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The Role of Kappa Opioid Receptors in the Sexual Behaviour of the Male Rat

Marco Leyton

**A Thesis
in
The Department
of
Psychology**

**Presented in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy at
Concordia University
Montréal, Québec, Canada**

August, 1991

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ABSTRACT

The Role of Kappa Opioid Receptors in the Sexual Behaviour of the Male Rat

Marco Leyton, Ph.D.
Concordia University, 1991.

The administration of opioid receptor agonists and antagonists is known to change male sexual behaviour; both facilitations and inhibitions have been reported. Little is known, however, about the specific opioid receptors that underlie these changes. In the current study, the role of central *kappa* (*k*) opioid receptors in male rat sexual behaviour was investigated.

The systemic administration of the selective *k* opioid receptor agonist U-50,488H was found to dose-dependently increase both copulation and ejaculation latencies as well as the inter-intromission interval (III), to decrease both locomotor activity and bodily grooming, and to induce both body flattening and lowered body temperature. U-50,488H-induced inhibitions of male sexual behaviour were prevented by systemic injections of naloxone and by intra-cranial injections of the *k* opioid receptor antagonist nor-binaltorphimine (NBNI). Injections of NBNI to either the ventral tegmental area (VTA) or the nucleus accumbens septi (NAS) increased female-directed behaviour, and prevented the systemic U-50,488H-induced decreases in female-directed behaviour and increases in both copulation latencies and III. Intra-medial preoptic area (mPOA) injections of NBNI also increased female-directed behaviour, and attenuated U-50,488H-induced decrements in female-directed behaviour as well as U-50,488H-induced increases in copulation latencies, ejaculation latencies, and III. Injections of NBNI into all three areas prevented U-50,488H-induced increases in body flattening; injections into the VTA prevented U-50,488H-induced decreases in bodily grooming. Following intra-cranial injection, NBNI also induced transient behavioural inhibitions suggestive of transient antagonism of *mu*

opioid receptors. Long-lasting antagonism of κ opioid receptors, as indicated by the prevention of U-50,488H-induced behavioural inhibitions, was apparent up to 26 days following intra-cranial infusions of NBNI. The decrements in both male sexual behaviour and locomotor activity induced by systemic U-50,488H administration were mimicked by U-50,488H injections directly into the VTA, NAS and mPOA. It was suggested that, pharmacologically, these results reflect U-50,488H-induced inhibitions of dopaminergic, and more speculatively, serotonergic activity; behaviourally, they might reflect the induction of a sexual refractory period.

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I would like to thank Jane Stewart who, in her role as graduate supervisor, has exemplified a clarity of thought and breadth of knowledge that is inspiring.

Others have also contributed to my understanding of both the present thesis - only nominally all mine - and the research of others. They include, but likely to my later embarrassment, are not limited to: Aldo Badiani, Doug Funk, Dena Davidson, Jon Druhan, Margaret Forgie, Kathryn Gill, John Mitchell, Dorothy Pocock, Heshmat Rajabi, Demetra Rodaros, and Cecilia de Leon.

Finally, to Jana; it's all over, I'm finished, I'm yours.

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When presented with a receptive female most male rats promptly begin a well-described copulatory series of pre-copulatory behaviours, mounts, intromissions, and eventually, ejaculation. Following a refractory period, during which the male is hyporesponsive to sexual stimuli, a new series can begin. The occurrence of copulation is clearly critical for reproduction. Less obvious, however, is that the timing and pattern of these behaviours seem also to be important for successful impregnation. The current thesis examines the roles that kappa (k) opioid receptors and their endogenous ligands might play in the initiation, and successful consummation of sexual activity in the male rat.

The Male Copulatory Series

In the presence of an estrous female, male rats become behaviourally excited, rapidly exploring both the immediate environment and the female. Typical investigations of the female, commonly referred to as either pre-copulatory or female-directed behaviours, include: chasing her; climbing over or crawling under her; anogenital sniffing, licking and nuzzling; and partial mounts when the male manipulates the female's flanks without actually making pelvogenital contact. The duration of these events prior to the initiation of mounting and intromitting varies between individual males, but it is typically prolonged in sexually naive males, and greatly attenuated in the sexually experienced animal. The majority, 70-90%, of these males will begin copulating within a few minutes of first being paired, and within 30 s on subsequent pairings with an estrous female. The remaining minority of reluctant copulators will eventually lose interest and begin to ignore the female. With repeated pairings, however, even these males will commonly display normal copulatory behaviour.

Throughout this period, the female is also an active participant (Beach, 1976). Estrous female rats in the presence of a male engage in a variety of solicitous behaviours that serve to both encourage copulation and facilitate mount approaches by ensuring that the male is oriented toward her; typically, the female will approach the male with wiggling

ears, display her perineal area to the male, and then run away in a characteristic hopping and darting motion only to abruptly stop again, rump in the air. With experience, most males will rapidly respond to these cues by initiating copulation.

A male's mount is characterized by the placement of his forelimbs on the female's rear flanks. This stimulation typically induces the female to exaggerate the perineal exposure by moving her tail, super-extending the spine to raise both rump and head to produce lordosis, and adjusting the perineal region's position toward the male's penis. The male responds by thrusting his pelvis toward the female, and rapidly making repeated contact with her perineum. If the male dismounts at this point, he is considered to have made a mount without penile insertion.

A mount with penile insertion, an intromission, is indicated by a longer, deeper pelvic thrust followed by a rapid dismount, distinctive back-kick, and retreating reverse run. Some mounts and most intromissions are followed by autogenital grooming. Repeated mounts can occur in rapid succession, but intromissions are usually evenly spaced throughout a copulatory series, occurring approximately once every 30 s. This separation of individual intromissions may indicate the induction of a brief refractory period, a transient inhibition of further copulating (Beach, 1956; Sachs, 1978; Sachs & Dewsbury, 1978). The exact pattern can vary between males, but is quite consistent within the same male.

Following roughly 12 intromissions the male will ejaculate. Ejaculations can be behaviourally distinguished from intromissions by a slower, deeper pelvic thrust followed by a spasmodic shudder that runs across the male's flanks. After a few seconds, the male releases his clasp of the female and rises to a standing position without actively dismounting while the female jumps away. Within a few seconds the male proceeds to autogenital grooming, and the lying, resting and body flattening that is characteristic of the post-ejaculatory refractory period. The former two behaviours are self-explanatory, but the latter behaviour deserves some greater explanation. Body flattening involves a genuine

flattening of the male's flexible rib cage and body on the floor; it is perhaps only slightly exaggerated to say that, to the viewer, the male suddenly appears to have become two-dimensional. Body flattening is also commonly seen when male's are overheated, and it is believed that the behaviour serves to cool the body. Likely related to this independent observation, ejaculation is associated with a brief, rapid rise in brain temperature.

The post-ejaculatory refractory period lasts from four to eight min, and is believed to be divisible into two distinct phases (Beach & Holz-Tucker, 1949; Larsson, 1959). During the initial "absolute refractory" phase, estimated to last approximately three quarters of the entire refractory period (Sachs & Barfield, 1974; Barfield & Geyer, 1975), it is considered impossible to sexually arouse the male. An additional, prominent characteristic restricted to this initial phase is that the male vocalizes an ultrasonic 22-kHz "song" (Barfield & Geyer, 1975). During the second "relative refractory" phase, males will normally continue not to copulate, however, there are procedures that can induce the male to do so: for example, either electric shock (Barfield & Sachs, 1968; Barfield & Geyer, 1975; Pollak & Sachs, 1975) or possibly the introduction of a novel estrous female (Pollak & Sachs, 1975).

Following this refractory interval, the male will reinitiate a new copulatory series. Repeated series are similar in kind to the first, but are distinguished by two general characteristics: the ejaculatory latencies are shorter with fewer intromissions preceding the ejaculation; and the subsequent refractory period is longer in duration than preceding versions. This cycle will repeat itself from roughly five to 10 times before the male is considered to be sexually exhausted. Sexual exhaustion is usually operationally defined as a refractory period that lasts at least 30-45 min (see Beach, 1956; Beach, Westbrook, & Clemens, 1966; Sachs & Meisel, 1988, for theoretical discussions and experimental reviews of both this and other male sexual behaviours described in this section).

The timing of copulatory events would appear to be crucial for determining the occurrence of an ejaculation. Beach (1956), the preeminent theoretician and empirical

observer of sexual behaviour in the male rat, argued compellingly that ejaculation was not the cumulative response to excitement from repeated intromissions. To the converse, it was suggested that a maximum level of ejaculatory excitement was achieved immediately from the first intromission, and that subsequent intromissions served to maintain this excitement, even briefly reducing it, until a necessary minimum period had past, after which the "ejaculatory threshold" would be crossed. Perhaps the most elegant support for this position was the observation that ejaculatory latencies were not delayed when the number of permitted intromissions was reduced; briefly removing the male after each intromission decreased the number of intromissions to precede ejaculation without lengthening the ejaculation latency (Beach, 1956; Beach & Whalen, 1959). Although the basic features of this hypothesis have been widely accepted, alternative explanations for these observations have been suggested. One appealing alternative notes that temporally spaced intromissions last longer than ad libitum intromissions (Bermant, Anderson, & Parkinson, 1969) suggesting that they may have greater excitatory power (Toates & O'Rourke, 1978). More specifically, these authors have argued that the brief inhibition that normally follows an intromission may be attenuated by forced spacing of intromissions.

Before undertaking a description of the neural events believed to regulate these different behaviours, a careful discussion of the theoretical controlling mechanisms will be helpful. Beach argued convincingly that sexual activity did not result from the engagement of a solitary mechanism (Beach, 1956; Beach & Jordan, 1956; Beach et al., 1966). Originally two, and later three (McGill, 1965; Beach et al., 1966), distinct, though often intermingling, mechanisms were judged necessary to explain the male rodent sexual pattern. More recently, Sachs (1978) has argued for the necessary inclusion of a fourth mechanism.

It was proposed that the period of initial sexual arousal, characterised by the previously described female-directed behaviours that precede the onset of copulation, was controlled by a distinct "sexual arousal mechanism" (SAM) (Beach, 1956; McGill, 1965).

The SAM served to instate the male's interest in, or appetitive motivation to engage with, the female. As suggested by Toates (Toates & O'Rourke, 1978; Toates, 1986), the degree of this sexual arousal depended on an integration of external incentive stimuli and internal, neuroendocrinological, intrinsic states.

The degree of activity of this SAM determined the temporal crossing of the copulatory threshold. The time to achieve that threshold - typically considered to be indicated by the mount latency - is generally regarded as the most direct measure of male sexual motivation. Once crossed, sexual behaviour was controlled by the copulatory mechanism (CM) (Beach, 1956), and mounting and intromitting could proceed to ejaculation. A certain minimum number of intromissions and amount of time appears to bring the male past the ejaculatory threshold controlled by the ejaculatory mechanism (EM) (McGill, 1965). The fourth hypothetical mechanism, a hit rate factor (Sachs, 1978; Dewsbury, 1979), was considered to control the number of mounts that were successful intromissions, and to be related to the post-ejaculatory refractory period.

Two observations suggested that the first copulatory series sensitized both the SAM and the CM of the second copulatory series. First, it was noted that the second series was shorter than the first, indicating that the CM had been sensitized. Secondly, removing the male between the first and second copulatory series resulted in a reduction in the initiation of copulation, relative to the first series, indicating that once the inhibitory refractory period was concluded males were quicker to reinitiate copulation (Beach & Whalen, 1959).

Neural Control of Copulatory Behaviour

Secondary environmental cues previously associated with rewarding incentive stimuli can come to elicit anticipatory, appetitive behaviours that prepare the animal for consummatory behaviours; appropriate interactions with the stimulus object. Remarkably, this ethological demarcation of animal behaviour has some parallel in anatomically distinct neurological circuitry. Substantial accumulated evidence indicates that appetitive aspects of

male sexual behaviour, related to the conceptual SAM, are largely determined by neural activity of the A10 mesolimbic dopamine (DA) pathway whereas consummatory components, related to the conceptual CM and EM, appear to be predominantly orchestrated in the medial preoptic area (mPOA), a terminal site of the A14 mesencephalic DA pathway.

The A10 DA system plays a well demonstrated role in the appetitive dimensions of numerous behaviours. The positive incentive motivational properties of both natural rewards such as food or water, and more contrived, but powerfully rewarding stimuli, such as drugs of abuse or electrical brain stimulation, critically involve the A10 DA system. An animal's inclination to approach these rewarding stimuli is increased when this pathway is activated by other means, and attenuated by pharmacological blockades or lesions. Surprisingly, it is only recently that its potential involvement in male sexual behaviour has been considered. Not surprisingly, studies now indicate that the appetitive components of male sexual behaviour are as modifiable by manipulations to mesolimbic DA as other appetitive behaviours have proven to be (Everitt, Cador, & Robbins, 1989; Band & Hull, 1990; Everitt, 1990; Mitchell & Stewart, 1990a).

The mPOA and Male Sexual Behaviour: The mPOA is the single brain region most clearly identified to play a critical role in the expression of male sexual behaviour. At the mPOA, testicular androgens necessary for sexual behaviour interface with the neural circuitry that guides the activity. Androgen sensitive neurons have been identified in the mPOA (Stumpf & Grant, 1975; Pfaff, 1980), and previously castrated male rats exhibiting low levels of sexual behaviour recover following the mPOA implantation of testosterone or estrogen (Davidson, 1966; Lisk, 1967; Christensen & Clemens, 1974; Morgantaler & Crews, 1978). Further, mPOA lesioned rats displaying diminished levels of copulatory activity also fail to show the typical elevation in testosterone in the presence of a sexually receptive female (Kamel & Frankel, 1978).

The medial preoptic nucleus (MPN), the mPOA subnucleus that appears to be most critically involved in male sexual behaviour, receives inputs from the VTA, NAS, amygdala, and numerous other limbic, infralimbic, insular cortical and brainstem regions (Simerly & Swanson, 1986).

In a particularly elegant series of experiments, Barry Everitt and his colleagues have developed an interesting methodology for discriminating between appetitive and consummatory dimensions of male sexual behaviour (Everitt, Fray, Kostarczyk, Taylor, & Stacey, 1987; Everitt & Stacey, 1987). Male rats were trained to bar press for stimuli previously associated with the presentation of an estrous female, and with sufficient responses, access to the female. In the first of these two papers, it was reported that males displayed less responding during their post-ejaculatory refractory period than prior to the first ejaculation, indicating that this period does not simply correspond to decreased consummatory variables, but also reflects a decrease in the male's motivation to engage with the female. Conversely, males allowed to copulate to sexual exhaustion continue to bar press upon being returned to the operant chamber, indicating that sexual exhaustion may more purely reflect a consummatory deficit (Everitt et al., 1987). In the latter paper, this same paradigm was employed to study the effect of mPOA lesions on male sexual behaviour. Compatible with much previous research, excitotoxic, N-methyl-D-aspartate (NMDA), lesions of the mPOA, that destroy neuronal cell bodies while sparing axons *en passant*, abolished mounts, intromissions and ejaculations. Conversely, these lesions did not diminish either female-directed behaviour or instrumental responding for access to estrous females. With repeated testing, however, males eventually displayed lower rates of responding, an extinction, suggesting that being in the presence of an estrous female without being able to copulate was no longer rewarding (Everitt & Stacey, 1987). These results show that mPOA lesions selectively disrupt consummatory dimensions of male sexual behaviour leaving intact motivation to engage with the female.

Findings from earlier studies support this demarcation between the functions of the mPOA and the mediation of appetitive motivation elsewhere, across a wide range of species. Male rats (Larsson & Heimer, 1964; Heimer & Larsson, 1966/67; Singer, 1968; Giantonio, Lund, & Gerall, 1970; Hendricks & Scheetz, 1973; Paxinos & Bindra, 1973; Chen & Bliss, 1974; Ginton & Merari, 1977; Kamel & Frankel, 1978; Szechtman, Caggiula, & Wulkan, 1978; Bermond, 1982; Hansen, Köhler, Goldstein, & Steinbusch, 1982; Miesel, 1982; Brackett & Edwards, 1984; Hansen & Drake & Hagelsrum, 1984; Edwards & Einhorn, 1986; Bitran, Hull, Holmes, & Lookingland, 1988), hamsters (Powers, Newman, & Bergondy, 1987; Floody, 1989), mice (Bean, Nunez, & Conner, 1981), guinea pigs (Phoenix, 1961), gerbils (Yahr, Commins, Jackson, & Newman, 1982; Commins & Yahr, 1984), cats (Hart, Haugen, & Peterson, 1973), dogs (Hart, 1974), goats (Hart, 1986), lizards (Wheeler & Crews, 1978), fish (Macey, Pickford, & Peter, 1974) and rhesus monkeys (Sлимп, Hart, & Goy, 1978) with mPOA lesions exhibit extremely low levels of copulatory behaviour, but typically continue to pursue and investigate the female as well as make poorly coordinated attempts to mount. Interestingly, in the presence of an estrous female, although not when tested either alone or with anestrus females, mPOA lesioned male rats display various displacement activities - increased grooming, scratching and drinking - suggesting that unexpressed sexual motivation remains; possibly, the denied sexual expression has been replaced by other behaviours (Hansen et al., 1982; Hansen & Drake & Hagelsrum, 1984).

Two particularly revealing behaviours that remained undiminished in male rhesus monkeys with mPOA lesions were masturbation, and lever pressing for access to a female (Sлимп et al., 1978). Intriguingly, these same lesioned males did exhibit abnormal presenting behaviour to females: all five tested males presented their rumps to females, and further, were even commonly mounted by them. In these animals it seems clear that an interest in sexual activity is maintained despite their inability to organize the appropriate behavioural repertoire for heterosexual contact. These findings reinforce suggestions that

lesions of the mPOA do not eliminate sexual arousal, but rather, interfere with the animals ability to integrate the information from sexual stimulation with the appropriate behavioural response.

The sexually debilitating effects of mPOA lesions can be partially reversed by the systemic injection of the ergot derivative, lisuride (Hansen et al., 1982a), a nonspecific monoamine receptor agonist that also facilitates mounting (Ferrari, Baggio, & Mangiafico, 1986) and ejaculatory behaviour (Ahlenius, Larsson, & Svensson, 1980; Ahlenius & Larsson, 1984a) in intact animals. The administration of lisuride to mPOA lesioned males does not completely reverse the lesion's effects, but it does increase the proportion of males that mount, intromit and ejaculate. Interestingly, when ejaculations do occur, they are commonly seen on the first or second intromission (Hansen et al., 1982a). This latter finding suggests that the mPOA may contain - in addition to its clearly facilitatory, even necessary and permissive role - mechanisms that normally act to inhibit ejaculation.

Few investigators have explored possible mPOA inhibitory systems, but two potentially interacting mechanisms have been suggested. First, a recent abstract indicates that mPOA serotonin (5-HT) receptors might regulate copulatory mechanisms; the mPOA infusion of flesinoxan - a 5-HT_{1a} receptor agonist - decreased the post-ejaculatory refractory period (Lumley, Markowski, Eaton, Thompson, Moses, Bazzett, & Hull, 1990). Compatibly, systemic administrations of the 5-HT_{1a} agonists 8-OH-DPAT (Ahlenius & Larsson, 1984a; Schnur, Smith, Lee, Mas, & Davidson, 1988; Ågmo, Fernández, & Picker, 1989), RDS-127 (Stefanick, Smith, Clark, & Davidson, 1982; Clark, Stefanick, Smith, & Davidson, 1983; Clark & Smith, 1986), and lisuride (Ahlenius et al., 1980; Ahlenius & Larsson, 1984a) also decrease this refractory period as well as decreasing both the ejaculation latency and the number of intromissions to precede ejaculation. Interestingly, these effects of 8-OH-DPAT are antagonized by naloxone (Ågmo et al., 1989) suggesting a 5-HT/opioid interaction, but not by neuroleptics (Ahlenius & Larsson, 1984a; Clark & Smith, 1986) suggesting a DA-independent

mechanism. The possibility that mPOA opioid receptor stimulation might inhibit male sexual behaviour has been explicitly examined; the details of these studies will be discussed below.

Outside of the mPOA, stimulation of 5-HT autoreceptors in the raphé nucleus somato-dendritic region produces similar enhancements of ejaculatory behaviour. Intra-raphé infusions of either 5-HT or 8-OH-DPAT decrease both the ejaculation latency and the number of mounts and intromissions to precede ejaculation (Hillegaart, 1991). Intra-NAS infusions - the NAS is a major terminal site for medial raphé 5-HT neurons (see Hillegaart, 1991) - of these serotonergic agonists had compatible effects. The stimulation of NAS 5-HT_{1a} receptors with 8-OH-DPAT decreased both the number of mounts and intromissions to precede ejaculation and the post-ejaculatory refractory period. In the converse experiment, intra-NAS infusions of 5-HT inhibited male sexual behaviour as reflected by the increased number of mounts and intromissions to precede ejaculation and the increased ejaculation latency (Hillegaart, 1991). Finally, the systemic administration of 5-HT₂ post-synaptic receptor antagonists, pirenperone and ketanserin, increases both copulation and ejaculation latencies as well as decreasing the number of mounts and intromissions (Mendelson & Gorzalka, 1985).

The Midbrain Dopamine System and Male Sexual Behaviour: In contrast to the many existing studies of the effects of mPOA lesions, relatively few studies are available to describe the effects of lesioning the mesolimbic DA system on sexual behaviour. Non-selective lesions of the posterior medial forebrain bundle (MFB) produce severe deficits in male copulatory behaviour (Hitt, Hendricks, Ginsberg, & Lewis, 1970; Paxinos & Bindra, 1973), but these may result from the destruction of mPOA efferents that also course through the MFB. Compatible with this interpretation, bilateral lesions of the dorsolateral tegmentum (DLT), a site adjacent to the VTA and substantia nigra (SN) that also receives efferent input from the mPOA via the MFB, severely reduce male copulatory behaviour similarly to mPOA lesions (Brackett & Edwards, 1984; Edwards & Einhorn, 1986;

Brackett, Iuvone, & Edwards, 1986). Moreover, unilateral lesions of the DLT combined with contralateral mPOA lesions replicate the deficits produced by bilateral lesions of either site (Brackett & Edwards, 1984).

Electrolytic lesions of the VTA that decrease NAS DA levels have been reported to significantly increase the latency to reinitiate mounting following the first ejaculation (the post-ejaculatory refractory period), and to non-significantly increase the first mount latency (198 ± 79 s vs 70 ± 9 s) without significantly altering other characteristics of the first ejaculatory series (Brackett et al., 1986). In contrast, two groups of investigators independently reported that VTA lesions shorten the refractory period without altering other measures of copulatory behaviour (Barfield, Wilson & McDonald, 1975; Clark, Caggiula, McConnell, & Antelman, 1975). This substantial discrepancy is puzzling and a resolution is not immediately evident. One possibility that should perhaps be considered is that the shortened refractory periods may not actually represent facilitated sexual activity. In contrast, they may, in fact, reflect diminished ejaculatory experience. In support, post-ejaculatory vocalizations were greatly reduced in these lesioned rats (Barfield et al., 1975; Clark et al., 1975); seminal fluid was often not apparent following the motor display usually indicative of ejaculation (Clark et al., 1975); and the males could be induced to immediately reinitiate copulatory activity after the "ejaculation" by tail pinch suggesting that the males had not entered the "absolute" refractory phase typically arising immediately following ejaculation, but instead had entered a "relative" refractory phase (Barfield et al., 1975). Taken together, one possible interpretation of these data is that these VTA lesioned males did not experience the full ejaculatory reflex, and therefore did not enter into the "absolute" refractory phase. This possibility is further bolstered by a recent report that ejaculation is associated with an abrupt release of NAS DA followed quickly by a sharp reduction in release (Pfaus, Newton, Blaha, Fibiger, & Phillips, 1989). Possibly VTA lesions disallow this DA release thereby preventing a subsequent refractory period, and in turn, misleadingly appear to facilitate sexual behaviour. Alternatively, the contrasting

reports of accelerated and extended returns to mounting might reflect differences in the lesions; contrasting increases (Le Moal, Cardo, & Stinus, 1969; Le Moal, Galey, & Cardo, 1975; Le Moal, Stinus, & Galey, 1976; Tassin, Stinus, Simon, Blanc, Thierry, Le Moal, Cardo, & Glowinski, 1978; Simon, Scatton, & Le Moal, 1980; Koob, Stinus, & Le Moal, 1981; Bunney, Massari, & Pert, 1984) and decreases (Koob et al., 1981) in locomotor activity have also been reported to follow small and large VTA lesions respectively. However, similar electrolytic lesion techniques were employed in all three sexual behaviour studies, and, the offered histology illustrations do not reveal marked differences in the lesioned areas.

At this time there are no reports of the effect of NAS lesions on copulatory behaviour. There is, however, a literature discussing the effects of pharmacological disruptions of NAS DAergic functioning that will be discussed below.

Although some inconsistencies are apparent, the general consensus from reported research strongly supports the supposition that increased levels of synaptic DA are associated with increased sexual behaviour. Most recently, this idea has been explicitly tested by measuring *in vivo* DA levels during sexual activity. This work indicates that both mesolimbic (Ahlenius, Carlsson, Hillegaart, Hjorth, & Larsson, 1987; Pfaus et al., 1989; Pfaus, 1990; Pleim, Matochik, Barfield, & Auerbach, 1990) and mPOA (Pfaus, 1990; Eaton, Moses, & Hull, 1991) synaptic DA and metabolite levels rise during copulatory behaviour, reaching a peak at ejaculation that then abruptly and sharply declines (Pfaus et al., 1989; Pfaus, 1990). A previous, more substantial body of evidence employing DA agonists, DA antagonists, DA lesions, and procedures that affect DA synthesis also indicates the importance of neural activity in ascending DA pathways.

Effects of Dopamine Lesions: The intra-ventricular infusion of 6-OHDA severely disrupts male sexual activity indicating that at least some brain catecholamine pathways are involved in this behaviour (Caggiula, Shaw, Antelman, & Edwards, 1976). Discrete infusions of 6-OHDA also indicate the involvement of the mPOA, a terminal region of the

A14 incerto hypothalamic DA pathway, and the A10 mesolimbic DA pathway in male sexual behaviour.

