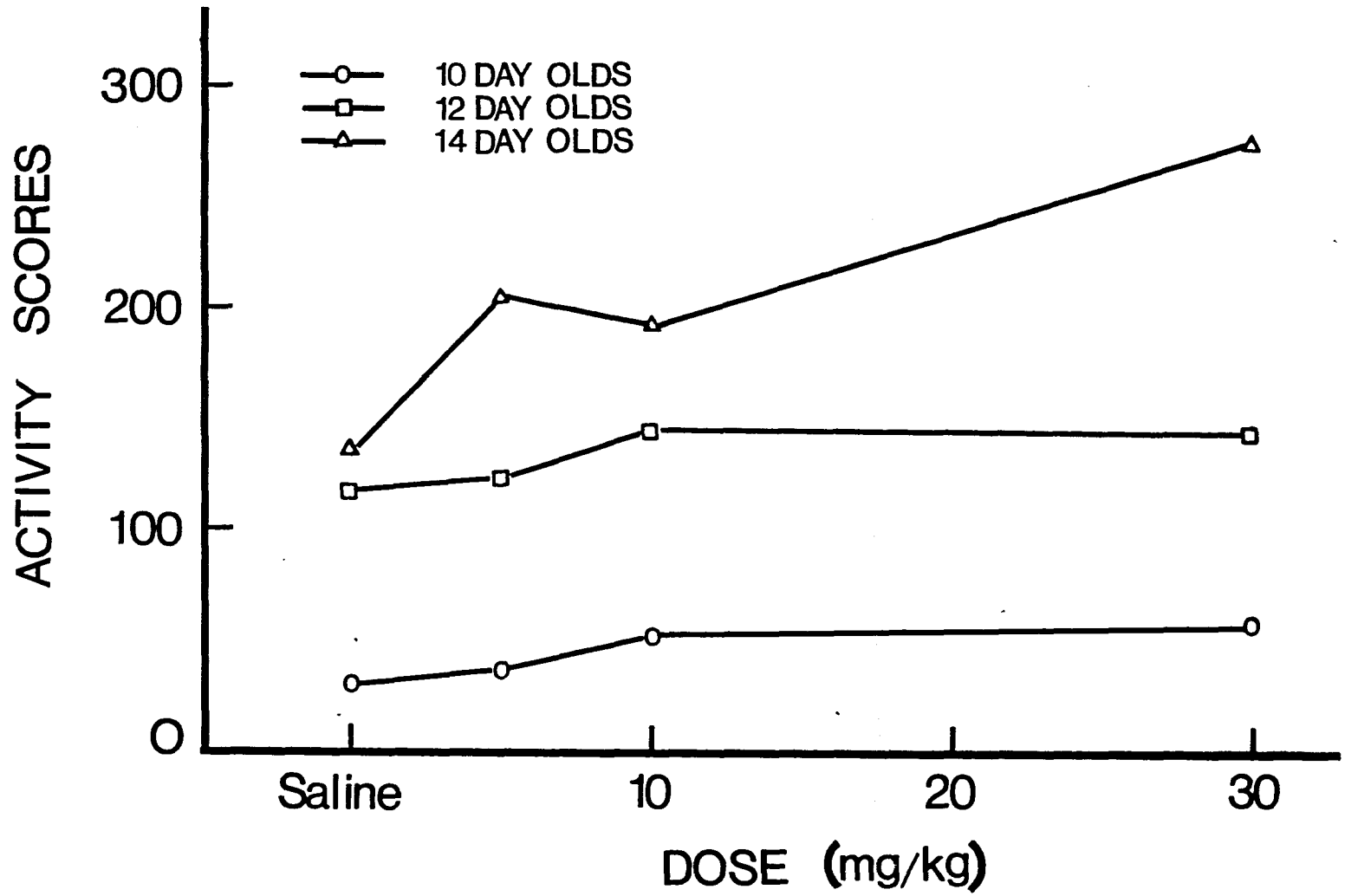

ACTIVITY SCORES
300
200
100
0
10 DAY OLDS
12 DAY OLDS
14 DAY OLDS
Saline
10
20
30
DOSE (mg/kg)