Selective mPOA DA lesions formed by co-administering systemic DMI, to protect noradrenergic neurons, with AMPT, a catecholamine synthesis inhibitor, and mPOA 6-OHDA, reduced both the proportion of males that intromitted and the number of ejaculations, as well as increasing both the ejaculation latency and the post-ejaculatory refractory period (Bitran et al., 1988; Bazzett, Lumley, Markowski, Bitran, Warner, & Hull, 1990).

Effects of Systemic Dopamine Agonists and Antagonists on Male Sexual Behaviour: Systemic injections of the DA agonists amphetamine (Bignami, 1966; Butcher, Butcher & Larsson, 1969; Leavitt, 1969; Ågmo & Fernández, 1989), cocaine (Leavitt, 1969), apomorphine (Tagliamonte, Fratta, del Fiaco, & Gessa, 1974; Gessa & Tagliamonte, 1975; Malmnäs, 1973, 1977; Paglietti, Pellegrini-Quarantotti, Mereu, & Gessa, 1978; Ahlenius & Larsson, 1984b), amfonelic acid (Ågmo & Fernández, 1989), pergolide (Ahlenius, Engel, Larsson, & Svensson, 1982), LY-163502 (Foreman & Hall, 1987), and the catecholamine precursor L-DOPA (Malmnäs, 1973, 1976; Gessa & Tagliamonte, 1974; Tagliamonte et al., 1974; Paglietti et al., 1978) have all been reported to facilitate male sexual behaviour. It is clear, however, that the effects of relatively extreme doses are often different from that which is seen following the administration of moderate doses. For example, low doses of either amphetamine, cocaine, L-DOPA or the D2 agonist LY-163502 stimulate, whereas higher doses disrupt sexual behaviour (Bignami, 1966; Leavitt, 1969; Malmnäs, 1976; Foreman & Hall, 1987). These inhibitory effects are likely attributable to difficulties in organizing behaviour when the animal is excessively activated and displaying stereotypies. Similarly, very low doses of the direct acting DA agonists apomorphine or LY-163502 inhibit male sexual behaviour, presumably through their actions on higher affinity autoreceptors (Ågmo & Fernández, 1989), whereas higher doses facilitate various measures, likely through direct stimulating actions on

postsynaptic DA receptors (Gessa & Tagliamonte, 1974; Malmnas, 1973, 1976; Paglietti et al., 1978; Foreman & Hall, 1987). Important for arguments that these are centrally mediated DAergic effects, the facilitations from the DA agonists can be blocked by centrally active neuroleptics (Tagliamonte et al., 1974; Malmnäs, 1976, 1977; Foreman & Hall, 1987), but are undiminished following the peripherally acting DA antagonist domperidone (Foreman & Hall, 1987).

Compatible with these reports, inhibitory effects on male sexual behaviour are induced by either DA antagonists (Malmnas, 1973; Tagliamonte et al., 1974; McIntosh & Barfield, 1984; Pehek, Thompson, Eaton, Bazzett, & Hull, 1988; Pfaus, 1990; Pfaus & Phillips, 1989, 1991) or monoaminergic depletors (Butcher et al., 1969), suggesting that sexual stimulation induces a release of DA that is important for the expression of sexual behaviour. In contrast, domperidone is without effect (Falaschi, Rocco, DeGiorgio, Frajese, Fratta, & Gessa, 1981; Pehek et al., 1988a).

Effects of Intra-Cranial Dopamine Agonists and Antagonists on Male Sexual Behaviour: The infusion of DA receptor agonists and antagonists directly into specific brain sites indicates even more clearly that changes in synaptic DA levels are important for engaging in sexual behaviour.

Compatible with the previously discussed lesion studies, DA release in the mPOA seems to regulate penile reflexes and consummatory dimensions of sexual behaviour. Changes in appetitive aspects of sexual behaviour are also sometimes reported following the stimulation or antagonism of mPOA DA receptors, however, this is perhaps not surprising given the nature of the other changes; altering penile reflexes and consummatory components are likely to indirectly alter an animal's interest in sexual behaviour.

The intra-mPOA infusion of post-synaptically active doses of apomorphine decreases the latency to ejaculate, as well as increases the number of ejaculations that are recorded during a given session (Hull, Bitran, Pehek, Warner, Band, & Holmes, 1986; Hull, Warner, Bazzett, Eaton, Thompson, & Scaletta, 1989). This stimulatory effect of

apomorphine is blocked by the co-infusion of the D1/D2 receptor antagonist alpha-flupenthixol (Pehek, Warner, Bazzett, Bitran, Band, Eaton, & Hull, 1988; Hull et al., 1989b). Compatibly, micro-infusions of either alpha-flupenthixol, haloperidol or the D1 antagonist SCH 23390 alone, decrease both the proportion of males that mount or ejaculate and the number of intromissions, as well as increase copulation latencies and the post-ejaculatory refractory period (Pehek et al., 1988a; Hull et al., 1989b; Pfaus, 1990; Pfaus & Phillips, 1991; Warner, Thompson, Markowski, Loucks, Bazzett, Eaton, & Hull, 1991). Interestingly, these effects are mimicked by intra-mPOA infusions of the D2 agonist LY-163502 (Hull et al., 1989b) suggesting that the ratio of mPOA D2/D1 receptor stimulation plays an important role in regulating consummatory dimensions of male copulatory behaviour. Alternatively, seemingly paradoxical actions of putatively selective ligands for DA receptor subtypes might reflect currently undetermined effects at the very recently identified D3, D4 and D5 receptor subtypes (for a recent discussion of newly identified DA receptors, see Lewin, 1991).

The DA neurons of the mPOA also appear to regulate penile reflexes. Intra-mPOA infusions of either the selective D2 antagonist raclopride or the mixed D1/D2 antagonist alpha-flupenthixol, inhibit penile reflexes (Hull, Eaton, Thompson, Bazzett, & Markowski, 1989; Warner et al., 1991). Compatible with these reports, the specific D1 agonist, thienopyridine, increases the number of erections and ejaculations (Markowski, Eaton, Lumley, Moses, & Hull, 1990; Hull, Eaton, Markowski, Lumley, Moses, Dua, & Loucks, 1991). More difficult to incorporate into this scheme is the report that the D2 agonist, LY-163502 (quinelorane), decreases erections and penile reflexes while increasing seminal emissions (Hull et al., 1989a; Hull, et al., 1991). As suggested by Markowski et al., (1990), these results might indicate that D1 receptor activation stimulates penile reflexes whereas D2 receptor activation stimulates sympathetic pathways to increase seminal emissions; in contrast to what is seen during the proportionately unmanipulated D1 and D2

receptor activation from endogenous DA, the selective activation of D2 receptors might increase seminal emissions to the detriment of erectile reflexes.

When administered at a dose that decreases penile reflexes, the intra-mPOA infusion of the D1/D2 antagonist alpha-flupenthixol has also been reported to decrease the number of times that males choose a box previously paired with an estrous female. The authors interpreted this to indicate that penile reflexes might be related to sexual motivation (Loucks & Hull, 1990; Warner et al., 1991). The mPOA has also been suggested to directly affect sexual motivation, independent of penile reflexes. Intra-mPOA infusions of neuroleptics decrease the display of anticipatory behaviours prior to the estrous female's introduction (Pfaus, 1990; Pfaus & Phillips, 1991), and reduce the frequency that males choose the female goal box in an X-maze (Warner et al., 1991).

Infusions of post-synaptically active doses of apomorphine into the NAS have revealed effects compatible with a role of mesolimbic DA systems in sexual motivation; intra-NAS apomorphine decreased the latency to begin intromitting in sexually experienced male rats (Hull et al., 1986). Although, as the authors noted, this effect was of only borderline significance [$p=.057$] it should be recognized that the intromission latencies of sexually experienced, gonadally intact males are already so swift as to be difficult to shorten. In spite of this difficulty, mount and intromission latencies have been significantly reduced by intra-NAS infusions of amphetamine (Everitt et al., 1989). In the converse experiment, intra-NAS infusions of haloperidol decreased anticipatory behaviours (Pfaus, 1990; Pfaus & Phillips, 1991). Further compatible with a role for NAS DA in the development and maintenance of incentive stimuli motivating properties, intra-NAS amphetamine infusions both increased the instrumental responding of male rats to gain access to secondary stimuli previously associated with an estrous female, and moreover, reversed amygdala lesion-induced decrements in instrumental responding for stimuli associated with estrous females (Everitt et al., 1989). As the authors discuss, this latter

finding indicates the importance of amygdala to NAS neuronal projections in the ability of conditioned stimuli to exert control over sexual behaviour.

Intra-VTA infusions of DA receptor agonists and antagonists also indicate the importance of this pathway. Infusions of apomorphine into the cell body region, where it presumably impinges on neuronally inhibitory autoreceptors, increased latencies to both the first intromission (Hull, Bazzett, Warner, Eaton, & Thompson, 1990) and to enter a female goal box (Hull et al., 1991) as well as increasing the number of failed mount attempts (Dua & Hull, 1990; Hull et al., 1991), and decreasing the number of ejaculations (Dua & Hull, 1990). Compatible with these reports, the intra-VTA infusion of alpha-flupenthixol, which presumably acted on somatodendritic autoreceptors to stimulate terminal DA release, decreased the intromission latency (Hull et al., 1990).

Opioids and Male Sexual Behaviour

There are three kinds of evidence to support the idea that the endogenous opioid systems play a role in the expression of male sexual behaviour. First, copulation induces analgesia that is apparent both during, and immediately after, ejaculation in male rats, whereas males are hyperalgesic later in the post-ejaculatory refractory period (Barfield & Sachs, 1968; Sachs & Barfield, 1974; Pollak, & Sachs, 1975; Szechtman, Simantov, & Hershhorn, 1979; Szechtman, Hershhorn, & Simantov, 1981). Although it cannot be stated conclusively that this analgesia is opioid mediated since no attempt was made to block these effects with opioid receptor antagonists, Szechtman and his colleagues (Szechtman et al., 1979, 1981) did find that midbrain opioid levels were diminished following 120 min of sexual activity. This finding may reflect a drained pool of endogenous opioids due to massive release, or it may be attributable to a reduced synthesis of the peptides. Supporting this idea that male copulation is accompanied by opioid release is a finding in male hamsters that plasma β -endorphin levels were elevated following repeated ejaculations (Murphy, Bowie, & Pert, 1979). Third, a substantial literature

reports that both opioid agonists and antagonists have both disruptive and facilitatory influences on male sexual behaviour. This literature is described in detail below.

Opioid Antagonists Facilitate Male Sexual Behaviour: Many studies report that opioid receptor antagonists stimulate sexual behaviour. High doses of systemic naloxone or naltrexone (2.0-30.0 mg/kg) have been demonstrated to decrease the latencies to mount, intromit and ejaculate (Pellegrini-Quarantotti, Paglietti, Bonanni, Petta, & Gessa, 1979; Myers & Baum, 1979, 1980; McIntosh et al., 1980); to evoke these behaviours in previous repeated non-copulators (Gessa, Paglietti, & Pellegrini-Quarantotti, 1979; McIntosh, Vallano, & Barfield, 1980); to decrease the number of intromissions necessary to induce ejaculation (Pellegrini-Quarantotti et al., 1979; Myers & Baum, 1979, 1980); to increase the proportion of males that ejaculate within five min of being paired with an estrous female (Hetta, 1977); to increase the time males spend copulating before reaching sexual exhaustion (Pfaus & Gorzalka, 1987a) and to induce penile erections in both rats (Ferrari & Baggio, 1982; Berendsen & Glower, 1986) and humans (Mendleson, Ellingboe, Keuhnle, & Mello, 1979). Additionally, the intrathecal administration of naloxone has been reported to decrease the number of intromissions necessary to elicit ejaculation (Wiesenfeld-Hallin & Sodersten, 1984). Finally, intra-mPOA infusions of naloxone 15 min before testing decreased both ejaculation latencies and the post-ejaculatory refractory period (Band, Warner, Pehek, Bitran, Holmes, & Hull, 1986). Although this last report is compatible with the above studies, the site specificity of the effect must be interpreted cautiously due to naloxone's notoriously lipophilic behaviour; potentially, the facilitatory effects might have been mediated anywhere in the brain. This warning acquires greater weight when noting the unexpectedly long latency preceding the induction of naloxone's influence.

Endocrinologically, opioid antagonists have been found to increase testosterone and lutenizing hormone (LH) levels - LH is an anterior pituitary gonadotroph hormone that stimulates the interstitial cells of the testis (Leydig cells) to produce androgens - in both rats (McConnell, Baum, & Badger, 1981) and rhesus monkeys (Abbott, Holman, Berman,

Neff, & Goy, 1984), and to increase LH in humans (Mirin, Mendelson, Ellingboe, & Meyer, 1976; Mendelson et al., 1979). Plausibly, these findings provide ready explanations for the increases in sexual behaviour that have been observed to follow the administration of opioid antagonists. Compatibly, systemic LH-releasing hormone (LHRH) injections elicit a facilitated male sexual response that is similar to that seen following naloxone administration (Myers & Baum, 1980). The authors of this report hypothesized that sexual stimulation elicits an endorphin release that attenuates LHRH release. Such an attenuation would extend both the ejaculation latency, and concurrently, the time in which the male can stimulate the female. This continued stimulation may be important for reproduction since pregnancy is less likely if ejaculation occurs too soon or is preceded by too few intromissions (Adler, 1969). For similar reasons naloxone's facilitatory actions produced at the spinal level may block a normal endogenous opioid-induced attenuation of penile sensory information.

The extremely high doses of naloxone employed in many of these studies might also account for some of its sexually facilitatory properties. High doses of naloxone have been reported to have DAergic-agonist like activity; systemic naloxone potentiated apomorphine-induced contraversive circling in unilaterally DA lesioned rats (Hirshhorn, Hiltner, Gardner, Cubells, & Makman, 1983). The authors attributed this finding to a possible naloxone-induced diminishment of endogenous opioid inhibition of striatal DA, and hence, an elevated release of DA that would impinge upon supersensitive receptors. However, as the authors themselves noted, the significance of this effect in the intact rat is likely to be negligible. Moreover, at least one study has found that the facilitatory effect of naloxone (30.0 mg/kg, i.p.) to delay the onset of sexual exhaustion was not significantly diminished by a pimozide (0.4 mg/kg, s.c.) pretreatment (Pfaus & Gorzalka, 1987a). This might indicate that DA is not involved in the facilitatory effects of high doses of naloxone, but the reasons for, and mechanisms of, these extended periods of sexual activity have yet to be determined. Moreover, these same authors report that pimozide alone did not affect

this variable. This latter finding stands in marked contrast to other reports confirming the sexually disruptive properties of neuroleptics.

Opioid Agonists Inhibit Male Sexual Behaviour: Compatible with these excitatory effects of opioid antagonists on sexual behaviour, the systemic administration of morphine can induce inhibition of male sexual behaviour that is apparent across a wide range of medium to high doses in different species. Moderate doses (1.0-6.0 mg/kg, i.p. or s.c.) have been reported to decrease both the frequency of mounting, intromitting and ejaculating (Hetta, 1977; McIntosh et al., 1980) and the proportion of tested animals that display these behaviours (Pfaus & Gorzalka, 1987a), as well as to increase the latencies to mount, intromit (Lieblich, Baum, Diamond, Goldblum, Iser, & Pick, 1985; Pfaus & Gorzalka, 1987a) and ejaculate (Lieblich et al., 1985a). These effects can be blocked by naloxone pre-treatments (McIntosh et al., 1980). Higher doses of morphine (5.0-20.0 mg/kg, i.p.) have even more potent effects, and diminish female-directed behaviour (Mumford & Kumer, 1979). Chronic treatments with extremely high doses (100.0 mg/kg, s.c. or i.p. for 4-5 weeks) can be particularly devastating: in these studies female-directed behaviour was disorganized, and mounting was all but abolished (Tokunaga, Mukari, & Hosoya, 1977; Mumford & Kumer, 1979).

Similar acute effects have been observed in both morphine treated mice (Landauer & Balster, 1982) and Pigtail Macaque monkeys (Crowley, Stynes, Hydingier, & Kaufman, 1974), and methadone injected hamsters (Murphy, 1981).

Intracerebroventricular (i.c.v) infusions of morphine elicit behavioural effects similar to systemic administrations indicating that these are central nervous system effects. In one study 1.0-5.0 μg given i.c.v dose-dependently decreased the proportion of males that mounted, and disrupted female-directed behaviour (Meyerson, 1981). These inhibitory effects were produced in the absence of motor impairments. Similarly, i.c.v. infusions of β -endorphin - a mixed mu, delta, epsilon receptor agonist - have been reported to decrease the proportion of males that mounted an estrous female (Meyerson & Terenius,

1977; McIntosh et al., 1980; Meyerson, 1981). Among those animals that did mount, the mount latencies were prolonged and fewer mounts were exhibited (Meyerson & Terenius, 1977). These inhibitory effects could be blocked by either naloxone or naltrexone pretreatments (Meyerson & Terenius, 1977; Meyerson, 1981).

Morphine also appears able to produce inhibitory actions on sexual behaviour at the level of the spinal cord; intrathecal morphine (1.0-10.0 μg , i.t.) has been found to increase the number of intromissions to precede ejaculation, and this effect is blocked by systemic naloxone pretreatment (Wisensfeld-Hallin & Sodersten, 1984). This inhibiting effect of morphine given i.t. likely results from the disruption of sensory information being relayed from the penis (Nunez, Gross, & Sachs, 1983).

The selective stimulation of delta receptors has also been found to disrupt sexual behaviour; i.c.v infusions of the long-lasting synthetic enkephalin DALA, a *delta* receptor agonist, increased both mount and intromission latencies at doses observed not to disrupt motor behaviour (Pellegrini-Quarantotti, Corda, Paglietti, Biggio, & Gessa, 1978).

Inhibitory effects of β -endorphin are also apparent when it is infused directly into the mPOA. Picomole doses of β -endorphin given into the mPOA can completely abolish mounting, intromitting and ejaculating (Hughes, Everitt, & Herbert, 1987, 1989; Stavy & Herbert, 1990). In contrast, this same treatment increased female-directed behaviour, suggesting that the decremental effects of mPOA β -endorphin were specific to consummatory aspects of the behaviour while leaving the appetitive components intact. These effects of β -endorphin were blocked by either systemic naloxone or intra-mPOA co-infusions of the specific *delta* receptor antagonist ICI 174864. Moreover, mPOA infusions of ICI 174864 alone augmented mount rates while decreasing post-ejaculatory refractory periods (Hughes et al., 1987). It would appear that endogenous mPOA *delta* receptor agonists function normally to attenuate sexual activity.

These inhibitory effects of intra-mPOA β -endorphin appear to be specific to sexual behaviour; mPOA β -endorphin did not affect the amount of time spent consuming a sweet solution (Hughes et al., 1987, 1989).

This specificity of effects resulting from mPOA infusions of β -endorphin has been further explored in an intriguing series of experiments (Stavy & Herbert, 1990). Replicating the earlier findings, mPOA infusions of β -endorphin were again observed to inhibit mounting, intromitting and ejaculating when the peptide was administered 3-6 min prior to being paired with an estrous female. In provocative contrast, if β -endorphin was infused following the first intromission, the drug was without effect; once the male had begun to intromit, intra-mPOA β -endorphin induced no disruptions. This nullification of β -endorphin's inhibitory effect by a single intromission was apparent up to two h after the drug infusion. This long-lasting effect of an intromission was not attributable to the metabolism of β -endorphin; following a six h delay the previous β -endorphin infusion again abolished mounting. Conversely, an intromission did not diminish the effect of β -endorphin if the male was re-paired with a different female. Allowing the male to mount a female with a taped vagina, so as to prevent penetration, was less effective than allowing a full intromission, and allowing the male limited interaction with the female separated by wire mesh was completely ineffective; β -endorphin fully inhibited mounting. These findings suggest mPOA infusions of β -endorphin disrupt the male's ability to associate interest in the female with the appropriate behavioural response; in the absence of adequate previous interactions with the female, the male is unable to "translate investigatory behaviour into mounting activity". Moreover, this may indicate that a normal function of the engaged mechanism is to regulate the association between incentive stimuli and appropriate behaviours (Stavy & Herbert, 1990).

At least some of the inhibitory effects seen following opioid administration are believed to be attributable to disrupted functioning of the hypothalamic-pituitary-gonadal axis; suppressions of serum LH and consequent decreases in circulating testosterone have

been reported following both acute and long-term systemic narcotic treatment, and disturbed functional and structural integrity of secondary sex organs are apparent following repeated administrations. The morphine-induced hormonal reductions are opioid antagonist reversible, and have been observed in both rats (Cicero, Meyer, Wiest, Olney, & Bell, 1975; Cicero, Meyer, Beli, & Koch, 1976; Cicero, Wilcox, Bell, & Meyer, 1976; Cicero, Bell, Meyer, & Schweitzer, 1977; Tokunaga et al., 1977; Cicero, Meyer, Gabriel, Bell, & Wilcox, 1980) and humans (Mendelson, Mendelson, & Patch, 1975; Mendelson, Meyer, Ellingboe, Mirin, & McDougle, 1975; Mirin et al., 1976; Mirin, Meyer, Mendelson, & Ellingboe, 1980). Interestingly, in rats, these opioid mediated hormonal changes are not apparent until puberty (Cicero, Schmoeker, Meyer, Miller, Bell, Cytron, & Brown, 1986). Morphine is believed to suppress LHRH and LH release by acting directly on the mediobasal hypothalamus and anterior pituitary gonadotrophs; the metabolism of testosterone is not disturbed in the secondary sex organs by repeated morphine treatments (Cicero et al., 1977), and morphine blocks the castration-induced elevation of LH (Cicero et al., 1980), but it has no effect on the hypophysectomy-induced decrease in secondary sex organ weight (Cicero et al., 1976).

Other aspects of morphine's inhibitory effect on male sexual behaviour might result from non-specific sedative actions produced by opioid receptor stimulation in the periaqueductal gray area (Broekkamp, Van den Boggard, Heyen, Rops, Cools, & Van Rossum, 1976). In contrast, the inhibitory effects seen following intra-mPOA infusions of β -endorphin seem to be specific disturbances of male sexual behaviour regulatory mechanisms. Interestingly, Hughes et al., (1989) have also reported that other peptides derived from the same precursor as β -endorphin, pro-opiomelanocortin (POMC), stimulate male sexual behaviour. This observation led the authors to suggest that one of the mechanisms by which castration reduces sexual behaviour might be through changes in the ratio of available POMC-derived peptides.

In summary, it seems possible that sexual stimulation evokes a release of endogenous opioids, both spinally and centrally, that acts to inhibit, or perhaps more accurately, extend, the pre-ejaculatory phase of male sexual behaviour. In contrast to this apparently reproductively beneficial release of endogenous opioids, it seems that sexual stimulation might also engage mechanisms that suppress the release of mPOA endorphins to prevent their consequent activation of *delta* receptors. This anatomically selective suppression of endorphin release might allow the male to better direct undifferentiated excitement toward a specific sexual goal and behaviour. The addition of exogenous opioids might both exaggerate the inhibitory effects of endogenous opioids, and further diminish sexual activity through the suppression of LHRH, LH and testosterone. Conversely, the systemic administration of opioid receptor antagonists might possibly foreshorten the pre-ejaculatory phase by blocking the effects of endogenous opioids spinally and centrally, as well as further facilitating sexual activity by stimulating the release of LH and testosterone.

Opioid Antagonists Inhibit Male Sexual Behaviour: Contrasting with the substantial literature described above, there is also evidence to indicate that opioid antagonists can both inhibit sexual behaviour and reduce the rewarding impact of ejaculating. These findings are perhaps most clear among castrated males in which the display of sexual behaviour is now a tenuous probability, but compatible findings are available among gonadally intact males as well.

Among castrated males the systemic administration of naloxone has been reported to increase mount, intromission and ejaculatory latencies (Lieblich et al., 1985a), and to decrease both the mount rate and proportion of animals that ejaculate (McConnell et al., 1981; Miller & Baum, 1987). Among castrated males maintained on low doses of testosterone, naloxone prevented the tail pinch-induced increases in both female-directed behaviour and the proportions of animals that mounted, intromitted or ejaculated. These findings suggest that tail pinch evoked a release of endogenous opioids that stimulated

sexual behaviour (Leyton & Stewart, 1990). Among gonadally intact male rats, systemic naloxone has been reported to increase the percentage of non-copulators among sexually naive males (Arcila, Berenfeld, & Ågmo, 1990); to increase the post-ejaculatory refractory period (McConnell et al., 1981; Szechtman et al., 1979, 1981; Sachs, Valcourt, & Flagg, 1981); to decrease the proportion of experienced male copulators that ejaculated (Ågmo & Berenfeld, 1990); to decrease penile flips (Sachs et al., 1981); and to potentiate the suppression of sexual behaviour induced by LiCl (Peters, Koch, & Blythe, 1988). Among both castrated and gonadally intact animals provided with a sweet solution to drink for 31 days - a procedure believed to elevate central levels of endogenous opioids, and that was found to elevate mount rates - systemic naloxone decreased mount rates (Lieblich, Shaviv, & Cohen, 1985). Finally, the intra-mPOA infusion of naloxone 15 min prior to testing has been reported to dose-dependently disrupt copulation (Band & Hull, 1987).

Unfortunately, this last finding should be interpreted with caution as naloxone is lipophilic and might have induced its inhibitory effects anywhere that it could spread. This caution seems especially warranted when one recalls that these same researchers had previously reported that intra-mPOA naloxone facilitates sexual behaviour (Band et al., 1986). The authors speculated that the discrepancies might be attributable to different baseline copulatory performance.

By employing the conditioned place preference paradigm, it has been demonstrated that naloxone attenuates the rewarding characteristics of a place previously associated with copulation; both the expression of a formerly developed place preference can be decreased when animals are tested with naloxone (Miller & Baum, 1987; Arcila et al., 1990), and if the animals are administered naloxone prior to the ejaculation, its development can be prevented. Moreover, in this latter condition, where animals were administered naloxone prior to copulation, a place aversion results instead (Ågmo & Berenfeld, 1990).

Similar findings have also been reported following the injection of opioid antagonists to primates. Among talapoin monkeys, naltrexone decreased the males'

inspection of females, the number of mounts, intromissions and ejaculations, and the degree of solicitation received from the females; apparently the uninterested males were also perceived as being less interesting to the females (Meller, Keverne, & Herbert, 1980).

Among rhesus monkeys, both naltrexone and naloxone were reported to inhibit male sexual behaviour. During two weeks of daily injections, the opioid antagonists were observed to decrease the number of males that engaged in sexual behaviour. Among those males that did act, the antagonists inhibited normal pre-mounting behaviour and reduced the number of ejaculations. During the 25 days following the drug administration period, these animals were found to display reduced levels of mounting and intromitting, and ejaculations were completely absent. These last observations further indicate that opioid antagonists can decrease the reward value of sexual behaviour, and therefore, make future sexual activity seem less appealing even to drug-free subjects (Abbott et al., 1984).

Opioid Agonists Facilitate Male Sexual Behaviour: Compatible with these findings, opioids have also been reported to potentiate some measures of male sexual behaviour. Using the same dose range employed by earlier investigators, Ågmo and Paredes (1988) have reported that morphine (2.5, 5.0 mg/kg i.p.) reduces both the latency to the second ejaculation and the post-ejaculatory refractory period. The probable explanation for this apparent discrepancy lies in the timing of injection. In the studies described earlier, systemic morphine injections were typically made 5-30 min before the test for sexual behaviour. Conversely, in the Ågmo and Paredes (1988) study, animals were injected 60 min prior to the test. This temporal difference is important given the now well established finding that the moderate to high doses of systemic morphine utilized in these studies elicit biphasic motor responses; initial depressant actions are followed later by elevations in locomotor activity (Vezina & Stewart, 1984). Compatible with this explanation, Ågmo and Paredes (1988) also reported that exclusively inhibitory effects of the drug were seen when animals were tested only five min after morphine injections.

The excitatory effects of morphine on sexual behaviour are most clearly seen when it is selectively administered intra-cranially so as to bypass masking inhibitory effects mediated elsewhere. Intra-VTA infusions of morphine elicit exclusively excitatory effects on sexual behaviour when administered to castrated males maintained on a schedule of low dose testosterone; this treatment dose-dependently increased both the proportion of time males spent engaging in female-directed behaviour and the proportion of animals that mounted, and these effects were blocked by systemic naloxone (Mitchell & Stewart, 1987, 1990a). Moreover, the repeated administration of intra-VTA morphine to either castrated or gonadally intact males produced conditioned elevations in female-directed behaviour, when tested following a saline infusion. Intact males also displayed a morphine-induced conditioned reduction in the latency to initiate mounting (Mitchell & Stewart, 1990b). These findings suggest that both morphine-induced and conditioned morphine-induced releases of DA increase sexual motivation.

Similarly, morphine infused into the NAS of gonadally intact males also elevated measures of appetitive aspects of sexual behaviour: intra-NAS morphine shortened the intromission latency, and elicited more ejaculations from reluctant copulators (Band & Hull, 1988, 1990). These effects were blocked by pretreatments with systemic naloxone. Morphine infused into the mPOA also stimulated sexual behaviour, but the effects suggested changes in both appetitive and consummatory aspects of sexual behaviour; intra-mPOA morphine decreased both the ejaculation latency and the number of intromissions to precede the ejaculation (Band & Hull, 1988, 1990).

Central administrations of dynorphin (DYN) fragments have also been reported to elevate sexual behaviour. The i.c.v. administration of leumorphin (DYN B(1-31)) has been observed to naloxone-reversibly and dose-dependently produce emissions of semen, following a 20-30 min delay, in urethane-anaesthetized male rats. This effect could not be replicated by either DYN A or DYN B(1-17). Conversely, seminal emissions could be produced by i.t. injections of leumorphin, and these same authors observed leumorphin-

like immunoreactivity at the lumbral and sacral regions of the spinal cord, indicating that the effect might be spinally mediated (Imura, Kato, Nakai, Tanaka, Jingami, Koh, Yoshimasa, Tsukada, Sakamoto, Morii, Takahashi, Tojo, & Sugawara, 1985).

Among castrated males maintained on low doses of testosterone, the intra-VTA infusion of DYN A₍₁₋₁₃₎ elicits increases in both female-directed behaviour and the proportion of males that mount (Mitchell & Stewart, 1987, 1990a). This effect of DYN was also blocked by systemic naloxone. In contrast to what is seen following repeated intra-VTA morphine administrations, the repeated intra-VTA infusion of DYN does not result in a conditioned elevation of female-directed behaviour, suggesting that, in spite of other behavioural similarities, the mechanism of action is different for the two opioids.

When DYN is infused into the mPOA the effects are also similar to those seen following intra-mPOA morphine treatments; both the latency to ejaculate and the number of intromissions necessary to evoke ejaculation are reduced (Band & Hull, 1988, 1990).

At this time it is unclear which receptor system is mediating these dynorphin-induced elevations in sexual behaviour. That their effects in the VTA could be blocked by naloxone pretreatments would seem to confirm that the effects were opioid, but the observation that these infusions did not elicit conditioned increases, as did morphine, would seem to indicate that, in spite of other similarities in their effects, DYN's actions were non- μ (*non- μ*). A second possibility that must be considered is that these effects resulted from the stimulation of *k* opioid receptors. DYN is the putative endogenous *k* opioid receptor agonist, and its affinity for this receptor site is much superior to morphine's as well as being somewhat greater than its own affinity for μ receptors. The reported effects of selective *k* receptor agonists do not allow one to unequivocally predict their influence on male sexual behaviour, but a substantial literature has accumulated describing many of their aspects anatomically, electrophysiologically, pharmacologically and behaviourally.

Dynorphin

Dynorphin Molecular Biochemistry: The multiple DYNs are a family of both opioid and non-opioid peptides derived from the propeptide precursor prodynorphin, also known as preproenkephalin B. The different members share in common the pentapeptide leu-enkephalin at their N-terminal, and are individually distinguished by different C-termini. Prodynorphin can be enzymatically cleaved into β -neo-endorphin, alpha-neo-endorphin, DYN A(1-17), and DYN B (rimorphin). Among others, DYN A can be further broken down into DYN A(1-5) (leu-enkephalin), DYN A(1-7), DYN A(1-8), DYN A(1-9), DYN A(2-9), and DYN A(1-13). Conversely, a C-terminal extended DYN B product, leumorphin, is also possible (Fallon & Leslie, 1986; Fallon & Ciofi, 1990).

The various DYNs are the putative endogenous ligands for the k opioid receptor (Chavkin & Goldstein, 1981; Quirion & Pert, 1981; Corbett, Patterson, McKnight, Magnan, & Kosterlitz, 1982; Garzón, Sánchez-Blázquez, Höllt, Lee, & Loh, 1983; Goldstein & James, 1983; Young, Walker, Houghton, & Akil, 1983; Garzón, Sánchez-Blázquez, Gerhart, Loh, & Lee, 1984; Gairin, Gouarderes, Mazarguil, Alvinerie, & Cros, 1985; Leslie, 1987; Mulder, Wardeh, Hogenboom, & Frankhuyzen, 1989). The DYNs' selectivity for k opioid receptors is not exclusive; they display only 1.2 to 30 times the affinity for k than for either μ or δ receptors, with the larger fragments displaying more, and the smaller fragments displaying less preferential affinity for the k receptor. Conversely, the much greater potency of DYNs at k receptors makes them approximately 1000 fold more effective at this binding site (Quirion & Pert, 1981; Garzón et al., 1983, 1984; Young et al., 1983; Chavkin, Henriksen, Siggins & Bloom, 1985; Mulder et al., 1989). The smaller peptides, DYN A(1-7), DYN A(1-8) and DYN A(1-9) act as inhibitory neurotransmitters, are rapidly degraded by peptidases, have only moderate opioid potency, and appear to have potent non-opioid effects (Walker, Moises, Coy, Baldrighi, & Akil, 1982; Moises & Walker, 1985; Robertson, Hommer, & Skirbol, 1987). Conversely, DYN A(1-13) and DYN A(1-17) are substantially more resistant to peptidases, display

significantly greater opioid receptor potency, and may act as neurohormones (Corbett et al., 1982). Oposing the substantially greater opioid potency of the larger fragments - DYN A(1-8) has been estimated to have only 3% of DYN A(1-17)'s opioid potency (Weber, Evans, & Barchas, 1982) - DYN A(1-8) immunoreactivity levels indicate that it is available in rat midbrain and striatum in 10 times greater concentrations than DYN A(1-17) (Weber et al., 1982; Dores, Lewis, Khachaturian, Watson & Akil, 1985).

The *k* receptors themselves have been suggested to have up to six receptor subtypes: *k1a*, *k1b*, *k2a*, *k2b*, *k3* and *R1* (Clark, Liu, Price, Hersh, Edelson, & Pasternak, 1989; Tiberi & Magnan, 1989; Rothman, Bykov, de Costa, Jacobson, Rice, & Brady, 1989, 1990; Devlin & Shoemaker, 1990; Kinouchi & Pasternak, 1990). Conversely, in a recent series of papers it has been argued that the nominal *k2* receptors are *epsilon*, rather than *k* (Nock, Rajpara, O'Connor, & Cicero, 1988; Nock, Giordano, Cicero, & O'Connor, 1990; Giordano, Nock, & Cicero, 1990; Rothman et al., 1990), and that the *R1* is a *sigma* receptor (Devlin & Shoemaker, 1990). These suggestions are based on the following observations. The anatomical distribution of *k* receptors was originally identified by using the 6,7-benzomorphans (e.g., ethylketocyclazocine and bremazocine). Although it is clear that these ligands bind to non- μ and non-*delta* receptors, not all of the receptors with which they do bind are necessarily *k*. Receptor mapping studies suggest that the *k2* receptors correspond closely to β -endorphin pathways whereas the *k1* receptors correspond more closely to dynorphin pathways. Further, the more recently developed highly specific *k* receptor agonists, U-50,488H and U-69,593 bind with much greater affinity to the *k1* sites than to the *k2*, particularly *k2b*. Taken together these observations have suggested to some writers that the *k2* and *R1* receptors are the *epsilon* and *sigma* binding sites for endogenous β -endorphin. Similarly, overlapping characteristics of the *k2b* and *k3* receptors suggest that these may be different names given by different writers to the same receptor. These arguments are appealing, but at this early time have yet to be widely considered. Certainly the functional significance of these different receptor subtypes remains largely

open to speculation, although some evidence suggests that DYN A binds with high affinity to both *k1a* and *k1b*, whereas DYN B and alpha-neo-endorphin display higher affinity for *k1a* than *k1b* receptors (Clark et al., 1989; Rothman et al., 1990).

Dynorphin Anatomy: The anatomical distribution of opioid receptors, cell bodies, and pathways receiving and carrying DYNs is well described (Watson, Khachaturian, Akil, Coy, & Goldstein, 1982; Weber et al., 1982; Chesselet & Graybiel, 1983; Quirion, Weiss, & Pert, 1983; Zamir, Palkovits, & Brownstein, 1983; Fallon, Leslie, & Cone, 1985; Haber & Watson, 1985; Lynch, Watt, Krall, & Paden, 1985; McGinty, 1985; Besson, Graybiel, & Quinn, 1986; Fallon & Leslie, 1986; Code & Fallon, 1986; Mansour, Lewis, Khachaturian, Akil, & Watson, 1986; Mansour, Khachaturian, Lewis, Akil, & Watson, 1987; Nock et al., 1988; Uhl, Navia, & Douglas, 1988; Gerfen & Young, 1988; Adler, Goodman, & Pasternak, 1990; Desjardins, Brawer, & Beaudet, 1990; Neal & Newman, 1991). This work makes it clear that DYNergic cell bodies, fibers of passage and receptors are widely distributed throughout the brain in patterns that are distinctively different from other opioid systems.

In a recent study that employed three preprodynorphin probes and two preproenkephalin probes, so as to avoid previous difficulties in cross-reactivity and quantification, DYN mRNA could be clearly detected in the striatum and was apparent in neurons different from those expressing enkephalin mRNA; only 1/40 examined striatal neurons displayed positive hybridization for the probes of both mRNA. Both DYN and enkephalin mRNA were most concentrated in medium-sized neurons (diameter < 30µm) of the ventral striatum (NAS) (Uhl et al., 1988). Similarly, a study employing *in situ* hybridization histochemistry with cDNA oligonucleotide probes also reported the presence of DYN expressing neurons in the striatum (Gerfen & Young, 1988), and these findings are consistent with other reports on rat (Weber et al., 1982; Watson et al., 1982; Fallon & Leslie, 1986; Anderson & Reiner, 1990), cat (Besson et al., 1986), bird (Anderson & Reiner, 1990), reptile (Anderson & Reiner, 1990) and human brain (Haber & Watson,

1985). Interestingly, in the striatonigral pathways of each of these studied non-human species, DYN has been commonly found to be co-localized with the excitatory neuropeptide substance P (SP) (Besson et al., 1986; Anderson & Rainer, 1990).

Lesion studies confirm the presence of a descending striatonigral DYN pathway. Striatal cell body ibotenic acid lesions deplete the SNR of DYN, SP and leu-enkephalin, but not met-enkephalin. Moreover, these same lesions produce parallel decrements in fibers labelled for DYN and SP as well as GABA (Christensson-Nylander, Herrera-Marschitz, Staines, Hökfelt, Terenius, Ungerstedt, Cuello, Oertel, & Golstein, 1986). These findings support two previously considered ideas: first, that DYN, SP and GABA neurons all project from the striatum to the SNR; and second, that in the SNR leu-enkephalin may be derived from prodynorphin.

In a more extensive study examining many more brain areas, Fallon and Leslie (1986) also identified evidence for DYN's presence in medium sized striatal cells. In their study, however, DYN B immunoreactivity was more concentrated in the dorsal striatum (caudate-putamen) than in the ventral striatum (NAS). Conversely, DYN A(1-8) labelled cell bodies were apparent in equal quantities to DYN B in the dorsal striatum, but were more plentiful than DYN B cell bodies in the NAS. Light to very dense DYN B and DYN A(1-8) labelled fibers were also apparent in the NAS. These DYN neurons project to SN-VTA sites, and extend local collaterals within the striatum itself (J. Fallon, personal communication to Trujillo, Day, & Akil, 1990). In the VTA - a likely projection site of NAS DYN cells - very sparse labelling of DYN cell bodies was apparent, but medium labelling of both DYN B and DYN A(1-8) fibers was detected. Perhaps noteworthy, the VTA might also receive DYN projections from both the amygdala, and lateral and medial hypothalamus (Fallon et al., 1985). In the mPOA, medium density immunoreactivity for both DYN B and DYN A(1-8) cells and fibers were found (Fallon & Leslie, 1986). Similar distributions for DYN A(1-13) cell body immunoreactivity have also been reported in the MPN (Zamir et al., 1983), the mPOA subnucleus most strongly implicated to have

regulatory functions in sexual behaviour. Finally, in the male hamster, and perhaps in the male rat, prodynorphin containing neurons in the medial nucleus of the amygdala, the lateral hypothalamus, and the medial bed nucleus of the stria terminalis have been identified to project to the mPOA (Neal & Newman, 1991). Interestingly, as had been seen in the striatonigral DYN containing neurons, these mPOA projecting DYN neurons also displayed co-localized SP.

The reported distribution of *k* receptors is compatible with the known anatomy for DYN cell bodies and pathways. Autoradiographic studies indicate that *k* receptors are densely distributed in both the NAS and mPOA, though, only lightly present in the VTA (Quirion et al., 1983; Lynch et al., 1985; Mansour et al., 1987). More recent work, utilizing *k* ligands that are more selective for *k1* binding sites, confirm the high density presence of *k1* receptors in the NAS and mPOA (Nock et al., 1988).

Mu receptors, for which DYN also displays considerable affinity, are similarly apparent in the VTA, NAS and mPOA. In contrast to the *k* receptors, however, there appear to be low μ densities in the mPOA, medium μ densities in the VTA, and high μ densities in the NAS (Mansour et al., 1987).

A recent study examining the pre- and postsynaptic distribution of opioid receptors in the spinal cord indicates that *k* receptors are evenly distributed between the two locales (Besse, Lombard, Zajac, Roque, & Besson, 1990). In contrast to this even distribution reported for *k* receptors, μ receptors were observed to be predominantly presynaptic (76%) (Besse et al., 1990). It should be cautioned, however, that although the proportion of receptors identified as *k*, relative to μ and *delta* receptors, was compatible with studies employing specific *k1* ligands, this study utilized a non-discriminating benzomorphan to competitively label *k* binding sites. As discussed above, the true significance of this distinction between *k* receptor subtypes is yet to be agreed upon.

Electrophysiology of Kappa Opioid Receptor Ligands: The various DYN peptides and synthetic *k* agonists have been reported to both increase and decrease neuronal firing

rates, but not all of these alterations are reversible by opioid antagonists. Naloxone reversible inhibitions in the brain stem, caudate and hippocampus have been reported to follow the administration of various benzomorphans or DYN A(1-13) (Bradley & Brookes, 1984). In contrast, DYN and synthetic *k* agonist-induced increases in hippocampal cell firing, that might be mediated through actions at μ receptors, have also been reported. DYN A(1-17), DYN B and U-50,488H increased the evoked response from hippocampal CA1 pyramidal cells, and these actions were blocked by the specific μ -receptor antagonist β -funaltrexamine. These observations indicate that these nominal *k* receptor opioid agonists might have induced physiological changes through activity at μ receptors. In further support, it was noted that the required effective doses for the putative *k* agonists were 1000 times greater than were necessary for μ or *delta* agonists to evoke similar responses (Chavkin et al., 1985). Compatible with this interpretation, DYN A(1-8) naloxone reversibly elevated pyramidal cell firing (Moises & Walker, 1985).

Potent non-opioid effects from these compounds have also been reported. DYN A(1-17) induced decreases in hippocampal cell firing that were not reversed by naloxone (Moises & Walker, 1985). Bath-applied superfusions of either U-50,488H or U-69,593 *in vitro* to hippocampal CA3 neurons simultaneously impaired and enhanced different characteristics of cell firing. Neither of these effects appeared to be mediated at opioid receptors; neither effect was blocked by co-administrations of either naloxone or the specific *k* receptor antagonist nor-binaltorphimine (NBNI) (Alzheimer & Bruggencate, 1990).

Electrophysiological studies in the substantia nigra also reveal both excitatory and inhibitory effects from nominal *k* agonists. Microiontophoretically applied DYN A(1-9) *in vivo* has been reported to decrease cell firing in 70% of tested SNR neurons, but to have no effect when directed at the SNC. This inhibition was found to be opioid mediated; systemic naloxone injections both blocked the DYN-induced decreased SNR cell firing as

well as further elevating the cell firing above baseline. This latter finding indicated that endogenous opioids tonically inhibit cell firing (Lavin & Garcia-Munoz, 1985).

Similarly, iontophoretically applied DYN A₍₁₋₈₎ has also been found to inhibit SNR cell firing. Interestingly, the same study also reported that SNR DYN A₍₁₋₈₎ attenuated GABA-induced decreases in cell firing (Robertson et al., 1987). Contrasting with the physiologically similar effects of larger DYN fragments, neither of the DYN A₍₁₋₈₎ cell firing changes were blocked by systemic administrations of the μ/k receptor antagonist Mr2266, indicating either non-opioid or, minimally, non- μ/k opioid receptor mediations.

More recently, it has been observed that SNC cells are inhibited by systemic injections of the specific *k* receptor agonist, U-50,488H. Conversely, these same DA cells are excited by morphine injections. Both of these effects are blocked by systemic naloxone injections, although 10 times more naloxone is required to antagonize U-50,488H. Since naloxone displays greater affinity for μ receptors than for *k* receptors (Smith, 1987) the differential dose-dependent inhibition is compatible with the differential antagonism of μ and *k* mediated effects. Most importantly, U-50,488H infusions directly into the caudate also inhibit SNC DA neurons suggesting that the *k* agonist's inhibitory effects may be, at least partially, provoked from the striatum (Walker, Thompson, Frascella, & Friederich, 1987). The following year these same researchers reported that U-50,488H infusions into the SNR were also able to significantly inhibit cell firing. On the other hand, intra-SNC U-50,488H infusions were without effect, reinforcing previous suggestions that *k* agonist's inhibitory influences are induced indirectly and not immediately at the somatodendritic level (Thompson & Walker, 1988).

These observations were followed one year later by a report indicating that the majority of SNR cells that are inhibited by either systemic or microiontophoretic administrations of U-50,488H also increase their firing in response to non-painful tactile stimuli. Furthermore, many of these touch-responsive, *k* agonist inhibited cells project to

either the superior colliculus or thalamus as parts of nigrotectal and nigrothalamic pathways (Thompson & Walker, 1989).

In summary, these electrophysiological findings would seem to indicate that both DYN and the more selective *k* agonists can indirectly inhibit ascending DA cells through actions initiated in their terminal fields. When infused directly into the cell body region *k* agonists would seem to inhibit non-DA neurons that are either intrinsic to the region (plausibly GABAergic) or contribute to the ascending nigrotectal and nigrothalamic pathways. Possibly, a *k* agonist-induced inhibition of descending striatonigral GABAergic cells might also indirectly inhibit ascending DA neurons.

Dynorphin Pharmacology: Pharmacological evidence compatible with the electrophysiologists' observation that both systemic and intra-striatal infusions of *k* receptor agonists inhibit SNC cell firing rates (Walker et al., 1987) is also available. The systemic administration of U-50,488H dose-dependently decreases extracellular NAS DA levels in locomoting rats (Imperato & Di Chiara, 1985; Di Chiara & Imperato, 1988). Compatibly, systemic U-50,488H also decreases DA synthesis in the NAS, striatum, median eminence, intermediate lobe, and neural lobe, and these inhibitions are blocked by i.c.v. pretreatments with the *k* antagonist NBNI (Manzanares, Lookingland, & Moore, 1991). Interestingly, *k* agonist-induced inhibitions of DA biosynthesis and release are seen when the neurons are pharmacologically activated either with neuroleptics (Manzanares et al., 1991), morphine (Wood & Thakur, 1982; Wood, 1984; Kim, Iyengar, & Wood, 1987) or anaesthesia (Herrera-Marschitz, Christensson-Nylander, Sharp, Staines, Hökfelt, Terenius, & Ungerstedt, 1986; Reid, Herrera-Marschitz, Hökfelt, Terenius, & Ungerstedt, 1988; Reid, O'Connor, Herrera-Marschitz, & Ungerstedt, 1990; Reid, Herrera-Marschitz, Hökfelt, Lindefors, Persson, & Ungerstedt, 1990; Spanagel, Herz, & Shippenberg, 1990), but not in untreated animals (Wood, Stotland, Richard, & Rackham, 1980; Wood, Kim, Cosi, & Iyengar, 1987; Kim et al., 1987; Manzanares et al., 1991); basal activity levels are not altered. Wood has suggested that some of these inhibitory effects exhibited

by synthetic *k* agonists are attributable to non-*k* receptor antagonist actions at $\mu 2$ opioid receptors (Wood et al., 1980; Wood, Charleson, Lane, & Hudgin, 1981; Wood, 1984; Kim et al., 1987; Wood et al., 1987), but U-50,488H would appear not to display this property (VonVoightlander, Lahti, & Ludens, 1983; Clark & Pasternak, 1988).

Intracerebroventricular infusions of either E-2078, a peptidase resistant dynorphin analog, or U-69,593 also decrease NAS DA in anaesthetized animals, and this too is blocked by NBNI (Spanagel, et al., 1990). Interestingly, the highest tested doses of either E-2078 or U-69,593 were ineffective, and as the authors speculated, this might indicate that the nominal *k* agonists have opposing effects on other receptor systems when administered at higher doses.

Evidence from accumulating pharmacological studies also support the supposition that intra-SNR infusions of either DYN or synthetic *k* receptor agonists, in the pmol to nmol range, inhibit the terminal release of DA. Unilateral infusions of SNR DYN B have been reported to increase post-mortem striatal levels of combined intra- plus extra-cellular ipsilateral DA and DOPAC, as measured by HPLC with electrochemical detection (Lohof, Matsumoto, Patrick, & Walker, 1986). These findings can be interpreted to indicate either increased DA synthesis and metabolism or, on the other hand, increased accumulation attributable to decreased release. The latter interpretation is suggested by studies employing *in vivo* microdialysis; these studies consistently report that extracellular striatal DA levels decrease following intra-SNR administrations of various DYN fragments (Herrera-Marschitz et al., 1986; Reid et al., 1988, 1990a,b). When the results of these studies are considered in the light of the previously described electrophysiological research of the same region, the hypothesis that intra-SNR infusions of DYN can indirectly inhibit adjacent SNC DA neurons via actions on opioid receptors seems well supported.

In contrast to, though not incompatible with the electrophysiological data indicating that SNC infusions of *k* agonists are without effect on cell firing, there is one report indicating that DYN can act directly on the DA neurons to regulate release: in rats with

unilateral SNR ibotenic acid cell body lesions, SN infusions of DYN A continued to reduce extracellular striatal DA levels (Reid et al., 1990b). It may be that DYN can exert inhibitory effects directly on the DA neuron.