FIG. 6

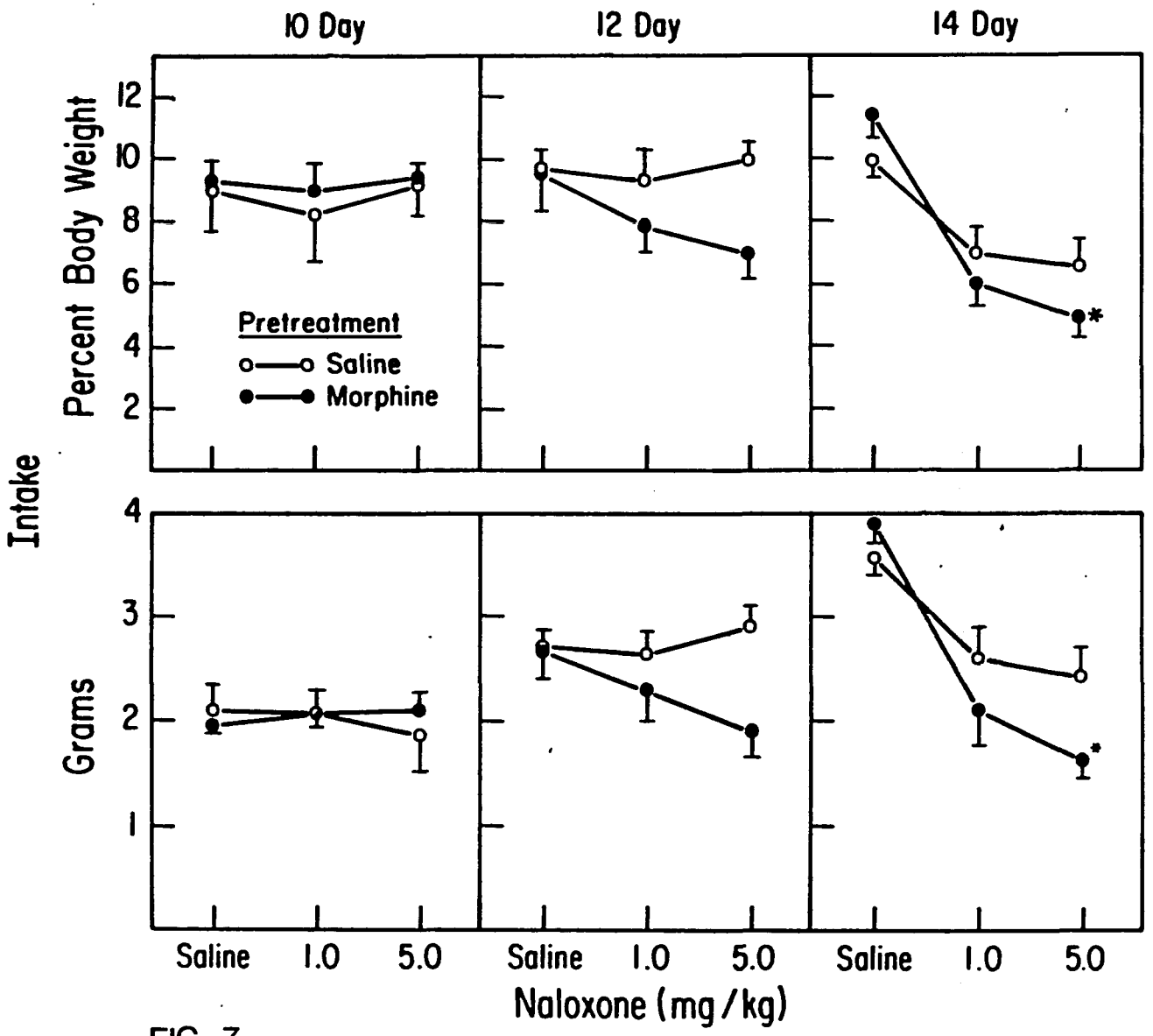

10 Day
12 Day
14 Day
Intake
Percent Body Weight
12
10
8
6
4
2
Pretreatment
Saline
Morphine
Grams
4
3
2
1
*
*
Saline
1.0
5.0
Saline
1.0
5.0
Saline
1.0
5.0
Naloxone (mg/kg)