In vitro studies indicate that *k* receptor agonists are also able to presynaptically inhibit DA release at various mesocorticolimbic terminal sites. DYN A(1-8), DYN A(1-13), DYN A(1-17), and the benzomorphans ketocyclazocine and bremazocine, concentration-dependently decrease stimulated, but not basal, DA release from striatal tissue (Mulder, Wardeh, Hogenboom, & Frankhuyzen, 1984, 1989; Clow & Jhamandas, 1987; Schoffelmeer, Rice, Jacobson, van Gelderen, Hogenboom, Heijna, & Mulder, 1988). Similar findings have been reported with the more selective *k* agonist U-50,488H, and these inhibitions of DA release are blocked by WIN 44,441-3 (Clow & Jhamandas, 1987), naloxone (Werling, Frattali, Portoghese, Takemori, & Cox, 1988; Heijna, Hogenboom, Bakker, Padt, Mulder, & Schoffelmeer, 1990a) and NBNI (Werling et al., 1988; Heijna, Schoffelmeer & Mulder, 1989; Heijna, Padt, Hogenboom, Portoghese, Mulder, & Schoffelmeer, 1990b; Heijna et al., 1990a). These effects are not peculiar to the dorsal striatum; similar decreases are seen in the NAS (Heijna et al., 1989, 1990a,b), olfactory tubercle (Heijna et al., 1989, 1990a,b), basal hypothalamus (Heijna et al., 1990a) and the frontal cortex (Werling et al., 1988; Heijna et al., 1989, 1990a,b). *In vitro veritas*.

These various electrophysiological and pharmacological findings indicate that the release of endogenous DYN regulates the release of DA. Interestingly, the converse would also seem to be true; the release of DA seems to regulate DYN. This possibility is indicated by a variety of findings: acute administrations of DA agonists deplete DYN in both the SN (Trujillo et al., 1987, 1990) and the striatum (Li, Sivam, McGinty, Jiang, Douglass, Calavetta, & Hong, 1988; Trujillo et al., 1987, 1990). Repeated activation of the nigrostriatal DA pathways appears to elevate the biosynthesis of DYN; repeated injections of amphetamine increase DYN immunoreactivity (IR) in both the SN (Li, Sivam & Hong, 1986; Hanson, Merchant, Letter, Bush, & Gibb, 1987; Li et al., 1988; Trujillo et al.,

1987, 1990) and the striatum (Li et al., 1986, 1988; Hanson, Merchant, Letter, Bush, & Gibb, 1987, 1988; Trujillo et al., 1987, 1990). Repeated apomorphine (Li et al., 1986; 1988) and repeated cocaine (Sivam, 1989) have similar effects. These DA agonist-induced increases in DYN are blocked by 6-OHDA lesions (Hanson et al., 1988; Sivam, 1989), haloperidol (Li et al., 1986), and specific D1 antagonists (Hanson et al., 1987; Sivam, 1989).

Compatible with the above reports, the repeated administration of a DA antagonist alone also decreases DYN-IR (Quirion et al., 1985), and 6-OHDA lesions decrease nigral immunoreactivity for DYN A and DYN B (Christensson-Marschitz et al., 1986). Moreover, repeat DA agonist treatments elevate (Li et al., 1988), and 6-OHDA lesions decrease, striatal DYN mRNA indicating that the changes in DYN-IR represent genuine alterations in DYN synthesis (Young, Bonner, & Brann, 1986).

More selective intrusions into the striatonigral and nigrostriatal pathways suggest that tonically released DA acts on D1 receptors situated on descending striatonigral prodynorphin neurons to stimulate DYN synthesis and release. Repeated, intermittent D1 agonist administration increases striatonigral prodynorphin mRNA in 6-OHDA lesioned rats (Gerfen, Engber, Mahan, Susel, Chase, Frederick, Monsama, & Sibley, 1990), and increases DYN-IR in both lesioned (Jiang, McGinty, & Hong, 1990) and intact rats (Nylander & Terenius, 1987; Jiang et al., 1990).

There also exists some evidence that GABA can regulate DYN. Striatal GABA_A receptor stimulation has been reported to increase prodynorphin mRNA (Jomary, Schwartz, & Llorens-Cortes, 1990). Compatible with mutual regulatory actions, *k* agonists decrease GABA release (Starr, 1985).

In summary, the pharmacological data suggests that tonic DA release stimulates the biosynthesis and release of DYN. Compatible with this interpretation, electrophysiological data suggests that this released DYN acts in a negative feedback pathway to inhibit the further release of DA. These hypotheses are supported by the behavioural data to be

discussed below. Interestingly, although clearly of highly speculative significance, schizophrenics - who have often been suggested to have overactive DA systems - may have reduced levels of cerebral spinal fluid DYN A(1-8) (Zhang, Zhou, Xi, Gu, Xia, & Yao, 1985). Notably, the patients were experiencing their first schizophrenic episode, and were medication-free at the time of testing.

Behavioural Effects of Dynorphin and Other Kappa Receptor Ligands: Both increases and decreases in locomotor activity have been reported following the administration of *k* agonists. Early investigations administered high doses of DYN i.c.v. and noted profoundly rigid, fixed postures, unlike what had been seen with other opioids (Walker et al., 1982). The mechanisms underlying these effects were unclear, and they were not blocked by systemic naloxone injections. In contrast, the i.c.v. infusion of lower doses elicited naloxone reversible increases in locomotor activity (Ukai & Kameyama, 1984). Early systemic administration studies produced equally confusing results; biphasic locomotor decreases and increases that were not reversible with naloxone were observed following bremazocine (Ruhland & Zeugner, 1983), and U-50,488H (Estall, de Costa, Rice, & Pert, 1988). This second study was able to shed some light when it also reported that the stimulatory effects were not disrupted by 6-OHDA lesions of the NAS. In contrast to the excitatory effects seen with μ or *delta* agonists, *k* agonist-induced behavioural excitation was apparently not DA mediated. Excitatory effects of U-50,488H have also been observed in both mice (Teskey & Kavaliers, 1988) and hamsters (Schnur & Walker, 1990) following the injection of a low dose; conversely, depressant effects were noted following higher doses. In contrast, another group reported no effect from the same low dose, but exclusively depressant effects from the higher dose, and this reduced locomotor activity was blocked by the mixed μ/k opioid antagonist Mr2266 (Ukai & Kameyama, 1985). Compatible with these findings, most recent investigations have also reported only decreases of motor activity to follow either systemic or i.c.v. administrations of *k* agonists, and these inhibitions were blocked by opioid antagonists (Castellano, Pavone, & Sansone,

1984; Ukai, Yamada, & Kameyama, 1984; Benton, 1985; Benton, Smoothy, & Brain, 1985; Imperato & Di Chiara, 1985; Tilson, McLamb, & Hong, 1986; Leighton, Johnson, Meecham, Hill, & Hughes, 1987; Dykstra, Gmerek, Winger, & Woods, 1987; Jackson & Cooper, 1988; Di Chiara & Imperato, 1988; Young, 1989). Moreover, systemic administrations of *k* agonists to rats have now been reported to decrease locomotor activity with a temporal pattern that is coincident with decreases in released NAS DA (Imperato & Di Chiara, 1985; Di Chiara & Imperato, 1988). The reasons for the discrepancies between the early and later studies are not immediately evident, but the following intra-cranial studies clearly indicate that both non-opioid and non-DAergic excitatory effects from *k* agonists are possible.

The preceding behavioural studies further establish the possibility that *k* opioid receptor agonists inhibit behavioural activity as a function of decreased NAS DA. They do not, however, suggest where in the brain the *k* agonists might be acting to induce these effects. There are studies that are informative to this question. Consistent with the previous electrophysiological and pharmacological studies, data also exists indicating that *k* agonists can induce behaviourally significant effects through actions either at the cell body region or at terminal sites.

The systemic injection of U-50,488H has been reported to attenuate the ipsilateral circling induced by methamphetamine injections to rats with unilateral 6-OHDA nigral lesions. Conversely, U-50,488H did not alter apomorphine-induced contralateral circling in lesioned animals (Ohno, Yamamoto, & Ueki, 1989). These findings indicate that U-50,488H can inhibit the methamphetamine stimulated release of DA from the intact side that would otherwise result in ipsilateral turning. Conversely, U-50,488H does not act post-synaptically to disrupt the contralateral circling induced by a direct acting DA agonist impinging on supersensitive DA receptors. This inhibition of DA release could possibly be induced through actions either at the cell body or in the terminal region.

There are two additional reasons to believe that *k* receptor stimulation in the SNR has behaviourally significant effects. First, intra-nigral infusions indicate that, at this site, *k* receptor agonists can induce both inhibitory DA-dependent, and excitatory DA-independent effects on motor activity. Unilateral infusions of either U-50,488H or DYN produce contralateral circling, a result that is indicative of motor stimulating effects (Herrera-Marschitz, Hökfelt, Ungerstedt, & Terenius, 1983; Herrera-Marschitz, Hökfelt, Ungerstedt, Terenius, & Goldstein, 1984; Morelli & Di Chiara, 1985; Christensson-Nylander et al., 1986; Herrera-Marschitz et al., 1986; Lohof et al., 1986; Friedrich, Friedrich & Walker, 1987; Matsumoto, Brinsfield, Patrick, & Walker, 1988; Matsumoto, Lohof, Patrick, & Walker, 1988; Tan & Tsou, 1988; Thompson & Walker, 1990), and most (Herrera-Marschitz et al., 1983, 1984; Morelli & Di Chiara, 1985; Matsumoto et al., 1988a,b; Tan & Tsou, 1988), though not all (Friederich et al., 1987), investigators find this circling to be blocked by systemic naloxone. There are three reasons to believe that this circling is DA-independent: 6-OHDA lesions potentiate, rather than prevent, *k* agonist-induced contralateral circling (Herrera-Marschitz et al., 1986; Matsumoto et al., 1988a,b); 6-OHDA plus systemic amphetamine results in ipsilateral turning, indicating a unilaterally dampened DA release by the *k* agonist (Herrera-Marschitz et al., 1984); and phenomenological descriptions of the circling (Tan & Tsou, 1988) indicate that it resembles the tight head to tail circling characteristic of DA-independent, GABAergic mechanisms (Waddington, 1980; Holmes & Wise, 1985). Since 6-OHDA lesions potentiate the contralateral circling, it appears that *k* receptor agonists can both stimulate motor activity through GABAergic systems, and inhibit motor activity through DA neurons.

Most available evidence indicates that this SN *k* receptor stimulation-induced inhibition of DA is mediated indirectly through actions on intermediate substrates. In contrast, there is one study reporting that SNC infusions of DYN can induce ipsilateral circling (Herrera-Marschitz et al., 1986) thereby indicating that DA neurons can be directly inhibited at their cell body. This finding is compatible with a lesion study from the same

laboratory also indicating that DYN may act directly on DA neurons to inhibit the synaptic release of DA (Reid et al., 1990).

The converse experiment provides a second major reason to believe that SNR DYN inhibits both DA and GABA neurons. In contrast to the contralateral circling that is seen following the SNR infusion of *k* agonists, lesion-induced depletions of SNR DYN produce asymmetric ipsilateral turning (Christensson-Nylander et al., 1986). Compatible with the idea that *k* agonists induce motor excitation by inhibiting ascending GABAergic projection neurons, it has also been reported that unilateral lesions of either the ventromedial thalamus or superior colliculus - SNR GABAergic projection sites - attenuate the circling induced by SN U-50,488H (Thompson & Walker, 1990). Moreover, as discussed above, these same researchers have found that SNR U-50,488H inhibits the cell firing of these neurons (Thompson & Walker, 1989).

In addition to these depressions of locomotor activity, systemic administrations of *k* agonists are also reported to suppress the display of grooming (Ukai & Kameyama, 1985; Jackson & Cooper, 1986a, 1988). In contrast, i.c.v. infusions of DYN A(1-13) increase grooming (Katz, 1980; Walker, Katz, & Akil, 1980; Ukai & Kameyama, 1984). The increased grooming elicited by relatively low doses of DYN A(1-13) are blocked by systemic naloxone (Ukai & Kameyama, 1984), whereas high dose-induced grooming seems to be naloxone resistant (Katz, 1980; Walker et al., 1980). These findings may suggest that *k* agonists act on peripheral mechanisms to decrease grooming, and on central substrates to increase grooming. Conversely, the discrepancy may reflect differences in the actions of DYN A(1-13), an endogenous peptide known to act on multiple opioid receptors, and synthetic *k* agonists that are more selective for the *k* receptor.

At this time the substrates of these grooming effects remain to be elucidated. One possibility is that the *k* agonist-induced suppression of grooming is mediated by reduced stimulation of D1 receptors; the administration of the D1 agonist SKF 38393, but not the D2 agonist quinpirole, induces excessive grooming. Conversely, the D1 antagonist SCH

23390, but not the D2 antagonist sulpiride, suppresses the excessive grooming that is typically seen in novel environments and following the administration of the neuropeptides ACTH, β -endorphin, bombesin, TRH, SP, and somatostatin (Greidanus, Maigret, Torn, Ronner, Van der Kracht, Van der Wee, & Versteeg, 1989). Central opioid receptors would also seem to be involved in neuropeptide-induced excessive grooming. Systemic injections of naloxone, but not of naloxone-metho-bromide which does not readily cross the blood-brain barrier, suppress neuropeptide-induced grooming (van Wimersma Greidanus, Maigret, & Merz, 1990).

K receptors may also be involved in thermoregulation. Synthetic k agonists induce hypothermia (Hayes, Skingle, & Tyers, 1985; Pillai & Ross, 1986; Adler et al., 1988; Bhargava, Ramarao, & Gulati, 1989; Cowan & Murray, 1989), that is partially attenuated by the prototypical opioid antagonist naloxone and completely prevented by the mixed μ/k antagonist Mr2266 (Pillai & Ross, 1986), but not by the selective μ antagonist β -funaltrexamine (Hayes et al., 1985). The site of action mediating this hypothermia is unclear; central infusions of synthetic k agonists seem not to alter body temperature, although i.c.v. infusions of DYN A(1-17) do induce hypothermia (see Adler et al., 1988).

Rewarding and Aversive Effects of Kappa Agonists: There are other reasons to believe that k agonists pharmacologically inhibit DA release to result in depressions of locomotor activity. Moreover, the decreases in motor behaviour that are observed following the administration of k agonists might reflect more than motor deficits; they might also reflect appetitively aversive properties of the opioid.

When examined in place-conditioning tests systemic injections of k receptor agonists are most commonly reported to induce place aversions. This is apparent following the administration of either the newer highly selective k agonists U-50,488H, U-69,593 or E-2078 (Mucha & Herz, 1985; Mucha, Millan, & Herz, 1985; Bechara & van der Kooy, 1987; Ableitner & Herz, 1989; Bals-Kubik, Herz, & Shippenberg, 1989; Shippenberg & Herz, 1987, 1991) or the less selective benzomorphans (Iwamoto, 1986). These aversive

effects seem to have both central and peripheral components: central actions are suggested by the finding that place aversions can be produced by i.c.v. infusions of either U-50,488H or E-2078 (Bals-Kubik, Herz, & Shippenberg, 1988; Bals-Kubik et al., 1989), whereas aversive peripheral effects in the gut are indicated by a report that vagotomy can induce place preference from systemic U-50,488H (Bechara & van der Kooy, 1987). At this time, the results from opioid receptor antagonist studies do not allow us to unequivocally state that the central aversive effects are mediated at *k*-opioid receptors; naloxone itself induces place aversions (Mucha et al., 1985; Iwamoto, 1986; Dymshitz & Lieblich, 1987; Bals-Kubik et al., 1989; Shippenberg & Herz, 1991) making it an inadequate choice to antagonize an opioid-mediated place aversion, and attempts have not been made to block them with specific *k*-opioid antagonists. On the other hand, the mixed μ/k benzomorphan antagonist Mr2266 partially attenuates the conditioned place aversion produced by systemic U-50,488H, and when the same dose of Mr2266 is administered alone it produces a place preference (Iwamoto, 1986; Bechara & van der Kooy, 1987). This place preference is likely due to its blockade of the *k* receptor as both naloxone (Iwamoto, 1986; Bals-Kubik et al., 1989) and the specific μ antagonist CTOP (Bals-Kubik et al., 1989) themselves elicit place aversions. Compatible with these findings are reports that i.c.v. infusions of the highly selective *k* antagonist NBNI non-significantly (Bals-Kubik et al., 1989), and the benzazocine *k* antagonist WIN 44,441 significantly (Iwamoto, 1986), also produce place preferences.

More recently, conditioned place aversions have also been obtained from discrete microinjections of either U-50,488H or the stable DYN analog E-2078 into the VTA, NAS and medial frontal cortex (MFC) (Bals-Kubik, Shippenberg, & Herz, 1990). These findings are compatible with the pharmacological data indicating that *k* agonists decrease synaptic DA release following their administration to either the cell body or terminal region of DA neurons.

Naloxone reversible place preferences have also been reported following the administration of *k* agonists. Some of these findings may be attributable to the use of multiple receptor agonist benzomorphans (Iwamoto, 1986) or DYN A(1-17) (Iwamoto, 1988), but other reports employing the much more selective U-50,488H (Bechara & van der Kooy, 1987) are less amenable to explanation. Possibly the rewarding effect of U-50,488H might be peculiar to a very narrow dose range, and in fact, it has been only reported in vagotomized animals administered 4.0 mg/kg. Moreover, among surgically intact animals this same dose produces neither place preferences nor place aversions (Mucha & Herz, 1985; Bechara & van der Kooy, 1987; Bals-Kubik et al., 1989). In contrast, both higher and lower doses exclusively produce place aversions (Mucha & Herz, 1985; Bechara & van der Kooy, 1987). These findings re-emphasize that even highly selective *k* agonists might dose-dependently engage different receptor systems. Whether these differential actions occur at different sub-classes of *k* receptors, different classes of opioid receptors or opioid versus non-opioid receptor systems remains undetermined at this time.

Studies employing electrical brain stimulation paradigms also indicate that *k* agonists have both facilitatory and inhibitory influences. At low doses, racemic ethylketocyclazocine increases rates of bar pressing for electrical stimulation of the lateral hypothalamus. In contrast, higher doses decrease bar pressing. A more selective examination of the isomeric forms of ethylketocyclazocine suggested that the positive effects were mediated by the (+) form whereas the inhibitory effects resulted from actions of the (-) form (Reid, Hubbell, Dunn, Hunter, & Costa, 1985). All of these effects could be blocked by systemic naloxone.

Perhaps the strongest evidence that endogenous *k* ligands can have behaviourally excitatory properties comes from feeding studies. Either antibodies for DYN A (Carr, Bak, Gioannini, & Simon, 1987; Carr, 1990; Carr & Bak, 1990) or the selective *k* antagonist NBNI (Carr, Bak, Simon, & Portoghese, 1989) infused i.c.v. inhibit lateral hypothalamic

stimulation-induced feeding suggesting that endogenous DYN normally acts to induce feeding. Similarly, i.c.v. administrations of NBNI dose-dependently decrease the consumption of a sweet solution (Calcagnetti, Calcagnetti, & Fanselow, 1990). Compatible with these reports, intra-VTA infusions of U-50,488H decrease the threshold necessary for stimulation-induced feeding (Wise, Jenck, Gratton, & Quirion, 1986; Jenck, Quirion, & Wise, 1987). Moreover, a substantial literature clearly indicates that feeding increases following intra-VTA infusions of either DYN (Hamilton & Bozarth, 1986, 1987, 1988) or U-50,488H (Hamilton & Bozarth, 1987; Badiani & Noel, 1991) as well as after systemic injections of various *k* agonists (Morley, Levine, Grace, & Kniep, 1982; Morley, Levine, Gosnell, & Billington, 1984; Levine, Morley, Gosnell, Billington, & Bartness, 1985; Morley, Levine, Kniep, Grace, Zeugner, & Shearman, 1985; Jackson & Cooper, 1985, 1986a,b, 1988; Cooper, Jackson, & Kirkham, 1985; Gosnell, Levine, & Morley, 1986; Imura, Nakao, Yanaihara, Katsuura, & Nakamura, 1986; Hewson, Hill, Hughes, Leighton, & Turner, 1987; Leighton, Hill, & Hughes, 1988; Ramarao & Bhargava, 1989). Finally, both spontaneous (Arjune & Bodnar, 1990; Levine, Grace, Billington, & Portoghese, 1990) and U-50,488H-induced feeding (Levine et al., 1990) are inhibited by i.c.v. infusions of the highly selective *k* antagonist NBNI.

In summary, it would appear that *k* agonists have functional significance through actions at multiple neuronal sites: 1. they can inhibit DA cells through opioid receptors both at the cell body region - either transynaptically or, possibly, also directly on the DA neuron - and presynaptically in the terminal region. Either action can lead to behavioural suppression; 2. they can inhibit non-DA - probably GABA - neurons at opioid receptors to produce behavioural excitation; 3. they might inhibit DA cells through a non-opioid mechanism producing behavioural inhibition; and 4. they might inhibit non-DA cells through a non-opioid mechanism that produces behavioural excitation. The presence of *k* receptor subtypes seems established (albeit their actual number remaining an ongoing investigation), but neither their electrophysiological, pharmacological nor behavioural

significance has been even initially explored. The extraordinary potency of DYNs and some synthetic *k* agonists coupled with the ultimately limited receptor selectivity of even the currently available *k* ligands has likely composed opioid effects at more than their nominal site; at sufficiently high, and not uncommonly employed, doses *k* agonists display substantial affinity for μ receptors. Finally, there are non-opioid effects of the presently used *k* receptor ligands.

The Present Experiments

The systemic and i.c.v. administration of various opioids produces both time- and dose-dependent excitatory and inhibitory behavioural effects. In contrast, infusions of either morphine or DYN directly into mesolimbic and preoptic area brain regions only increase male sexual behaviour, whereas intra-mPOA β -endorphin only inhibits it. At this time, the opioid receptors for, and brain site specificity of, these effects remain unclear. In the present study the possible inhibitory role of *k* opioid receptors in these behaviours has been investigated. These experiments have taken one of two forms: first, male rats were tested for sexual behaviour following systemic injections of the *k* receptor agonist U-50,488H in combination with either systemic injections of naloxone or intra-cranial infusions of the specific *k* receptor antagonist NBNI; and second, rats were tested following intra-cranial infusions of U-50,488H and challenged with systemic naloxone. Following these same drug treatments in separate tests, male rats were also monitored for locomotor activity, a behavioural measure considered indicative of an animal's motivation to interact with, and by implication interest in, the surrounding environment.

CHAPTER TWO

Behavioural Effects of Systemic U-50,488H

The studies reviewed in the previous chapter lead to the conclusion that endogenous opioid systems of the mammalian brain play both excitatory and inhibitory roles in the evocation of normal male sexual behaviour. At this time, it seems reasonable to speculate that the facilitatory effects of μ opioid agonists on male sexual behaviour seen when they are injected into the VTA are at least partially attributable to excitatory effects on mesolimbic DA neurotransmission. The mechanisms mediating the excitatory effects of intra-VTA DYN remain unclear. Although the behaviourally stimulating effect produced by DYN and synthetic *k* agonists in circling studies appear to result from inhibitions of ascending GABA neurons, the mechanism mediating the effect of DYN on sexual behaviour and feeding is poorly understood. These diverse findings make it difficult to predict what effect the systemic administration of a selective *k* opioid receptor agonist might have on male sexual behaviour, but the weight of evidence reviewed in the Introduction suggests that *k* opioid receptor stimulation would decrease both locomotor activity and male sexual behaviour.

The experiments to be reported in this chapter examined the effects of systemic injections of the selective *k* opioid receptor agonist, U-50,488H, on male sexual behaviour and locomotor activity. In part, to replicate the obtained findings, and in addition to investigate the differential dose effects following i.p. versus s.c. routes of administration, different groups of rats were tested for both behaviours following the systemic administration of U-50,488H given either i.p. or s.c.

Previous investigators have reported that systemic U-50,488H decreases body temperature (see Adler et al., 1988) and produces body flattening (Brent, Chahl & Johnston, 1990), a behaviour that is believed to cool the body and is commonly seen to follow ejaculation. Interestingly, mPOA temperature has been reported to gradually increase during copulatory behaviour and rapidly decrease immediately following ejaculation (Blumberg, Mennella, & Moltz, 1987). Parallel, though less consistent,

changes were seen in peripheral body temperature (Blumberg et al., 1987). To investigate this issue further, a fifth experiment was conducted to investigate possible changes in body temperature that might be seen following U-50,488H administration.

Methods

Subjects

Sixty-seven male and 18 female Wistar rats obtained from Charles River Canada Inc. (USA) were housed individually in a controlled environment (temperature of $21 \pm 1^\circ\text{C}$; reverse cycle, lights on from 800 h to 2000 h) with food and water available ad libitum. The males weighed 275-300 g, and the females weighed 200-220 g, upon arrival at the laboratory. The males chosen for use in the experiments were selected from a larger pool of males on the basis of three 30-min tests for sexual behaviour with an estrous female. All tests were separated by three or four days. Only those males that ejaculated at least once on both of the final two tests were retained as experimental subjects.

Surgery

The females were ovariectomized under methoxyflurane anaesthetic (Metophane, Pitman-Moore Ltd./M.T.C. Pharmaceuticals, Mississauga, Ontario) and allowed a minimum of one week to recover from surgery before being paired with males for testing. Sexual receptivity was induced by subcutaneous (s.c.) injections of 10.0 μg estradiol benzoate (Sigma) in 0.1 ml peanut oil 48 and 24 h before testing, and 0.5 mg progesterone s.c. (Sigma) in 0.2 ml peanut oil 4 to 6 h before testing.

Apparatus

The tests for sexual behaviour were conducted in nine boxes (50 x 28 x 37 cm), formed from pressed wood on three sides and the floor, a wire screen ceiling and a removable Plexiglas front, located in a room dimly lit by red lights. A red-light sensitive camera (Panasonic CCTV camera, model WV-1460), and a video cassette recorder (Sony Betamax VCR, model SLO-420) were used to record the sessions. At later dates, the video

tapes were observed twice. During one viewing, both the latencies to begin copulating and to ejaculate as well as the occurrence of additional mounts and intromissions were recorded for each ejaculatory series performed during the 30-min test. During a second viewing, males were observed for 2 to 3 s once every 30 s, and the predominantly displayed behaviour was recorded; hence a total of 60 observations per male were recorded from the 30-min session.

The locomotor activity tests were conducted in eight activity boxes equipped with photocell beams. The boxes (40 x 30 x 25 cm) had transparent Plexiglas front and rear walls, pressed wood side walls, and a removable wire screen ceiling; the floors were constructed from stainless steel rods spaced at approximately 1.5 cm intervals. Two evenly spaced photocell beams cut across the width of the box, and a third pair monitored from the sides. The photocells were 4.0 cm off the rod floor. Breaking a beam was recorded as an activity count. The data were collected from all eight boxes using an Apple IIe computer.

Body temperature was measured with six temperature sensitive transmitters and Mini-Mitter receivers (Data Sciences, Inc., Model No. RA1010). The data were collected by a computer data collection system (TEC MASTER Data Train, Model No. V242A).

U-50,488H (trans-(±)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidiny)-cyclohexyl]-benzeacetamide methane sulphonate) (U-50,488H) (Lahti, VonVoigtlander & Barsuhn, 1982; VonVoigtlander, Lahti & Ludens, 1983; Takemori, Ikeda & Portoghese, 1986; Clark & Pasternak, 1988; Cheng, Lu, Lee & Tam, 1990), a generous gift from Upjohn, was dissolved in physiologically isotonic saline (0.9%) and injected in a volume of 1.0 ml/kg.

Procedure

In Experiments 1A and 1B, males were injected with either U-50,488H or saline and placed together in a carrying bucket for 15 min. Males were then placed in individual mating boxes where they waited an additional five min before being paired with an estrous female for a further 30 min. The tests for sexual behaviour were conducted every three or four days.

In Experiment 1A, 12 males were first observed on two counterbalanced tests with both U-50,488H (8.0 mg/kg, i.p.) and saline. These tests were followed by two additional counterbalanced tests with both U-50,488H (16.0 mg/kg, i.p.) and saline.

In Experiment 1B, 25 males were observed on three counterbalanced tests with 5.0 and 10.0 mg/kg U-50,488H given s.c. or saline. All sexual behaviour tests occurred during the dark phase of the cycle (1400 h to 1800 h).

In Experiment 2A, 22 males - the 12 from Experiment 1A plus 10 additional males from a separate replication of Experiment 1A not described here - were tested for locomotor activity. The animals were randomly divided into three groups and tested for 90 min in the photocell boxes immediately following i.p. injections of either 8.0 or 16.0 mg/kg U-50,488H or saline vehicle.

In Experiment 2B, 39 males - the 25 from Experiment 1B plus 14 from a replication of Experiment 1B not described here - were tested for locomotor activity. The animals were randomly divided into four groups and tested for 90 min in the photocell boxes immediately following s.c. injections of either 0.5, 5.0 or 10.0 mg/kg U-50,488H or saline vehicle. All locomotor activity tests occurred during the animals' dark phase (1200 h to 1800 h).

In Experiment 3, 6 additional rats chosen from the original large population of available animals were implanted with temperature sensitive transmitters in the peritoneal cavity. Four days after this surgery, the animals were moved to a separate reverse cycle room (lights on from 800 h to 2000 h; temperature of $21\pm 1^{\circ}\text{C}$; food and water available ad libitum) and housed in six individual sound attenuated boxes equipped with the Minimitter receivers. The animals remained in these boxes for seven days receiving saline injections (1.0 ml/kg) on each of the first six days at 1400 h. On the seventh day, also at 1400 h, all animals were injected with 5.0 mg/kg U-50,488H given s.c.

Statistical Analysis

The proportion of animals in each condition that mounted, intromitted or ejaculated was analysed by Cochran's Q statistic. The latency to mount, intromit and ejaculate (time from first intromission to first ejaculation), and the mean number of intromissions, ejaculations, and inter-intromission intervals (III) were each analysed by repeated measures ANOVAs. When animals did not mount or intromit they were assigned a score of 1800 sec. When animals did not ejaculate they were assigned either the time from their first intromission to the end of the session or a score of 1800 sec if they did not intromit. For refractory periods, animals were assigned the time from their first ejaculation to either the first mount of their second ejaculatory series or the end of the session if they ejaculated once but did not reinitiate mounting. The locomotor activity scores were analysed by between measures ANOVAs. Post hoc analyses, when performed, were with Newman-Keuls tests. The changes in body temperature scores were analysed with a repeated measures ANOVA.

Results

Experiment 1A: Tests for Male Sexual Behaviour Following Intra-Peritoneal U-50,488H

On four separate tests for sexual behaviour, 12 males were administered either saline or one of two doses of U-50,488H (8.0 or 16.0 mg/kg, i.p.). The mean of the data from the two saline tests was compared to the data obtained following U-50,488H.

Copulatory Behaviour: As can be seen in Fig 1a-c, injections of U-50,488H given i.p. decreased the proportion of males that copulated. U-50,488H significantly decreased the proportion of males that mounted [$Q(2)=13.38$, $p<.01$], intromitted [$Q(2)=21.56$, $p<.01$], and ejaculated [$Q(2)=15.51$, $p<.01$].

The latencies for males to initiate different aspects of an ejaculatory series were also increased by U-50,488H. As depicted in Fig. 1d-f, U-50,488H increased the latency to mount [$F(2,22)=14.532$, $p=.0001$], intromit [$F(2,22)=22.651$, $p<.00001$] and ejaculate

[$F(2,22)=18.018, p<.00001$]. For all three of these measures only the 16.0 mg/kg dose of U-50,488H differed significantly from saline [$p<.01$]. Although longer, the copulatory latencies with the 8.0 mg/kg dose did not differ significantly from saline [$p>.05$], and they were significantly shorter than those with the higher dose [$p<.01$]. As described in the Methods section, when a male did not copulate, it was assigned a default score of 1800 s for mount, intromission and ejaculation latencies. Among those animals that did copulate, the mean mount latencies were (mean \pm SEM) 24.2 \pm 6.8 s, n=12; 37.8 \pm 13.4 s, n=10; and 75.0 \pm 57.6 s, n=3, following 0.0, 8.0 and 16.0 mg/kg U-50,488H given i.p. Among intromitting animals, the mean intromission latencies were 35.8 \pm 8.0 s, n=12; 57.4 \pm 24.2 s, n=9; and 13.0 \pm 0.0 s, n=1, for the three test respective doses. Among ejaculating males, the three mean ejaculation latencies were 414.6 \pm 47.4 s, n=12; 503.8 \pm 158.9 s, n=9; and 316.0 \pm 0.0 s, n=1.

As illustrated in Fig. 1g, U-50,488H decreased the number of ejaculations observed during the 30-min test sessions [$F(2,22)=14.634, p=.0001$]. However, only the higher dose resulted in significantly fewer ejaculations than saline [$p<.01$]. When one examines only those males that did copulate, the mean number of ejaculations was 2.6 \pm 0.1, n=12; 2.3 \pm 0.4, n=10; and 1.0 \pm 1.0 n=3 following saline and the two U-50,488H doses respectively.

Figure 2 illustrates the number of intromissions to precede the first ejaculation, among those males that did ejaculate. As shown, 8.0 mg/kg U-50,488H decreased the number of intromissions prior to the first ejaculation [$t(7)=3.30, p<.02$], compared to the saline treatment. A statistical comparison between the 16.0 mg/kg dose and other treatments was not possible as only one animal ejaculated. U-50,488H also precipitated a parallel decrease in the mean number of mounts to precede ejaculation (data not illustrated (SAL: 11.2 \pm 1.9, n=12; U-50,488H: 8.0 mg/kg: 6.9 \pm 2.4, n=8; U-50,488H: 16.0 mg/kg: 4.0 \pm 0.0, n=1)), and as a result, the hit rate - the proportion of mounts that were

intromissions - was not altered (data not illustrated {SAL: 0.58 ± 0.04 , $n=12$; U-50,488H: 8.0 mg: 0.62 ± 0.04 , $n=8$; U-50,488H: 16.0 mg/kg: 0.83 ± 0.0 , $n=1$ }).

Figure 3 shows the mean inter-intromission intervals (III) in the first copulatory series of males that intromitted at least twice. Because few animals intromitted in all three test conditions, statistical comparison with repeated measures was not possible. It can be seen, however, that when animals did intromit, 8.0 mg/kg of U-50,488H given i.p. induced non-significant increases in the III [$t(8)=1.11$, $p=.2983$], relative to saline. Only one animal intromitted following the higher test dose.

Although a repeated measures ANOVA analysing the post-ejaculatory refractory period was not informative due to small numbers of animals, among those males that did begin a second ejaculatory series, it was apparent that U-50,488H did not alter this measure {SAL: 373.4 ± 18.6 s, $n=12$; U-50,488H 8.0 mg: 354.5 ± 18.1 s, $n=8$; U-50,488H 16.0 mg: 376.0 ± 0.0 s, $n=1$ }.

Non-Copulatory Behaviour: The mean percent of observations (out of 60 possible; 2/min) that various non-copulatory behaviours were recorded during the 30-min sessions is shown in Fig. 4. Significant reductions in the frequency of female-directed behaviour [$F(2,22)=13.506$, $p<.0002$], bodily grooming [$F(2,22)=58.813$, $p<.00001$], and genital grooming [$F(2,22)=52.643$, $p<.00001$] were produced by both doses of U-50,488H [$p<.01$]. Only bodily grooming, however, was more potently inhibited by the higher dose than the lower dose of U-50,488H [$p<.01$]. U-50,488H was also observed to significantly increase the frequency of lying down [$F(2,22)=18.449$, $p<.00001$], body flattening [$F(2,22)=14.137$, $p<.0002$], and sniffing behaviour [$F(2,22)=22.488$, $p<.00001$].

Figure 5 depicts a finer analysis of female-directed behaviour in the first two min of the 30-min test sessions. A repeated measures Drug x Min ANOVA yielded significant effects of both Drug [$F(2,22)=7.296$, $p<.004$] and Min [$F(1,11)=66.165$, $p<.00001$], reflecting the greater occurrence of female-directed behaviour both in the first, relative to

Figure 1 The proportion of males that mounted (1a), intromitted (1b) and ejaculated (1c); the mean (+ 1 S.E.M.) latency to mount (1d), intromit (1e) and ejaculate (1f); and the mean (+ 1 S.E.M.) number of ejaculations (1g) following the systemic injection of either saline, 8.0 mg/kg or 16.0 mg/kg U50 given i.p. during tests for sexual behaviour in Experiment 1A.

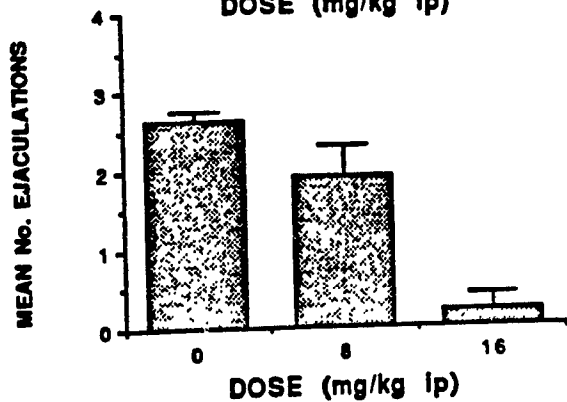
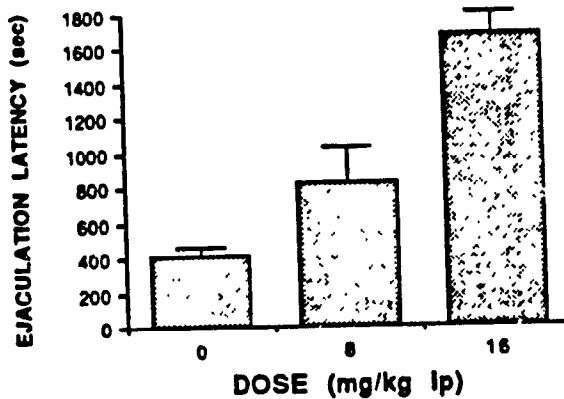
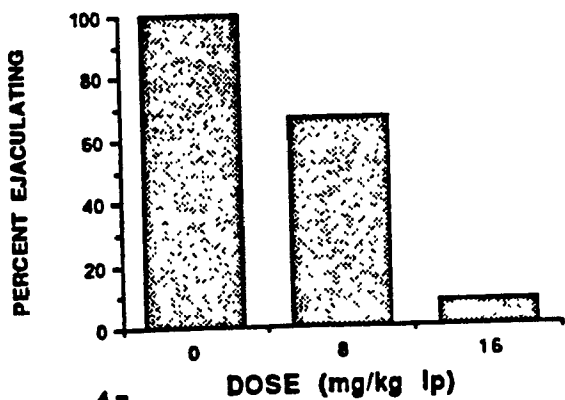
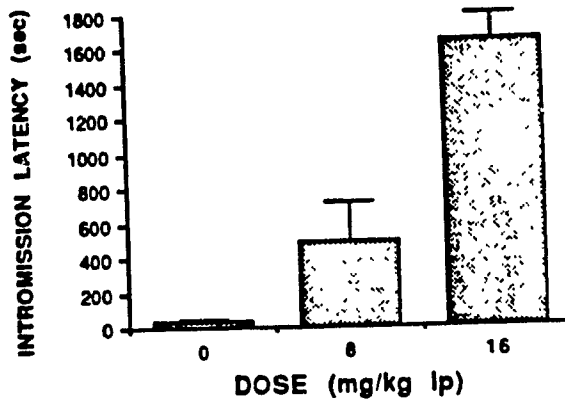
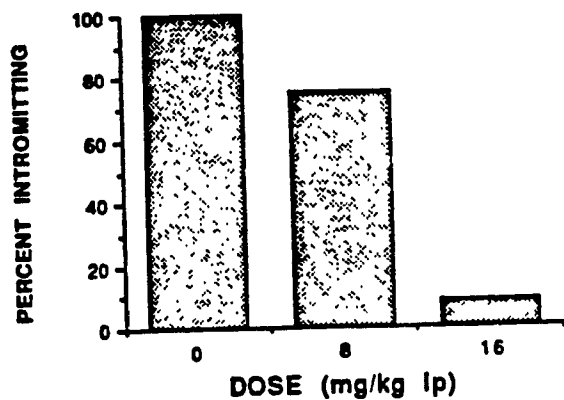
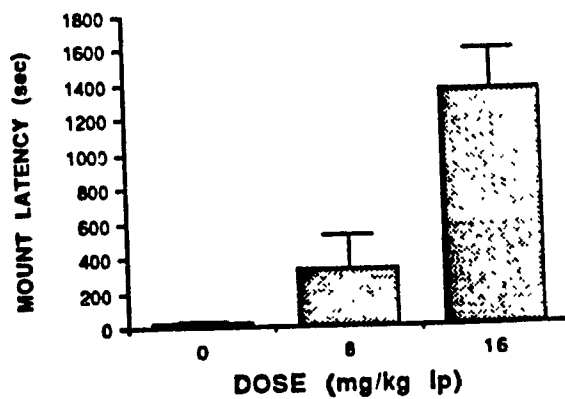
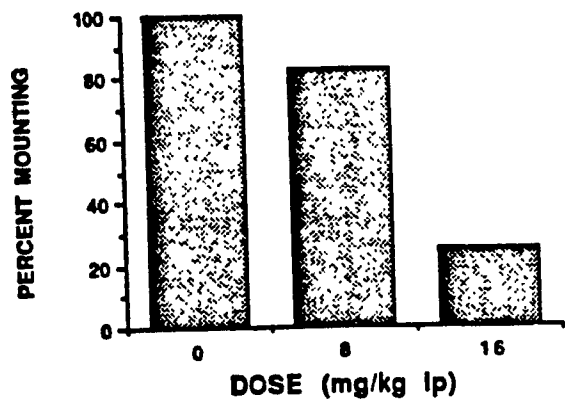


Figure 2 The mean (+ 1 S.E.M.) number of intromissions to precede the first ejaculation following the systemic injection of either saline, 8.0 mg/kg or 16.0 mg/kg U50 given i.p. during tests for sexual behaviour in Experiment 1A.

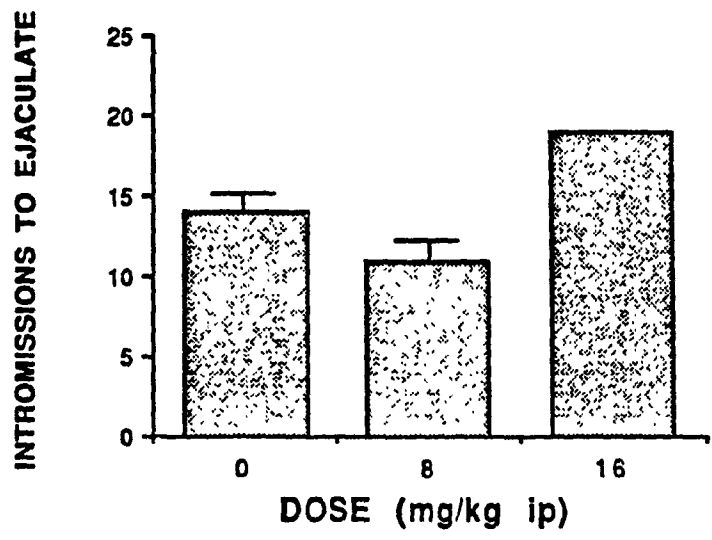


Figure 3 The mean (+ 1 S.E.M.) inter-intromission interval prior to the first ejaculation following the systemic injection of either saline, 8.0 mg/kg or 16.0 mg/kg U50 given i.p. during tests for sexual behaviour in Experiment 1A.

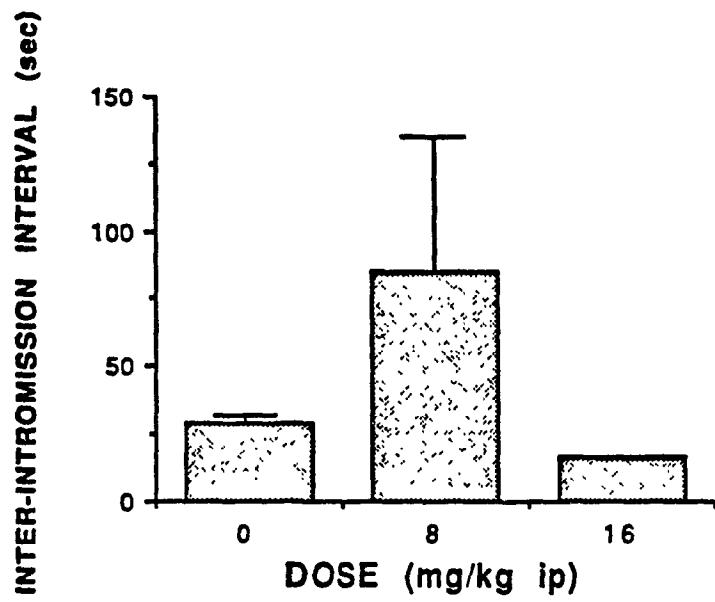


Figure 4 The mean (+ 1 S.E.M.) proportion of observations that non-copulatory behaviours were recorded to occur following the systemic injection of either saline, 8.0 mg/kg or 16.0 mg/kg U50 given i.p. during tests for sexual behaviour in Experiment 1A. (FDB: female-directed behaviour; L: laying down; LL: body flattening; Sn: sniffing; Gr: body grooming; GG: genital grooming; W: walking; R: rearing).

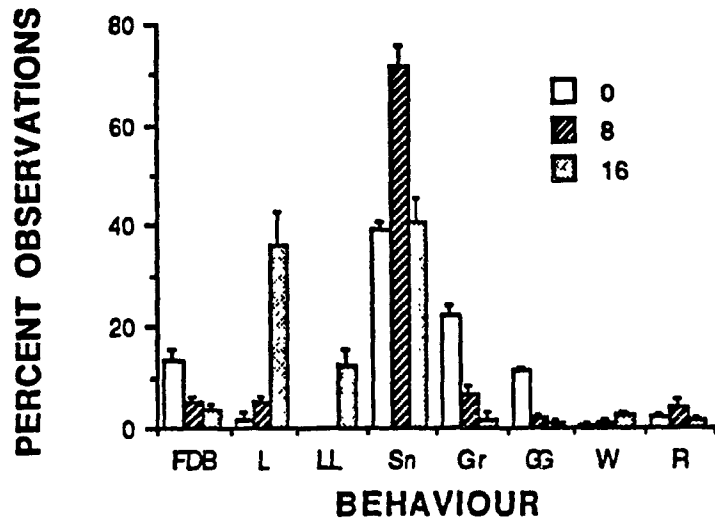
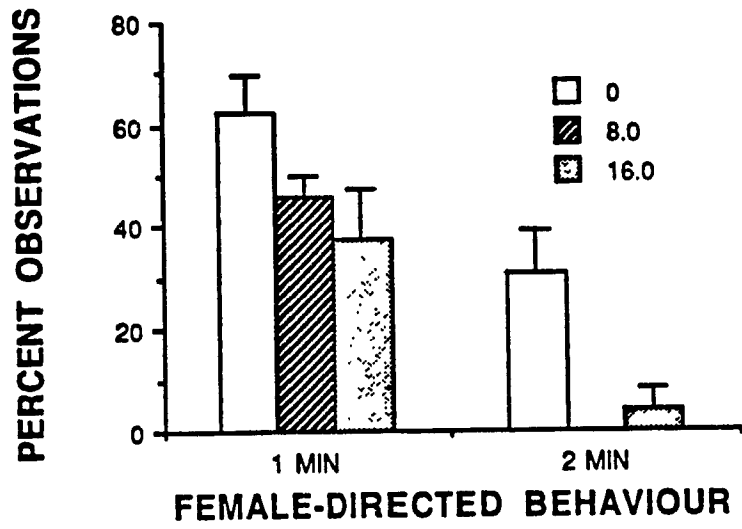


Figure 5 The mean (+ 1 S.E.M.) proportion of observations that female-directed behaviour was recorded to occur during the first and second min following the systemic injection of either saline, 8.0 mg/kg or 16.0 mg/kg U50 given i.p. during tests for sexual behaviour in Experiment 1A



the second min and following saline, relative to U-50,488H injected males. Moreover, while acknowledging the small number of observations upon which the calculations are made (2/min/animal), it appears that female-directed behaviour was not significantly decreased by either dose of U-50,488H during the first min of testing [$p > .05$], but was significantly decreased by both doses during the second min [$p < .05$].

As described, when the males were administered the higher U-50,488H dose, copulatory behaviour was all but abolished. Interestingly, these treated males were occasionally mounted by the estrous females. More striking, when the females mounted these males, rare, but unmistakable, displays of lordosis were seen. Fifty percent of these U-50,488H treated males exhibited lordosis at least once, and half of these males responded to the female's mounts with a mean of 6.0 lordotic displays.

Experiment 1B: Tests for Male Sexual Behaviour Following Sub-Cutaneous U-50,488H

On three counterbalanced tests for sexual behaviour, 25 males were administered either saline, 5.0 or 10.0 mg/kg U-50,488H given s.c.

Copulatory Behaviour: When U-50,488H was administered s.c. the subsequent profile of depressed sexual behaviour was very similar to that previously seen following i.p. injections; overall, however, the drug was more effective at lower doses when given s.c. As illustrated in Fig. 6a-c, s.c. injections of U-50,488H dose-dependently decreased the proportion of males that mounted [$Q(2)=32.99$, $p < .01$], intromitted [$Q(2)=33.09$, $p < .01$], and ejaculated [$Q(2)=33.43$, $p < .01$], relative to that seen in the saline test.

As depicted in Fig. 6d-f, U-50,488H dose-dependently increased the latency to mount [$F(2,48)=50.167$, $p < .00001$], intromit [$F(2,48)=51.078$, $p < .00001$], and ejaculate [$F(2,48)=54.852$, $p < .00001$]. For all three measures the latencies recorded following the 10.0 mg/kg dose were longer than those from the 5.0 mg/kg dose [$p < .01$], which, in turn, were longer than those seen following saline [$p < .01$]. These same increases were apparent even when only the scores of those males that did copulate were examined. Among these

animals, the mean mount latencies were SAL: 53.1 ± 26.5 s, $n=25$; U-50,488H: 5.0 mg/kg: 222.3 ± 121.6 s, $n=14$; U-50,488H: 10.0 mg/kg: 9.7 ± 1.2 s, $n=3$. Mean intromission latencies were SAL: 68.7 ± 30.7 s, $n=25$; U-50,488H: 5.0 mg/kg: 236.7 ± 134.1 s, $n=13$; U-50,488H: 10.0 mg/kg: 44.3 ± 18.2 s, $n=3$. Mean ejaculation latencies were SAL: 368.3 ± 38.1 s, $n=24$; U-50,488H: 5.0 mg/kg: 669.5 ± 157.8 s, $n=13$; U-50,488H: 10.0 mg/kg: 596.0 ± 79.8 s, $n=3$.

As shown in Fig. 6g, U-50,488H dose-dependently decreased the number of ejaculations during the 30-min test sessions [$F(2,48)=60.091$, $p<.00001$]. The difference between drug and saline treatment was significant at both doses [$p<.01$]. When only those males that did copulate were examined, the same inhibitory effects could be seen. Both the 5.0 (1.6 ± 0.4 , $n=14$) and 10.0 mg/kg U-50,488H (1.7 ± 0.3 , $n=3$) doses decreased the mean number of ejaculations, relative to the saline test (2.6 ± 0.2 , $n=25$).

Among those males that did ejaculate, fewer intromissions preceded ejaculation when animals were administered U-50,488H. These data are depicted in Fig. 7, but a repeated measures t-test between the saline and 5.0 mg/kg U-50,488H dose was the only informative statistical comparison because of insufficient subject numbers [$t(8)=2.48$, $p<.04$]. This decrease in the number of intromissions was paralleled by a decrease in the mean number of mounts to precede ejaculation (data not illustrated {SAL: 9.0 ± 1.2 , $n=24$; U-50,488H: 5.0 mg/kg: 3.7 ± 0.85 , $n=9$; U-50,488H: 10.0 mg/kg: 4.0 ± 1.0 , $n=2$ }), and therefore, the hit rate was unaltered (data not illustrated {SAL: 0.67 ± 0.03 , $n=24$; U-50,488H: 5.0 mg/kg: 0.75 ± 0.05 , $n=9$; U-50,488H: 10.0 mg/kg: 0.79 ± 0.12 , $n=2$ }).