FIG 7

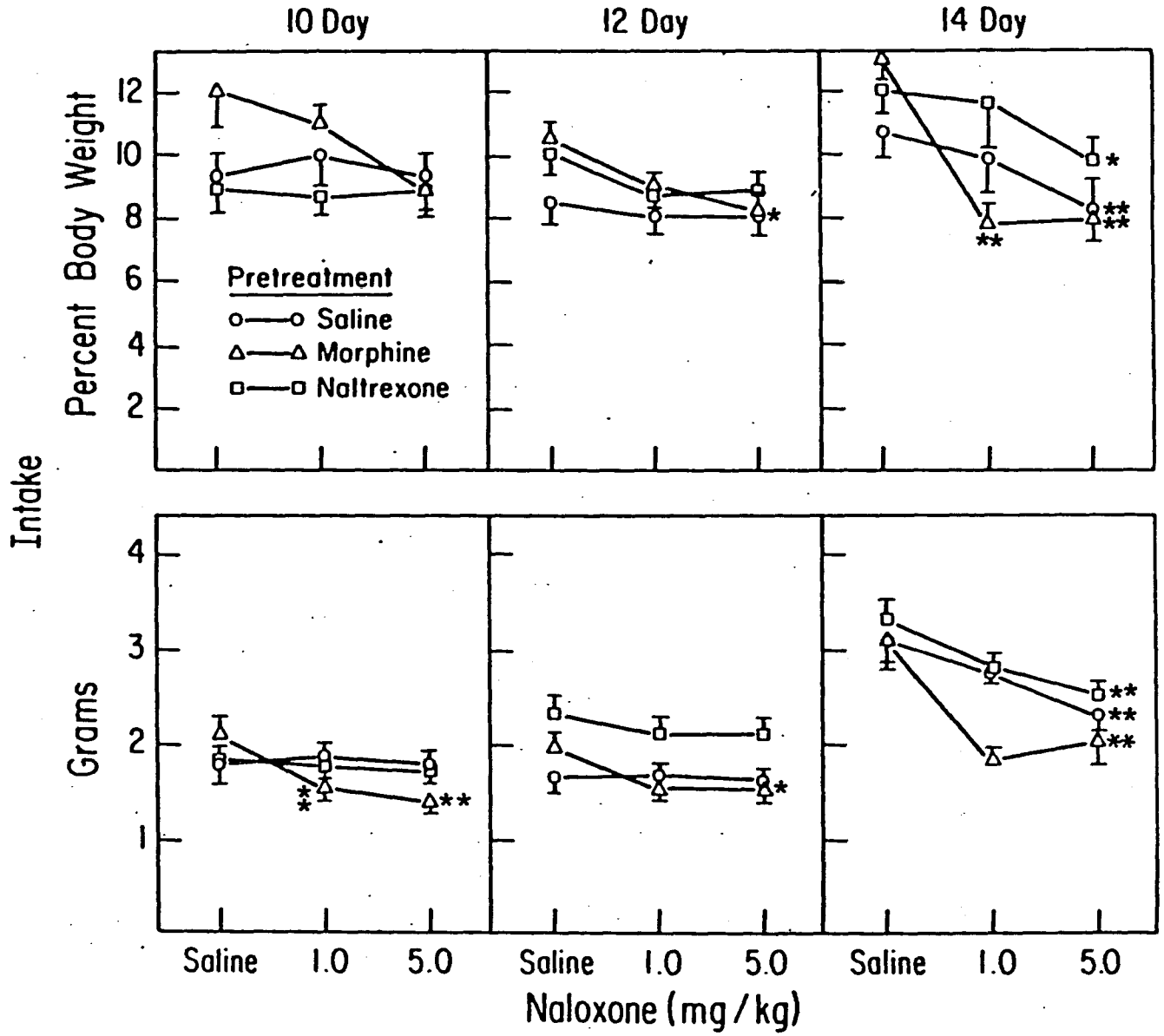

10 Day
12 Day
14 Day
Intake
Percent Body Weight
Grams
Pretreatment
Saline
Morphine
Naltrexone
Saline
1.0
5.0
Naloxone (mg/kg)

FIG 8