Figure 8 shows the mean III from the first copulatory series in each drug condition. Only three of the males given the higher dose intromitted precluding an informative statistical analysis of the three doses. Interestingly, however, a repeated measures t-test done on the III scores of males that did intromit during both the saline and lower U-50,488H dose test suggested a significant effect of drug [$t(12)=3.22$, $p<.008$]; U-50,488H increased the III, relative to saline.

As in Experiment 1A, a statistical analysis of the refractory periods from the few animals that ejaculated following all three drug treatments was not informative. It was apparent, however, that among those animals that did begin a second ejaculatory series, U-50,488H did not convincingly increase the post-ejaculatory refractory period (SAL: 369.2 ± 14.1 s, $n=24$; U-50,488H 5.0 mg: 420.5 ± 30.1 s, $n=8$; U-50,488H 10.0 mg: 566.0 ± 232.4 s, $n=3$).

Non-Copulatory Behaviours: The mean percent of observations (out of 60 possible; 2/min) that various non-copulatory behaviours were recorded during the 30-min sessions is shown in Fig. 9. Significant dose-dependent reductions in the frequency of female-directed behaviour [$F(2,48)=49.448$, $p<.00001$], bodily grooming [$F(2,48)=72.671$, $p<.00001$], and genital grooming [$F(2,48)=68.935$, $p<.00001$] were produced by both doses of U-50,488H [$p<.01$], and both female-directed behaviour and genital grooming were more profoundly suppressed by the higher than the lower dose [$p<.01$]. U-50,488H also produced dose-dependent increases in lying down [$F(2,48)=98.453$, $p<.00001$], body flattening [$F(2,48)=21.830$, $p<.00001$] and sniffing behaviour [$F(2,48)=7.270$, $p<.002$].

Figure 10 depicts the changing occurrence of female-directed behaviour during the first two min of the 30-min test session. A repeated measures ANOVA for Drug x Min yielded significant effects for both Drug [$F(2,48)=20.388$, $p<.00001$] and Min [$F(1,24)=28.174$, $p<.00001$], reflecting the decreases in female-directed behaviour that were induced by U-50,488H, and those seen from the first to the second min. Moreover, it appears that only the highest test dose significantly decreased female-directed behaviour during both the first and second min [$p<.01$]; female-directed behaviour among males administered 5.0 mg/kg U-50,488H was not significantly diminished [$p>.05$] until the second min of testing [$p<.05$].

As in Experiment 1A, some of the U-50,488H-injected males from Experiment 1B were mounted by females and responded with mild, but unmistakable, lordosis. These

Figure 6 The proportion of males that mounted (6a), intromitted (6b) and ejaculated (6c); the mean (+ 1 S.E.M.) latency to mount (6d), intromit (6e) and ejaculate (6f); and the mean (+ 1 S.E.M.) number of ejaculations (6g) following the systemic injection of either saline, 5.0 mg/kg or 10.0 mg/kg U50 given s.c. during tests for sexual behaviour in Experiment 1B

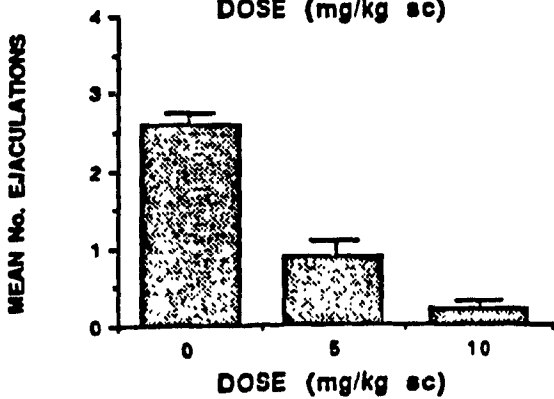
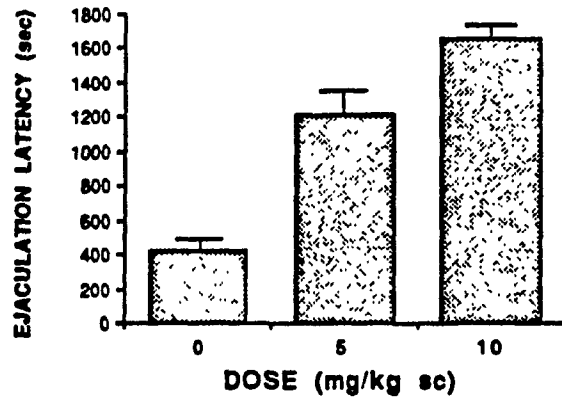
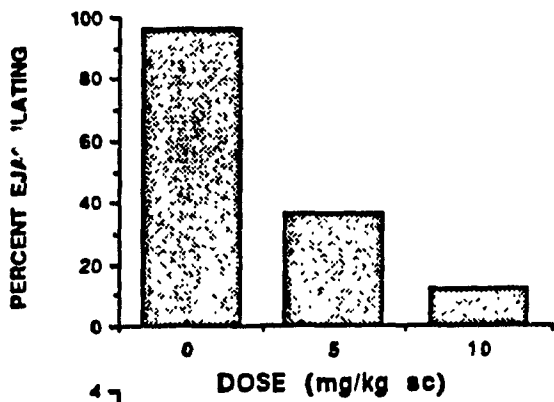
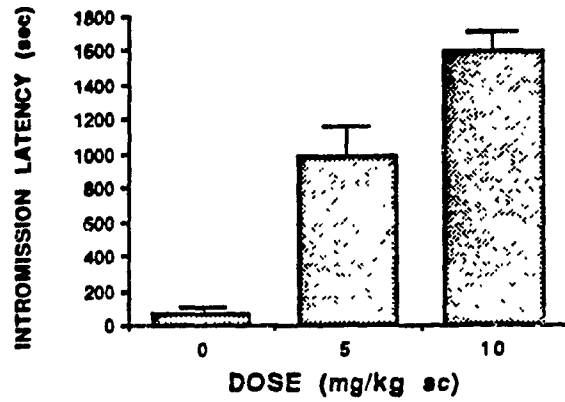
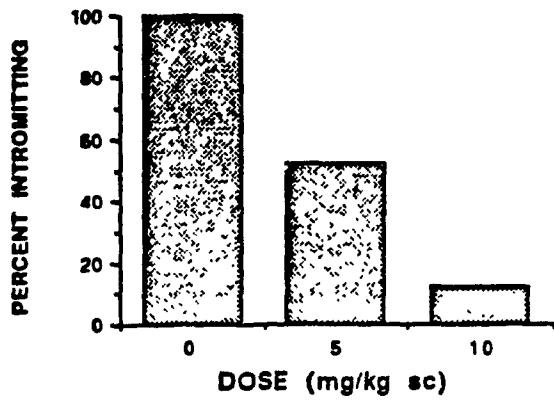
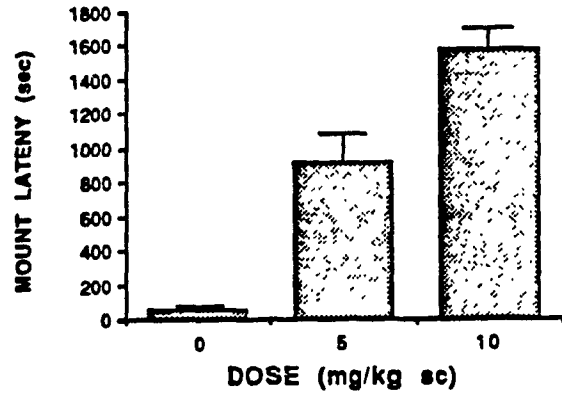
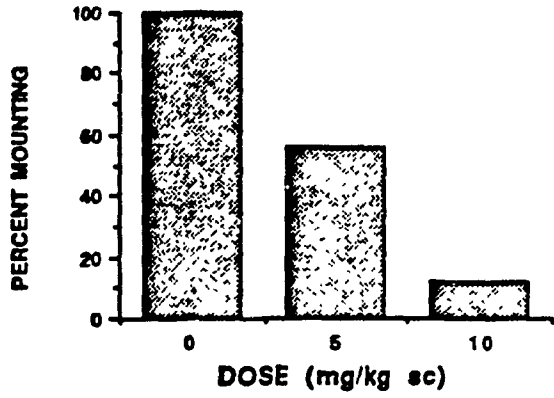


Figure 7 The mean (+ 1 S.E.M.) number of intromissions to precede the first ejaculation following the systemic injection of either saline, 5.0 mg/kg or 10.0 mg/kg U50 given s.c. during tests for sexual behaviour in Experiment 1B

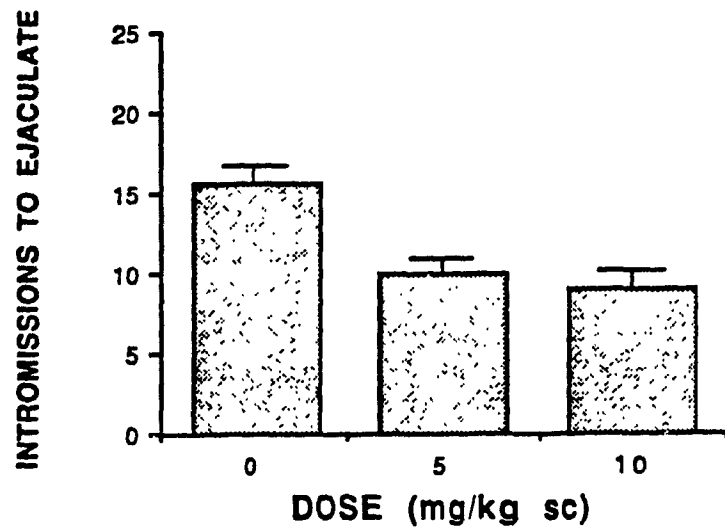


Figure 8 The mean (+ 1 S.E.M.) inter-intromission interval prior to the first ejaculation following the systemic injection of either saline, 5.0 mg/kg or 10.0 mg/kg U50 given s.c. during tests for sexual behaviour in Experiment 1B

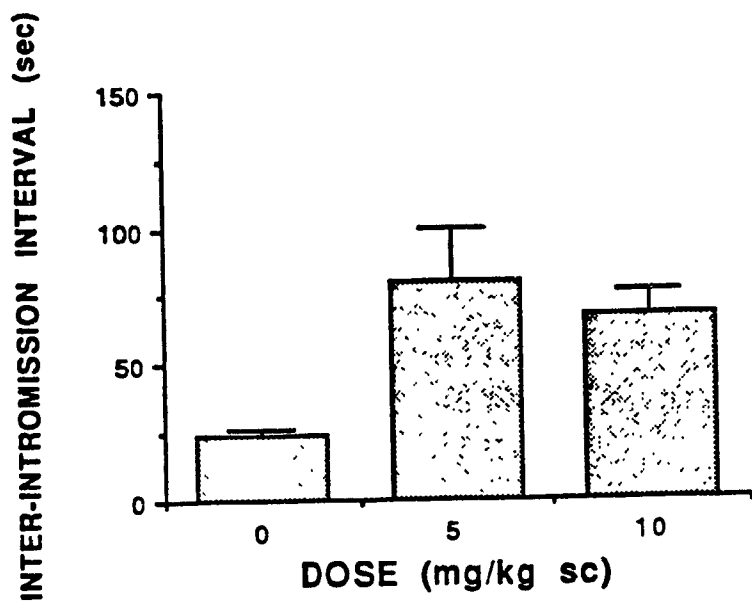


Figure 9 The mean (+ 1 S.E.M.) proportion of observations that non-copulatory behaviours were recorded to occur following the systemic injection of either saline, 5.0 mg/kg or 10.0 mg/kg U50 given s.c. during tests for sexual behaviour in Experiment 1B. (FDB: female-directed behaviour; L: laying down; LL: body flattening; Sn: sniffing; Gr: body grooming; GG: genital grooming; W: walking; R: rearing)

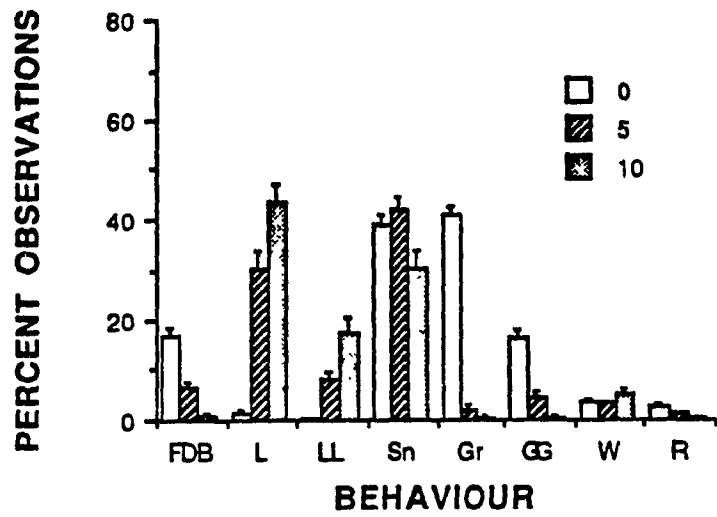
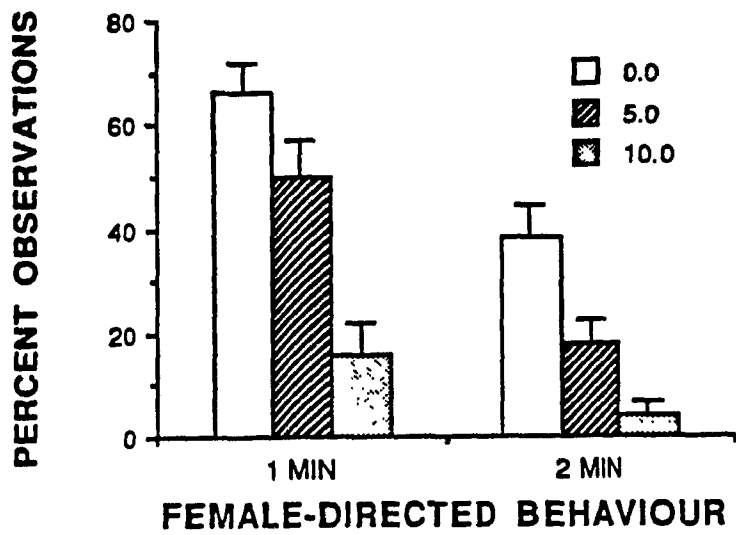


Figure 10 The mean (+ 1 S.E.M.) proportion of observations that female-directed behaviour was recorded to occur during the first and second min following the systemic injection of either saline, 5.0 mg/kg or 10.0 mg/kg U50 given s.c. during tests for sexual behaviour in Experiment 1B.



displays were not common; only 16% of males treated with the higher dose, and only 4% of males administered the lower dose showed lordosis. Although such displays were rare following U-50,488H injections, they were never seen after saline.

Experiment 2A: Test for Locomotor Activity Following Intra-Peritoneal U-50,488H

On one test for locomotor activity in novel photocell boxes, 22 males were administered either saline, 8.0 or 16.0 mg/kg U-50,488H given i.p.

Locomotor Activity: As shown in Fig. 11a, the i.p. administration of U-50,488H dose-dependently decreased locomotor activity. When the activity scores for each 30-min interval were subjected to a two-way, between-within, ANOVA for Dose x Time, the analysis yielded main effects for both Dose [$F(2,19)=11.049$, $p<.0006$] and Time [$F(2,38)=6.671$, $p<.003$]. Post hoc analyses indicated that both 8.0 mg/kg [$p<.05$] and 16.0 mg/kg U-50,488H given i.p. [$p<.01$] significantly decreased locomotor activity, and that the two U-50,488H doses were significantly different from each other [$p<.05$]. There was no Dose x Time interaction [$F(4,38)=1.704$, $p=.1693$], indicating that the temporal pattern of locomotor activity was the same for each dose.

Experiment 2B: Tests for Locomotor Activity Following Sub-Cutaneous U-50,488H

On one test for locomotor activity in novel photocell boxes, 39 males were administered either saline, 0.5, 5.0 or 10.0 mg/kg U-50,488H given s.c.

Locomotor Activity: Fully compatible with the locomotor depressant actions of U-50,488H administered i.p., Fig. 11b illustrates that s.c. injections of U-50,488H also dose-dependently decreased locomotor activity. The ANOVA yielded main effects for both Dose [$F(3,43)=23.125$, $p<.00001$] and Time [$F(2,86)=47.256$, $p<.00001$]. Post hoc analyses indicated that both 5.0 and 10.0 mg/kg of U-50,488H given s.c. decreased locomotor activity significantly below the levels observed following either saline [$p<.01$] or 0.5 mg/kg of U-50,488H [$p<.01$]. Conversely, there was no significant effect of 0.5

mg/kg U-50,488H on activity [$p > .05$]. Overall, 10.0 mg/kg U-50,488H did not decrease locomotor activity more potently than 5.0 mg/kg, but it did produce a faster onset of inhibition as reflected in a significant Dose x Time interaction [$F(6,86) = 3.157, p < .008$].

Experiment 3: Tests for Body Temperature Following Sub-Cutaneous U-50,488H

Over six days, six males were monitored continuously for body temperature while receiving one saline injection at the same time each day. On the seventh day, all animals were administered 5.0 mg/kg U-50,488H given s.c. The changes in body temperature following the systemic injections on days six and seven are described below.

Body Temperature: Figure 12a,b shows the body temperature changes resulting from injections of either saline or 5.0 mg/kg U-50,488H given s.c. The scores were plotted and analysed as the change in temperature, from the 15 min point immediately preceding the injection to the 15 and 30 min time points following injection. As can be seen, modest increases in body temperature were recorded following the saline injection whereas U-50,488H both prevented this presumably handling-induced increase and precipitated a decrease in body temperature. These changes in body temperature were apparent at both 15 and 30 min post-injection, but the differences between the saline and U-50,488H temperature scores were greater at the latter time. Reflecting these changes the analysis yielded a significant main effect for Drug [$F(1,5) = 4.234, p < .03$], but not for Time [$F(1,5) = .026, p = .8786$], and a significant Drug x Time interaction [$F(1,5) = 47.348, p < .002$]. Simple main effects analyses confirmed that the effect of drug was significant at both 15 [$F(1,5) = 6.644, p = .050$] and 30 min [$F(1,5) = 13.710, p < .02$], and that both the saline injection-elicited increase [$F(1,5) = 60.176, p < .002$] and the U-50,488H-induced decrease [$F(1,5) = 9.752, p < .03$] in body temperature were greater at 30 than at 15 min.

Figure 11 The mean (± 1 S.E.M.) 30-min locomotor activity scores following the systemic injection of either saline, 8.0 mg/kg or 16.0 mg/kg U50 given i.p. (11a) in Experiment 2A, and following, injections of either saline, 0.5 mg/kg, 5.0 mg/kg or 10.0 mg/kg U50 given s.c. (11b) in Experiment 2B.

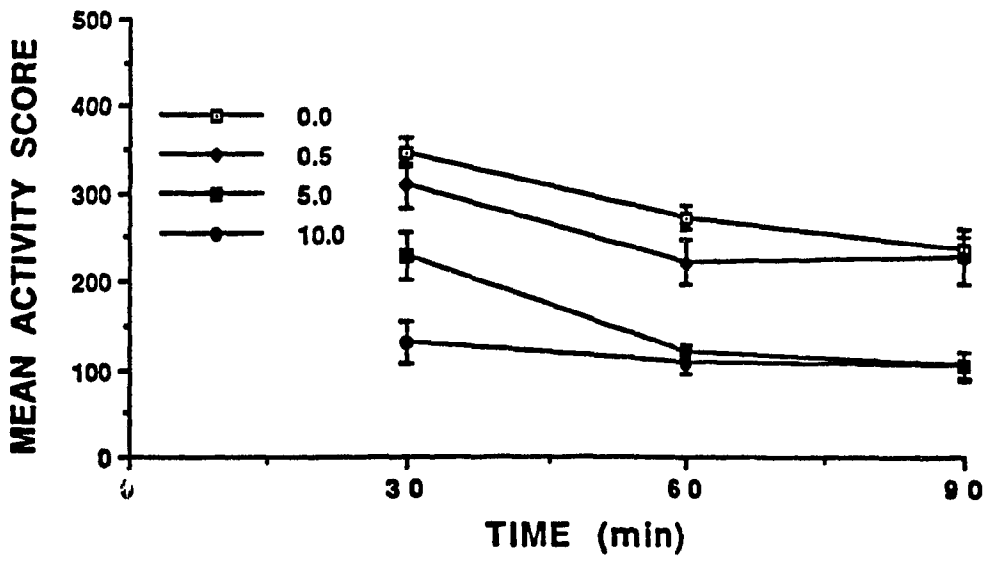
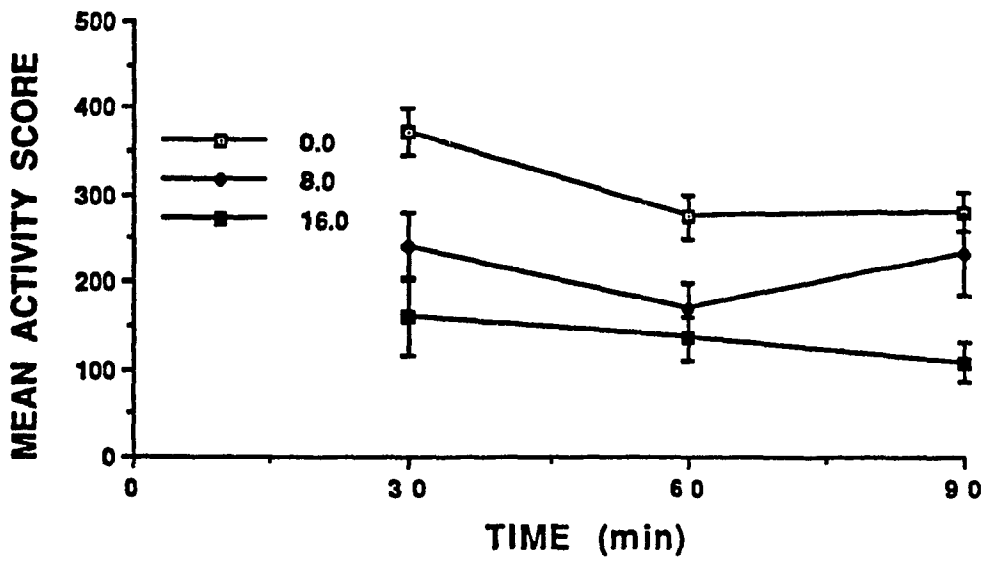
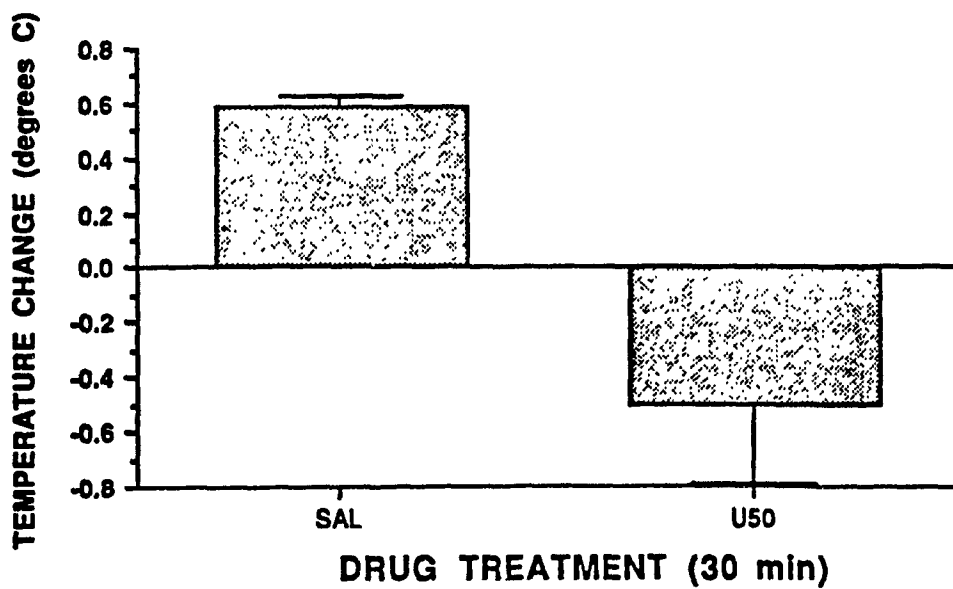
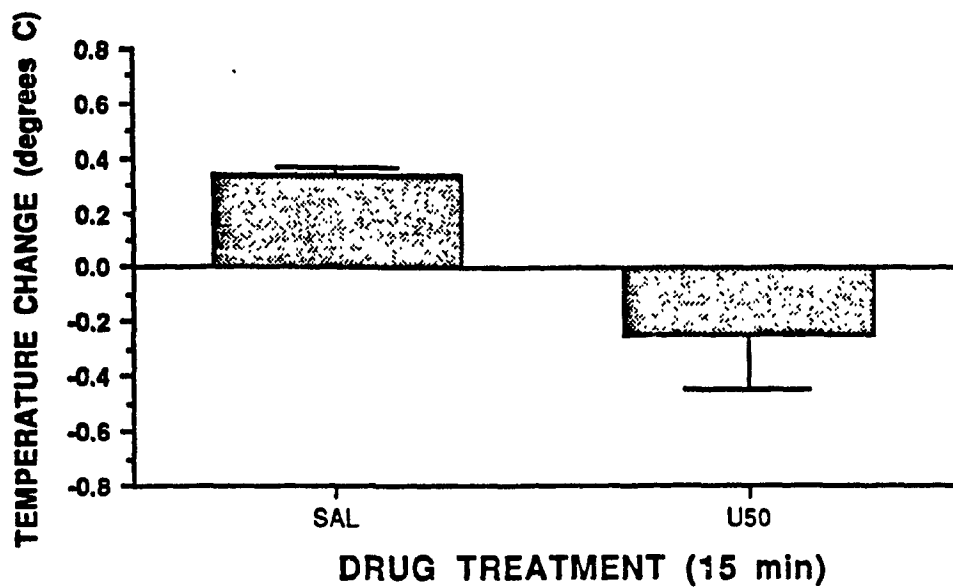


Figure 12 The mean (+ 1 S.E.M.) change in body temperature 15 (12a) and 30 min (12b) following the systemic injection of either saline or 5.0 mg/kg U50 given s.c. in Experiment 3.



Discussion

Within the testing parameters of the present study, *k* opioid receptor stimulation induced by the systemic administration of U-50,488H had only inhibitory effects on behaviour; no excitatory effects were evident. U-50,488H produced a broad range of behavioural inhibitions, decreasing both male sexual behaviour and the exploration of novel activity boxes. These parallel findings are compatible with earlier suggestions that *k* receptor stimulation decreases DA neurotransmission; synaptic levels of mesolimbic DA are known to vary the positive incentive value that animals will attach to environmental stimuli (see Everitt, 1990). Classically, this interest in appetitive incentive stimuli is measured by observations of the animal's investigatory and approach behaviour. Consummatory dimensions of behaviour - the stimulus-appropriate interaction with the incentive stimulus (e.g., eating and consuming food as opposed to approaching and sniffing it) - are most commonly measured by the actual performance of the behaviour.

Copulatory Behaviour: In Experiments 1A and 1B, U-50,488H, given either i.p. or s.c., potently inhibited sexual behaviour measures. Both appetitive and consummatory mechanisms appear to have been affected, but as is often a problem in studies of sexual behaviour, it is difficult to dissociate these two effects. Indicative of a relatively pure appetitive deficit, U-50,488H decreased the display of female-directed behaviour. Interestingly, this U-50,488H-induced attenuation of female-directed behaviour was not immediately apparent; during the first min after the estrous female had been introduced to the male, female-directed behaviour was not significantly diminished; statistically significant decrements appeared later. This observation is reminiscent of the changes in feeding behaviour that are seen to develop following systemic injections of neuroleptics (Wise & Colle, 1984; Wise & Raptis, 1986; Koehling, Colle & Wise, 1988). In both these feeding studies and the present experiments, the drug treatments had little effect on the animal's initial contact with the incentive stimulus, but did disrupt the maintenance of interactions with the stimulus; as increased time was taken to finish eating the provided

food, so did female-directed behaviour appear less often, more distantly and less commonly progressing to copulatory behaviour. These observations suggest that the recorded behavioural deficits do not result from motor incapacitation - the animals display an undiminished capacity to initially engage with the stimulus - but rather, reflect decreases in the ability of the stimulus characteristics of the estrous female to maintain the interest of the male. Compatibly, the latencies to finally initiate mounts and intromissions were significantly prolonged by systemic injections of U-50,488H.

In addition to these U-50,488H-induced decrements in the appetitive motivation to both interact with the female and initiate copulation, consummatory deficits in male sexual behaviour were also seen; among copulating males, U-50,488H prolonged the ejaculation latency. It would seem that even among those males that had surmounted the copulatory threshold, disruptions to the theoretical CM remained. Most probably related to these increases in mount, intromission and ejaculation latencies, U-50,488H-injected animals also ejaculated less often during the 30-min test sessions.

Systemic injections of U-50,488H were also found to decrease both the number of mounts and intromissions that preceded ejaculation. This decrease was surprising; the number of copulations prior to ejaculation typically remain constant within the same animal, and decreases are usually associated with increases in sexual excitement (see Sachs & Meisel, 1988). Interestingly, castration decreases the number of intromissions that precede ejaculation, but in contrast to that seen in the present experiments following the administration of U-50,488H, this castration-induced decrease is accompanied by an equal increase in the number of mounts (e.g., Beach & Holz-Tucker, 1949), and might reflect changes in penile reflexes (Meisel, O'Hanlon & Sachs, 1984).

Copulatory Behaviour: Neurochemical Substrates: These decrements in male copulatory behaviour induced by systemic U-50,488H closely resemble those seen to follow neuroleptic injections given both systemically and intra-cranially into either the NAS or mPOA (e.g., Pfaus & Phillips, 1989, 1991). DA receptor antagonists decrease pre-

copulatory anticipatory behaviours, increase the latencies to initiate copulation and decrease the proportion of males that copulate. Given the ability of k opioid agonists to inhibit DA neurotransmission (e.g., Walker et al., 1987; Di Chiara & Imperato, 1988; Heijna et al., 1989, 1990a,b; Reid et al., 1988, 1990a; Spanagel et al., 1990; Manzanares et al., 1991), decreased DA release seems a probable explanation of these behavioural attenuations.

Other U-50,488H-induced changes in copulatory behaviour do not so clearly indicate decreased DA neurotransmission. Systemic U-50,488H injections decreased the number of mounts and intromissions to precede ejaculation without accompanying decreases in the ejaculation latency. The mechanisms through which these effects might be mediated are unclear, although alterations in both DA and 5-HT neurotransmission are suggested. For example, both systemic (e.g., Pfaus, 1990; Pfaus & Phillips, 1989, 1991) and intra-mPOA (Hull et al., 1989b; Pfaus, 1990; Pfaus & Phillips, 1989, 1991) neuroleptic injections decrease the number of intromissions to precede ejaculation, but these changes appear to result from decreases in penile erections (Pehek et al., 1988a; Hull et al., 1989a, 1991; Markowski et al., 1990). In contrast, the unaltered hit rate observed in the present study suggests that penile reflexes are undisturbed by U-50,488H (Experiments 1A and 1B).

Some of the effects seen after U-50,488H are similar to those seen when 5-HT agonists and antagonists are administered. For example, the administration of 5-HT_{1a} autoreceptor agonists, either systemically (Ahlenius et al., 1980; Stefanick et al., 1982; Clark et al., 1983; Ahlenius & Larsson, 1984b; Clark & Smith, 1986; Schnur et al., 1988; Ågmo et al., 1989) or intra-cranially into the raphé (Hillegaart, Ahlenius & Larsson, 1989; Hillegaart, 1991) or NAS (Hillegaart, 1991), also decreases the number of intromissions to precede ejaculation. Furthermore, systemically administered 5-HT₂ post-synaptic antagonists increase both copulation and ejaculation latencies as well as decrease the number of mounts and intromissions (Mendelson & Gorzalka, 1985).

These similarities between the altered copulation characteristics seen to follow both U-50,488H administration and decreased 5-HT activity suggest at least two alternative interpretations. First, U-50,488H might be inhibiting serotonergic post-synaptic activity; U-50,488H/5-HT interactions have been reported in tests of analgesia (VonVoigtlander, Lewis & Neff, 1984; Ho & Takemori, 1989, 1990), and the effects of 8-OH-DPAT on sexual behaviour are antagonized by naloxone (Ågmo et al., 1989). Secondly, the U-50,488H-induced decreased intromissions and increased copulation latencies might only superficially resemble those produced by 5-HT_{1a} agonists and 5-HT₂ antagonists; possibly both their functional and physiological significance are distinct. Thus, the neural system mediating the U-50,488H-induced alterations in copulatory behaviour remains untested; changes in latencies to both copulate and ejaculate as well as the number of mounts and intromissions could result from inhibitions of either 5-HT or DA neurotransmission, or both.

Flattening Behaviour and Body Temperature: As has been reported previously in guinea pigs (Brent et al., 1990), U-50,488H induced striking body flattening reminiscent of that seen during the post-ejaculatory refractory period. The previous investigators also reported that U-50,488H-induced flattening was prevented by systemic administrations of NBNI, indicating that the behaviour is mediated by *k* receptor stimulation. Why such a behaviour might be evoked is less clear. One possibility is that body flattening reflects an attempt to dissipate increased body heat, however, the results of Experiment 3 did not unequivocally support this idea. Replicating the findings of other investigators (e.g. Adler et al., 1988), U-50,488H was found to produce only hypothermia. These data suggest that U-50,488H-induced body flattening is initiated for other reasons - either thermoregulation-independent mechanisms or from increases in brain temperature that are not detected in the peripheral body cavity - and possibly, that the U-50,488H-induced decrease in body temperature is produced by flattening.

One other pharmacological treatment that is known to induce body flattening is the central infusion of either 5-HT or the 5-HT_{1a} autoreceptor agonist 8-OH-DPAT into either the raphé or the NAS (Hillegaart et al., 1989; Hillegaart, 1991). Moreover, dorsal raphé infusions of either 5-HT or 8-OH-DPAT also decrease body temperature (Hillegaart, 1991). Interactions between *k* opioid agonists and 5-HT have been observed previously; both *in vitro* (Ho & Takemori, 1989a) and analgesia studies (VonVoigtlander et al., 1984; Ho & Takemori, 1989b, 1990) suggest that U-50,488H increases 5-HT release, and repeated administrations of U-50,488H increase 5-HT₁ receptor density (Gulati, Ramarao & Bhargava, 1989). In contrast, the behavioural syndrome of increased copulation latency, decreased mounts and intromissions to precede ejaculation, decreased motor activity, decreased body temperature and increased body flattening induced by systemic U-50,488H (present study) most closely resemble a mixture of the effects produced by the administration of 5-HT_{1a} autoreceptor agonists (Ahlenius et al., 1980; Stefanick et al., 1982; Clark et al., 1983; Ahlenius & Larsson, 1984b; Clark & Smith, 1986; Schnur et al., 1988; Ågmo et al., 1989; Hillegaart et al., 1989; Hillegaart, 1991) that most probably decrease 5-HT release, and 5-HT₂ post-synaptic receptor antagonists (Mendelson & Gorzalka, 1985). Perhaps U-50,488H inhibits selective 5-HT post-synaptic mechanisms.

Interestingly, another parallel between post-ejaculatory behavioural immobility (Barfield & Geyer, 1975) and the behaviour of U-50,488H-injected animals (Young, 1989) is the appearance of slow-wave, sleeplike EEG. Possibly, the post-ejaculatory slow-wave EEG associated with body flattening is instigated by a release of DYN. Possibly, U-50,488H engages this process.

Grooming: A profound suppression of grooming was also seen in Experiments 1A and 1E. Given the reduction in copulatory behaviour it is not surprising that there would be less genital grooming. An explanation for the reduced bodily grooming, however, is less readily apparent. As discussed earlier, bodily grooming is induced by selective D1 receptor agonists. D1 antagonists decrease the grooming that is typically seen

either in novel environments or following the administration of various neuropeptides (Greidanus et al., 1989). If, as substantial evidence suggests, *k* agonists do decrease the release of DA, then the *k* agonist-induced reduction of bodily grooming might result from diminished D1 receptor stimulation.

Lordosis: Males administered either 16.0 mg/kg U-50,488H given i.p. or 5.0 and 10.0 mg/kg U-50,488H given s.c. were occasionally mounted by the female. In response, some of these males displayed lordosis. The display of masculine sexual behaviour by normal females is not uncommon; throughout the estrous cycle, female rats mount castrated males, sexually sluggish gonadally intact males, and even other females (Beach, 1942; Beach & Rasquin, 1942; Beach, 1976). Similarly, the display of female-typical sexual behaviour by male rats has also been reported. Both prepuberally castrated estrogen administered males (Larsson, Södersten, Beyer, Morali & Pérez-Palacios, 1976) and prenatally stressed males (Ward, 1972,1977; Herrenkohl & Whitney, 1976; Dahlof, Hard & Larsson, 1977; Dunlap, Zadina & Gougis, 1978; Meise, Dohanich & Ward, 1979; Gotz & Dornier, 1980; Rhees & Fleming, 1981) will display lordosis, and although it is less common for untreated males to do so, there are many reports that they do. In female rats, the display of lordosis has been suggested to vary with levels of brain DA; 6-OHDA lesions and neuroleptic treatments that decrease postsynaptic DAergic efficacy reduce proceptive behaviours while augmenting sexual receptivity (Caggiula, Antelman, Chiodo & Lineberry, 1979; Caggiula, Herndon, Scanlon, Greenstone, Bradshaw & Sharp, 1979). Caggiula suggested that these findings reflected the broader role of brain DA systems in both the initiation of action and responsivity to environmental stimuli.

In the tests for sexual behaviour, U-50,488H-injected males were presented with both the visual and olfactory cues from the estrous female and the tactile stimulation from being mounted by the female. It is possible then, that the U-50,488H-induced decrease in DA neurotransmission disinhibited the male's potential to display female-typical lordosis. Interestingly, it has been reported that the i.c.v. administration of DYN potentiates lordosis

in female rats (Suda et al., 1986; Pfaus & Gorzalka, 1987b). At this time, however, no data concerning the effects of more selective *k* agonists have been reported.

Locomotor Activity: Increases and decreases in both male sexual behaviour (e.g., Hull et al., 1986; Ahlenius et al., 1987; Ågmo & Fernández, 1989; Everitt et al., 1989; Pleim et al., 1990) and locomotor activity (e.g., Kalivas & Miller, 1985; Ahlenius, Hillegaart, Thorell, Magnusson & Fowler, 1987; Carr & White, 1987) are associated with parallel alterations in mesolimbic DA neurotransmission. This similarity is believed to reflect the focal role that the mesolimbic DA pathway plays in the regulation of appetitive motivation to approach incentive stimuli (see Wise & Rompré, 1989; Everitt, 1990). Following reports of the efficacy of *k* opioid agonists to decrease DA neurotransmission (e.g., Walker et al., 1987; Di Chiara & Imperato, 1988; Reid et al., 1988, 1990a; Spanagel et al., 1990; Manzanares et al., 1991) the present thesis tested the hypotheses that U-50,488H would decrease both locomotor activity and male sexual behaviour. As discussed above, U-50,488H dose-dependently decreased male sexual behaviour. In Experiments 2A and 2B, systemic U-50,488H was seen to dose-dependently decrease locomotor activity, and this is compatible with most previous reports examining the motor activity effects of *k* opioid agonists (Castellano et al., 1984; Benton, 1985; Benton et al., 1985; Imperato & Di Chiara, 1985; Ukai & Kameyama, 1985; Dykstra et al., 1987; Leighton et al., 1987; Di Chiara & Imperato, 1988; Jackson & Cooper, 1988; Young, 1989). The substrate for U-50,488H's locomotor inhibiting action has yet to be unequivocally identified, however, the association between U-50,488H-decreased NAS DA release and locomotor activity (Imperato & Di Chiara, 1985; Di Chiara & Imperato, 1988) makes the mesolimbic DA system a compelling candidate. Interestingly, in light of the other strikingly similar behavioural effects induced by both systemic U-50,488H administration and 5-HT receptor ligands, infusions of 5-HT into the dorsal raphe decrease, and into the median raphe increase, locomotor activity (Hillegaart et al., 1989).

Overall, these experiments are compatible with the suggestion that U-50,488H acts on central *k* receptors in the mesolimbic and incertohypothalamic pathways to reduce DA release and decrease both the estrous female's stimulus characteristics ability to maintain the interest of the male and the consummatory functioning to express various male sexual behaviours. Additionally, intriguing parallels between the behavioural effects of 5-HT neural suppression and U-50,488H administration suggests that the *k* agonist might also inhibit 5-HT actions particularly in the NAS. It would be of interest to know whether selective characteristics of the U-50,488H-induced behavioural profile could be differentially reversed by the administration of selective DA and 5-HT post-synaptic agonists.

Although data from the present experiments are compatible with the induction of behaviourally inhibitory effects from central *k* opioid receptor stimulation, other interpretations are possible. Included among these are the aversive effects of *k* opioid stimulation in the periphery (Bechara & van der Kooy, 1987), and the non-opioid effects seen by others on DA systems (Robertson et al., 1987). In order to examine these possibilities, the effects of direct central injections of U-50,488H were examined and systemic injections of U-50,488H were co-administered with systemic naloxone.

CHAPTER THREE

U-50,488H Sites of Action: Opioid vs Non-Opioid; Central vs Peripheral

In the preceding group of experiments, it was demonstrated that systemically administered U-50,488H reduced both locomotor activity and male sexual behaviour. However, it was not determined whether these effects were opioid mediated. The experiments presented in this chapter addressed this question by co-administering systemic injections of U-50,488H and the opioid receptor antagonist naloxone. To investigate the possibility of central mediation of the observed U-50,488H-induced behavioural changes, intra-cranial injections of U-50,488H were administered to male rats tested for both sexual behaviour and locomotor activity. Three central sites were chosen based on studies demonstrating the importance of DA neurotransmission to sexual behaviour. *In vivo* studies indicate that DA release in the NAS (Pfaus et al., 1989; Pfaus, 1990), terminal region of the mesolimbic DA pathway, and the mPOA (Pfaus, 1990; Eaton et al., 1991), terminal region of the incertohypothalamic DA pathway, increases during copulation reaching a peak at the moment of ejaculation. As well, pharmacological studies further indicate that changes in DA receptor stimulation in either of these terminal regions (e.g., Hull et al., 1991; Pfaus & Phillips, 1991) or the VTA (e.g., Hull et al., 1991), cell body region of the mesolimbic DA pathway, can alter male sexual behaviour. Qualitatively different effects of U-50,488H injections to the different brain sites would suggest that different aspects of the behavioural changes induced by systemic injections of U-50,488H are mediated at different brain loci. Naloxone antagonism of the behavioural changes induced by systemic injections of U-50,488H would indicate that these changes result from the stimulation of opioid receptors.

The systemically administered dose of U-50,488H was 5.0 mg/kg given s.c. This dose was chosen because in Experiments 1B and 2B it was seen to induce behavioural deficits without incapacitating the animal; these animals were less active, but phenomenologically normal. In contrast, animals administered higher doses were

considerably less active, muscularly flaccid and unresponsive to the initial presentation of an estrous female. The centrally administered U-50,488H doses were based on the dose range of intra-SN *k* agonists demonstrated to induce circling (Herrera-Marschitz et al., 1986; Matsumoto et al., 1988a,b; Tan & Tsou, 1988) and decrease striatal DA release (Reid et al., 1988a,b, 1990).

Methods

Subjects

Male Wistar rats weighing 260-280 g upon arrival were obtained from Charles River Canada Inc. (USA), and were housed individually as in Experiment 1. Nineteen of these males were then selected following a screen to ensure that the males displayed consistent levels of sexual behaviour. The same 19 males served as subjects in all four of the following experiments.

Surgery

Following their selection from the larger population, all animals were stereotaxically implanted with chronic bilateral guide cannulae (26-gauge stainless steel tubing) aimed at either the VTA (A/P -3.6, L \pm 0.6, D/V -8.9), NAS (A/P +3.4 \pm 2.8, L, D/V -7.4), or mPOA (A/P +2.4, L \pm 0.2, D/V -6.7), positioned 1.0 mm above the final injection site. The incisor bar was set at 5.0 mm superior to the interaural line. The animals were then given one week to 10 days to recover prior to beginning testing.

Histology

Following the experiment, all animals were perfused transcardially with saline and 10% formalin solution under deep anaesthesia. Brains were stored in a 10% formalin solution for at least three days. Histological verification of cannulae tip placement was subsequently made on 30 μ m thionin-stained coronal sections

Apparatus

The apparatus used was the same as described in Experiment 1.

Procedure

In Experiment 4A, the male rats were tested for sexual behaviour following 5.0 mg/kg U-50,488H given s.c. plus counterbalanced administrations of either 2.0 mg/kg or 5.0 mg/kg naloxone or saline given i.p. The males were administered U-50,488H 15 min, and naloxone 10 min, prior to being placed in the mating boxes. As previously, the males then waited a further 5 min before being paired with an estrous female for 30 min. All sexual behaviour tests were 3 to 4 days apart.

In Experiment 4B, the same male rats were tested for locomotor activity in the photocell boxes following three counterbalanced tests of either two systemic injections of saline, 5.0 mg/kg U-50,488H given s.c. plus saline given i.p. or U-50,488H plus 2.0 mg/kg naloxone given i.p. The U-50,488H was administered to animals 5 min prior to their being placed in the box; the naloxone was injected immediately before testing began. Animals were then monitored for 90 min every other day. These four tests were preceded by two drug-free 60-min tests in the photocell boxes in order to habituate the animals to the testing environment.

In Experiment 5A, the same male rats were injected intracranially with one of five doses of U-50,488H (0.0, 0.0005, 0.05, 0.5, 5.0 nmol/0.5 μ l/side) in counterbalanced sexual behaviour tests twice a week. Drugs were infused over 45 s in freely moving animals. The injectors were then left in place for an additional 75 s. Males were then placed in individual mating boxes for 5 min before being paired with an estrous female for 30 min.

When the results from Experiment 5A were analysed, it became apparent that there were behavioural effects produced by the lower doses of U-50,488H that were not reproduced by the highest test dose. To investigate for the possibility of multiple and opposing effects by U-50,488H exerted on different receptor systems these five counterbalanced tests were followed four weeks later by one further test for sexual

behaviour. The 17 remaining healthy males were injected with 2.0 mg/kg naloxone given i.p. immediately before an intracranial infusion of U-50,488H (5.0 nmol/0.5 μ l/side). As previously, the males waited 5 min in the mating boxes prior to being paired with an estrous female for 30 min.

In Experiment 5B, the male rats were tested every two to four days for locomotor activity in the photocell boxes for 60 min following the co-administration of either 2.0 mg/kg naloxone or saline vehicle given i.p., plus one of five intracranial doses of U-50,488H (0.0, 0.0005, 0.05, 0.5, 5.0 nmol/0.5 μ l/side). All tests were counterbalanced.

Statistical Analyses

The proportion of animals from each condition that mounted, intromitted and ejaculated was analysed by Cochran's Q statistic. In Experiment 4A, in which animals were systemically administered U-50,488H, the latency for animals to mount, intromit and ejaculate, and the mean number of ejaculations, intromissions and the III were each analysed with one repeated measures ANOVA. In Experiment 5A, where animals were intra-cranially administered U-50,488H, these same measures were analysed with separate repeated measures ANOVAs for each of the three brain sites examined. When animals did not mount or intromit they were assigned a cut off score of 1800 sec. When animals did not ejaculate they were assigned either the time from their first intromission to the end of the session or a cut off score of 1800 sec if they did not intromit. For refractory periods, animals were assigned the time from their first ejaculation to either the first mount of the second ejaculatory series or the end of the session if they ejaculated once but did not reinitiate mounting. The locomotor activity scores in both Experiments 4B and 5B were analysed by repeated measures ANOVAs. Post hoc comparisons, when performed, were made with Newman-Keuls tests.

Results

Experiment 4A: Tests for Male Sexual Behaviour Following the Co-Administration of U-50,488H with Naloxone

Eighteen males were repeatedly tested on separate occasions for sexual behaviour following four different drug conditions. In three counterbalanced tests, animals received either 5.0 mg/kg U-50,488H given s.c. plus saline given i.p., U-50,488H plus 2.0 mg/kg naloxone given i.p., or U-50,488H plus 5.0 mg/kg naloxone. These data were compared to the same animals' saline control test in Experiment 5A.

Copulatory Behaviour: As shown in Fig. 13a-c, the inhibiting effects of 5.0 mg/kg U-50,488H given s.c. obtained in Experiment 1B were replicated. U-50,488H significantly decreased the proportion of males that mounted [$Q(3)=20.52, p<.01$], intromitted [$Q(3)=23.08, p<.01$] and ejaculated [$Q(3)=20.47, p<.01$]. The important observation of this experiment was that these U-50,488H-induced effects were prevented by both tested doses of naloxone.

The repeated measures ANOVA indicated that the latencies to mount [$F(3,51)=13.036, p<.0001$], intromit [$F(3,51)=12.820, p<.0001$], and ejaculate [$F(3,51)=11.628, p<.0001$] were all significantly increased by systemic U-50,488H [$p<.01$], and these inhibitions were attenuated by both doses of naloxone [$p<.01$] (Fig. 13d-f).

The mean number of ejaculations recorded in each 30-min session [$F(3,51)=8.524, p<.0002$] was also decreased by the injection of U-50,488H [$p<.01$], and this decrease was attenuated by both the 2.0 mg/kg [$p<.01$] and 5.0 mg/kg dose of naloxone [$p<.05$] (Fig. 13g).

Figure 14 illustrates the number of intromissions to precede the first ejaculation among those males that did ejaculate at least once during the 30-min test session. Replicating the findings of Experiment 1B, U-50,488H tended to decrease the number of intromissions that precede an ejaculation, however, when the data from the few ($n=5$)

Figure 13 The proportion of males that mounted (13a), intromitted (13b) and ejaculated (13c); the mean (+ 1 S.E.M.) latency to mount (13d), intromit (13e) and ejaculate (13f); and the mean (+ 1 S.E.M.) number of ejaculations (13g) following the systemic injection of either saline, 5.0 mg/kg U50 given s.c., U50 plus 2.0 mg/kg naloxone given i.p. or U50 plus 5.0 mg/kg naloxone during tests for sexual behaviour in Experiment 4A.

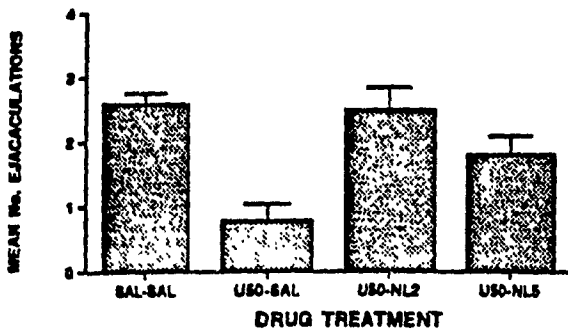
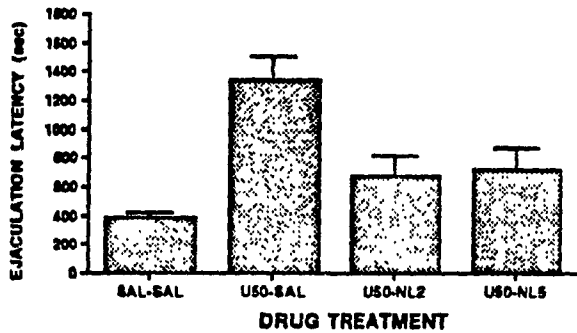
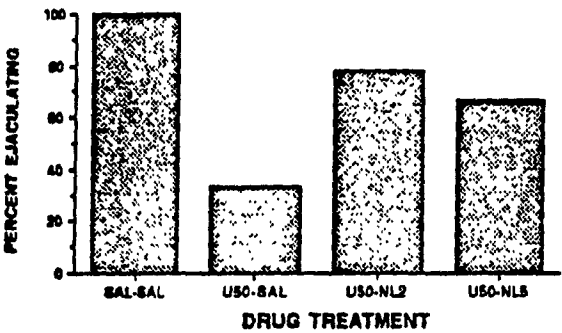
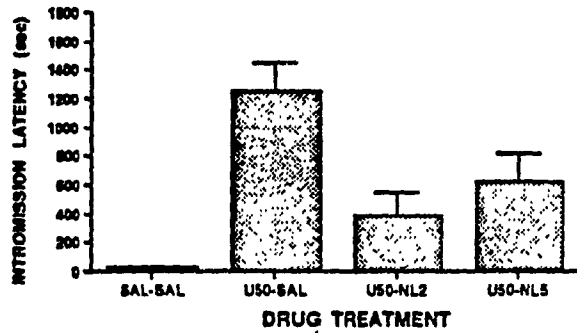
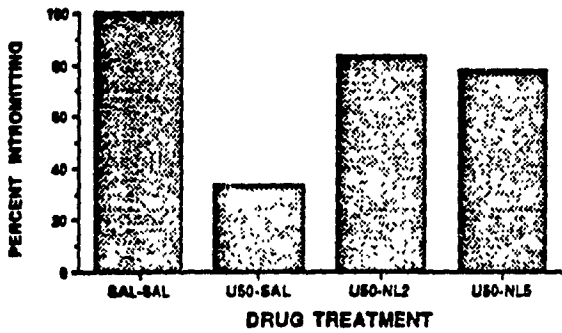
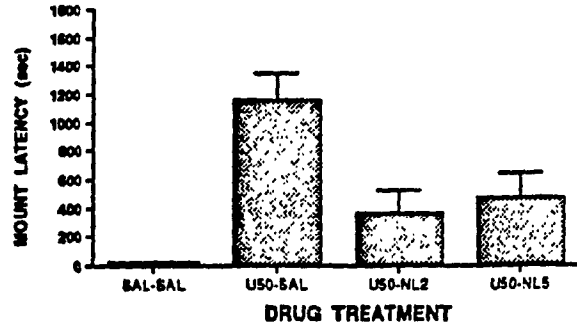
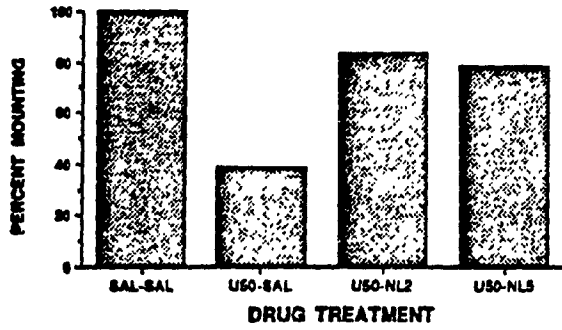


Figure 14 The mean (+ 1 S.E.M.) number of intromissions to precede the first ejaculation following the systemic injection of either saline, 5.0 mg/kg U50 given s.c., U50 plus 2.0 mg/kg naloxone given i.p. or U50 plus 5.0 mg/kg naloxone during tests for sexual behaviour in Experiment 4A.

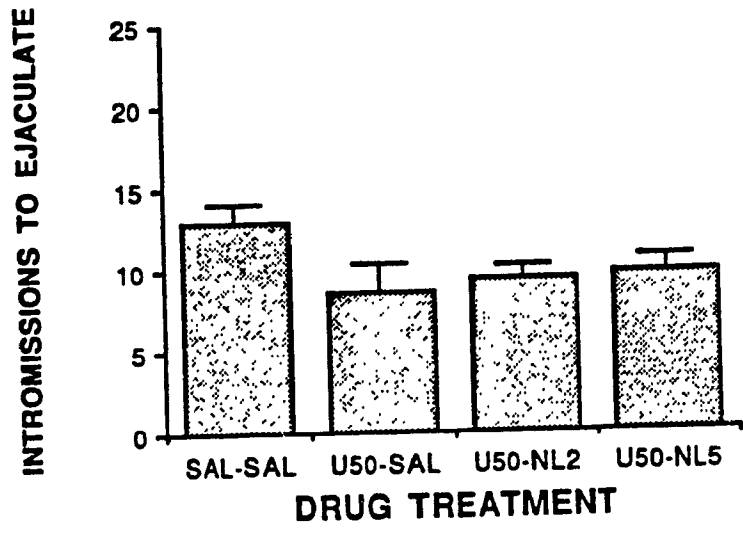


Figure 15 The mean (+ 1 S.E.M.) inter-intromission interval prior to the first ejaculation following the systemic injection of either saline, 5.0 mg/kg U50 given s.c., U50 plus 2.0 mg/kg naloxone given i.p. or U50 plus 5.0 mg/kg naloxone during tests for sexual behaviour in Experiment 4A.

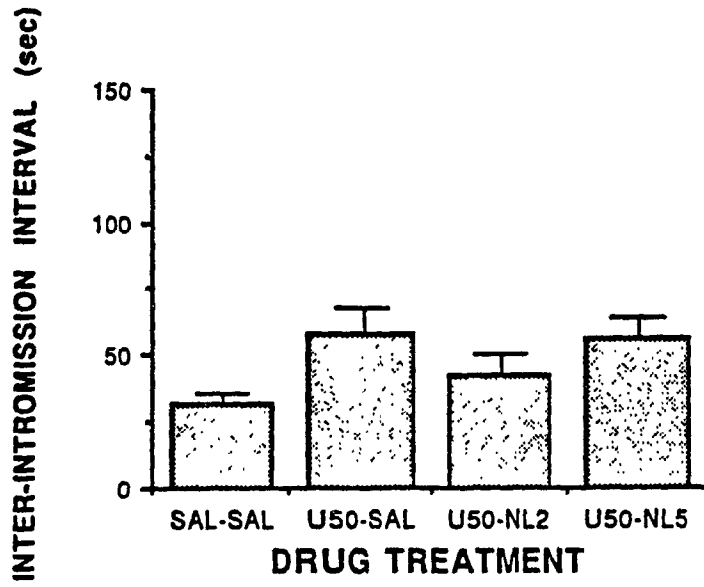


Figure 16 The mean (+ 1 S.E.M.) proportion of observations that non-copulatory behaviours were recorded to occur following the systemic injection of either saline, 5.0 mg/kg U50 given s.c., U50 plus 2.0 mg/kg naloxone given i.p. or U50 plus 5.0 mg/kg naloxone during tests for sexual behaviour in Experiment 4A. (FDB: female-directed behaviour; L: laying down; LL: body flattening; Sn: sniffing; Gr: body grooming; GG: genital grooming; W: walking; R: rearing).

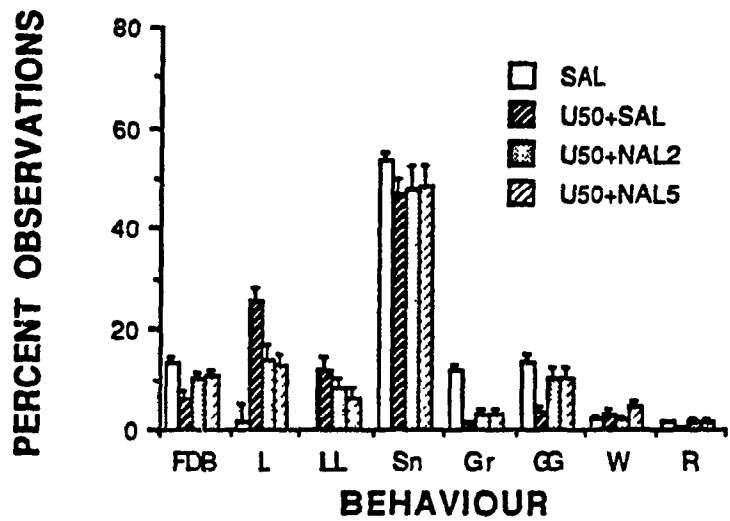
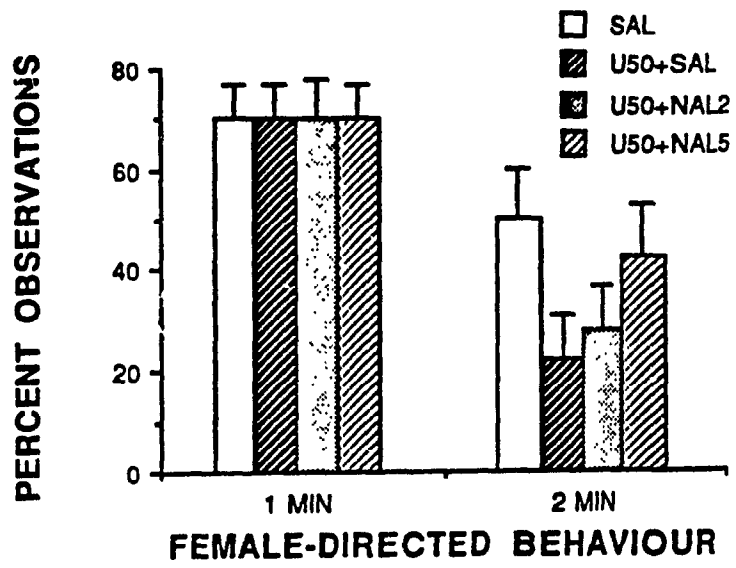


Figure 17 The mean (+ 1 S.E.M.) proportion of observations that female-directed behaviour was recorded to occur during the first and second min following the systemic injection of either saline, 5.0 mg/kg given s.c., U50 plus 2.0 mg/kg naloxone given i.p. or U50 plus 5.0 mg/kg naloxone during tests for sexual behaviour in Experiment 4A.



males that ejaculated during all four test conditions were analysed by a repeated measures ANOVA, this difference was not statistically significant, and was only weakly prevented by naloxone [$F(3,12)=2.328$, $p=.1262$].

Similarly, when the data from the five males that intromitted at least twice under all four drug conditions were analysed, the different drug treatments were found to non-significantly change the III in a pattern similar to that seen in Experiment 1B [$F(3,15)=2.469$, $p=.1019$]. As can be seen in Fig. 15, U-50,488H tended to increase the III, whereas naloxone non-significantly attenuated this effect.

Too few individual animals reinitiated copulatory behaviour following an ejaculation in all four tests to allow an informative repeated measures statistical analysis, but it was apparent that the different drug treatments did not alter this refractory period. The post-ejaculatory refractory periods of animals that began a second ejaculatory series were: SAL: 362.6 ± 14.0 s, $n=18$; U-50,488H: 343.5 ± 38.0 s, $n=6$; U-50,488H+NAL 2.0 mg: 319.9 ± 20.9 s, $n=14$; U-50,488H+NAL 5.0 mg: 353.2 ± 11.4 s, $n=12$.

Non-Copulatory Behaviours: Figure 16 shows the mean percent of observations (out of 60 possible) for which various non-copulatory behaviours were recorded during the 30-min test sessions. As in Experiment 1A and 1B, U-50,488H altered the frequency of non-copulatory behaviours; not all of them, however, were naloxone reversible. Female-directed behaviour [$F(3,51)=5.578$, $p<.003$], genital grooming [$F(3,51)=5.228$, $p<.004$] and bodily grooming [$F(3,51)=34.786$, $p<.00001$] were all reduced by U-50,488H, relative to saline, [$p<.01$], but only the former two behaviours were prevented by naloxone [$p<.05$]. U-50,488H also increased the occurrence of lying down behaviour [$F(3,51)=17.943$, $p<.00001$], relative to saline [$p<.01$], and this was partially attenuated by both doses of naloxone [$p<.01$]; there was a higher incidence of lying down in U-50,488H treated than in saline treated animals [$p<.01$] even after naloxone injection. U-50,488H also increased the occurrence of body flattening [$F(3,51)=8.696$, $p<.0002$],

relative to saline [$p < .01$], but this was not significantly prevented by either dose of naloxone [$p > .05$].

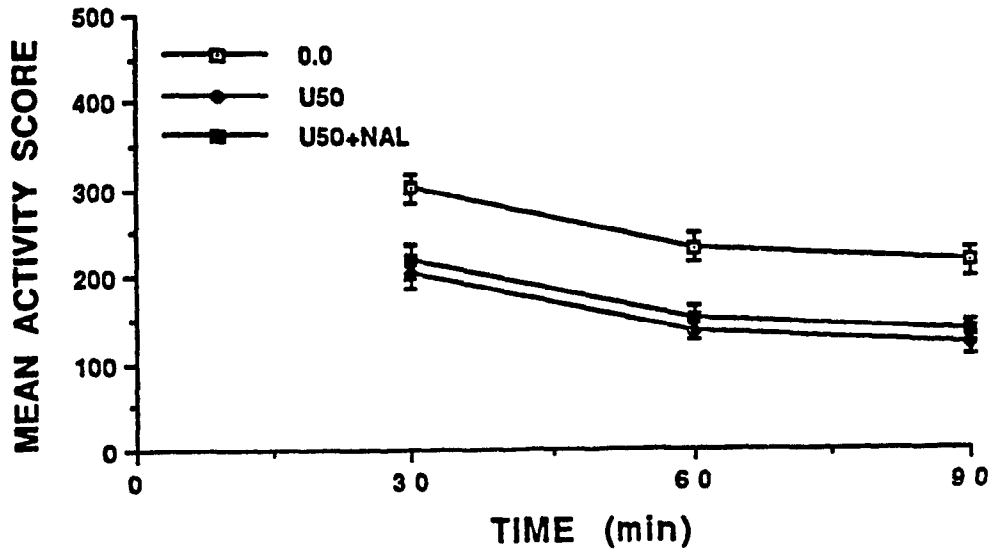
Figure 17 depicts the changing occurrence of female-directed behaviour during the first two min of the 30-min test. A repeated measures ANOVA yielded a significant main effect of Min [$F(1,17)=48.077$, $p < .00001$], but not of Drug [$F(3,51)=0.895$, $p = .4503$], reflecting the decrease in female-directed behaviour from the first to the second min. Post hoc tests revealed that although there was no difference between Drug treatments during the first min [$p > .05$], during the second min of testing female-directed behaviour was significantly decreased in U-50,488H treated animals, relative to saline [$p < .05$], and that this decrease was prevented by both doses of naloxone.

Experiment 4B: Tests for Locomotor Activity Following the Co-Administration of U-50,488H with Naloxone

On three counterbalanced tests, 18 animals were monitored for locomotor activity in photocell boxes following the co-administration of either two systemic injections of saline; 5.0 mg/kg U-50,488H given s.c. plus saline given i.p.; or U-50,488H plus 2.0 mg/kg naloxone given i.p.

Locomotor Activity: The 30-min activity scores from the 90-min tests were subjected to a two-way, repeated measures ANOVA. The analysis yielded significant main effects for Drug [$F(2,34)=43.394$, $p < .0001$] and Time [$F(2,34)=51.302$, $p < .0001$]. As illustrated in Fig. 18, 5.0 mg/kg U-50,488H given s.c. produced the expected decrease in locomotor activity [$p < .01$], relative to saline. Unexpectedly, however, this U-50,488H-induced decrease was not attenuated by 2.0 mg/kg naloxone given i.p.; the activity scores following the co-administration of U-50,488H plus naloxone were not significantly different from U-50,488H alone [$p > .05$], and they remained significantly less than those seen following saline [$p < .01$]. Further, the Drug x Time interaction was not significant

Figure 18 The mean (± 1 S.E.M.) 30-min locomotor activity scores following the systemic injection of either saline, 5.0 mg/kg given s.c., U50 plus 2.0 mg/kg naloxone given i.p. or U50 plus 5.0 mg/kg naloxone in Experiment 4B.



[$F(4,68)=0.019$, $p=.9993$] indicating that the temporal pattern of activity was similar with each drug treatment.

Experiment 5A: Tests for Male Sexual Behaviour Following Intra-Cranial Infusions of U-50,488H

On five counterbalanced tests, 19 animals were repeatedly tested for sexual behaviour following the intra-cranial administration of either saline or one of four doses of U-50,488H. Following these tests, 18 of these same animals were tested for sexual behaviour one additional time following the co-administration of 2.0 mg/kg naloxone given i.p. plus 5.0 nmol/0.5 μ l/side U-50,488H.

Histological verification of the cannulae tips confirmed accurate placements, and on this basis no animals were removed from the analysis. The cannulae placements of 15 recovered brains are presented in Figs. 19-21.

Copulatory Behaviour: The intra-cranial infusion of U-50,488H into discrete brain sites produced some, but not all, of the behavioural effects seen to follow systemic administration in Experiments 1A, 1B and 4A. Intra-cranial infusions of U-50,488H into either the VTA or the NAS increased copulatory latencies and decreased the proportion of animals that either copulated or ejaculated. Intra-mPOA infusions of U-50,488H did not induce many behavioural inhibitions, but both significant reductions in female-directed behaviour and a trend to increase ejaculation latencies were found. Surprisingly, increasingly potent dose-response effects were not observed following the intra-cranial infusions of U-50,488H. Within the VTA, the unusual dose-responses might reflect the differential activation of *k* receptor mediated excitatory and inhibitory effects; opposing effects have been reported in other behavioural studies and these were discussed earlier. Within the NAS, the absence of a progressively effective dose-response to U-50,488H might also reflect opposing excitatory and inhibitory effects of the drug; alternatively, it might only indicate a ceiling effect; copulatory behaviour was abolished in many animals

administered intra-NAS U-50,488H necessitating the assignment of a maximum cut-off score.

The following results section, with the accompanying Figs. 22-27, describe in more detail the changes to copulatory behaviour that were observed following intra-cranial infusions of U-50,488H.

Among animals administered intra-VTA infusions of U-50,488H, both mount [$F(4,20)=4.293$, $p<.02$] and intromission [$F(4,20)=3.901$, $p<.02$] latencies were increased, relative to saline. Post hoc tests revealed that this increase was statistically significant only following the lowest test dose, 0.0005 nmol/0.5 μ l/side [$p<.05$]. Although these increased copulation latencies were partially attributable to the fact that some males did not copulate at all, even in those males that did mount, copulation latencies tended to be increased, but not in a dose-dependent manner. Among mounting animals administered intra-VTA infusions of 0.0005 nmol/0.5 μ l/side U-50,488H, mean mount latencies were 465.8 ± 256.5 s, $n=4$ compared to 22.7 ± 6.0 s, $n=6$, following saline infusions.

Intra-VTA infusions of U-50,488H also significantly decreased the proportion of males that ejaculated [$Q(4)=10.05$, $p<.05$], and significantly increased the ejaculation latencies [$F(4,20)=7.071$, $p<.002$] following either 0.0005 nmol [$p<.01$] or 0.5 nmol/0.5 μ l/side U-50,488H [$p<.01$]. Again, although the increased ejaculation latencies were partially attributable to the decreased number of VTA treated animals to ejaculate, even among those males that did ejaculate, ejaculation latencies tended to be increased, but not dose-dependently. Among ejaculating animals administered intra-VTA infusions of either 0.0005 or 0.5 nmol/0.5 μ l/side U-50,488H, ejaculation latencies were 565.7 ± 196.2 s, $n=3$; and 681.0 ± 229.7 s, $n=4$; compared to 372.0 ± 37.5 s, $n=6$ following saline. Interestingly, among those animals that completed a second ejaculatory series, intra-VTA infusions of U-50,488H tended to decrease the second ejaculation latency. In ascending dose order, U-50,488H elicited second series ejaculation latencies of 169.0 ± 91.0 s, $n=2$;

122.3±13.7 s, n=6; 171.3±4.4 s, n=4; and 171.2±40.7 s, n=6, compared to a relatively slow 234.4±12.7 s, n=5, seen following saline.

In contrast to the negligible changes in mount and intromission latencies that had been observed when males were co-administered the highest dose of U-50,488H intra-VTA with systemic naloxone, a repeated measures t-test showed that this combined treatment unexpectedly decreased the ejaculation latency, relative to saline treatment [$t(4)=5.70$, $p<.005$].

Among animals administered intra-NAS infusions of U-50,488H, both mount [$F(4,24)=3.922$, $p<.02$] and intromission latencies [$F(4,24)=7.322$, $p<.0006$] were increased, relative to saline. Post hoc tests revealed that the mount latencies were significantly increased only by 0.5 nmol/0.5 μ l/side [$p<.05$] whereas the intromission latencies were significantly increased by both 0.0005 and 0.5 nmol/0.5 μ l/side of U-50,488H [$p<.05$]. Compatibly, intra-NAS infusions of U-50,488H also significantly decreased the proportion of males that initiated intromitting during the 30-min test [$Q(4)=15.28$, $p<.01$]. This increase in non-copulators partially accounted for the increase in intromission latencies, however, even among those animals treated with intra-NAS U-50,488H that did intromit, a trend to prolong that latency was evident. Among those animals that did intromit at least once during the 30-min test session, intra-NAS infusions of U-50,488H produced intromission latencies of 320.5±292.2 s, n=5; 352.2±221.2 s, n=6; 25.0±0.0 s, n=1 and 67.8±23.5 s, n=7, compared to 29.1±6.5 s, n=7, following saline.

Intra-NAS infusions of U-50,488H also significantly decreased the proportion of animals that ejaculated [$Q(4)=13.93$, $p<.01$] as well as the mean number of ejaculations [$F(4,24)=5.948$, $p<.002$] following all but the highest dose of U-50,488H [$p<.05$], relative to saline. Again, these intra-NAS U-50,488H-induced inhibitions were partially attributable to the decrease in the number of animals to copulate, but the same trend was evident even among those males that did copulate. Among copulating animals administered

Figure 19 Histological dissection of 30 μ m VTA slices for animals from Experiments 4A, 4B, 5A and 5B. The coronal sections are from the atlas of Pellegrino, Pellegrino, & Cushman (1979). Numbers to the left indicate mm from bregma.

-3.2

-3.8

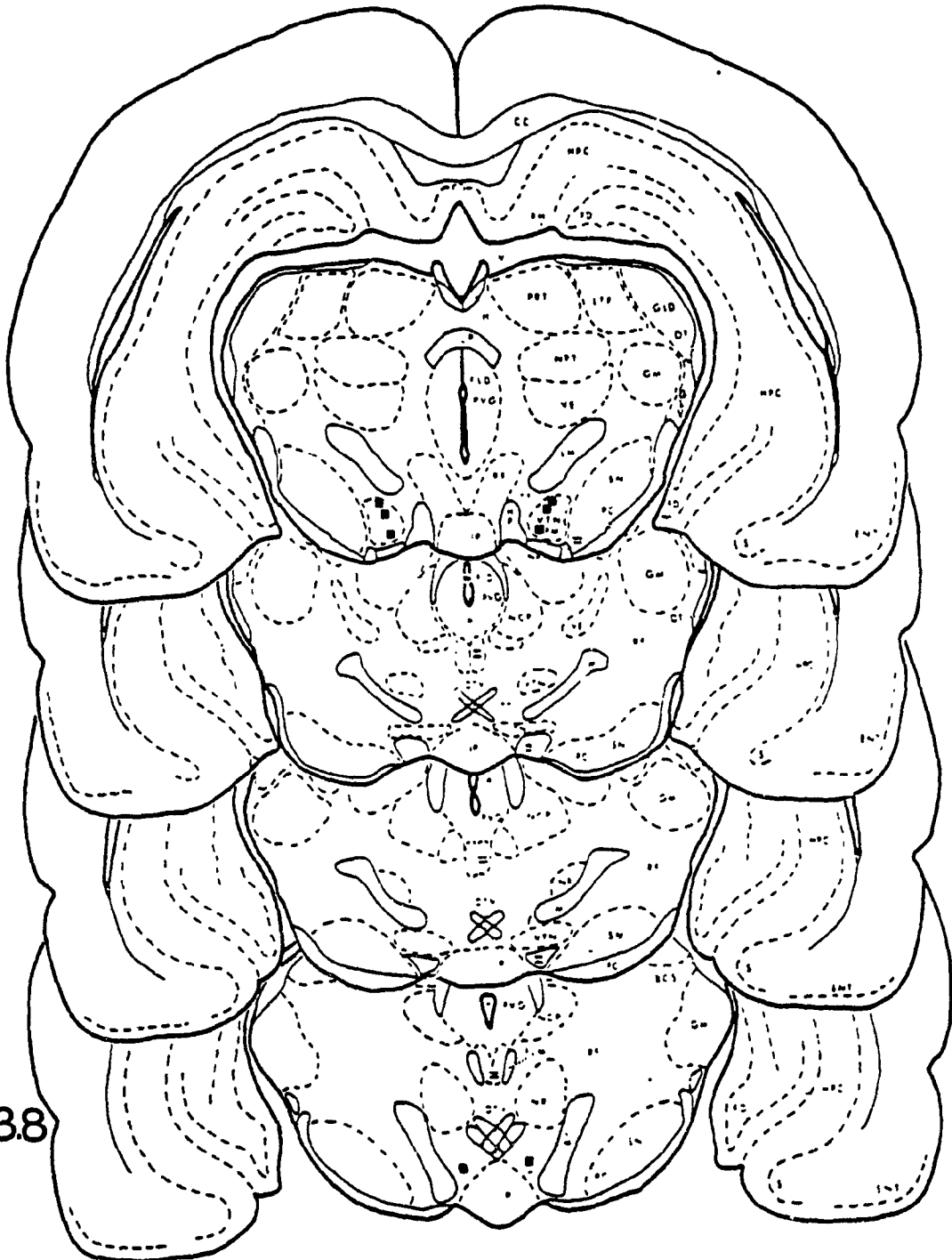
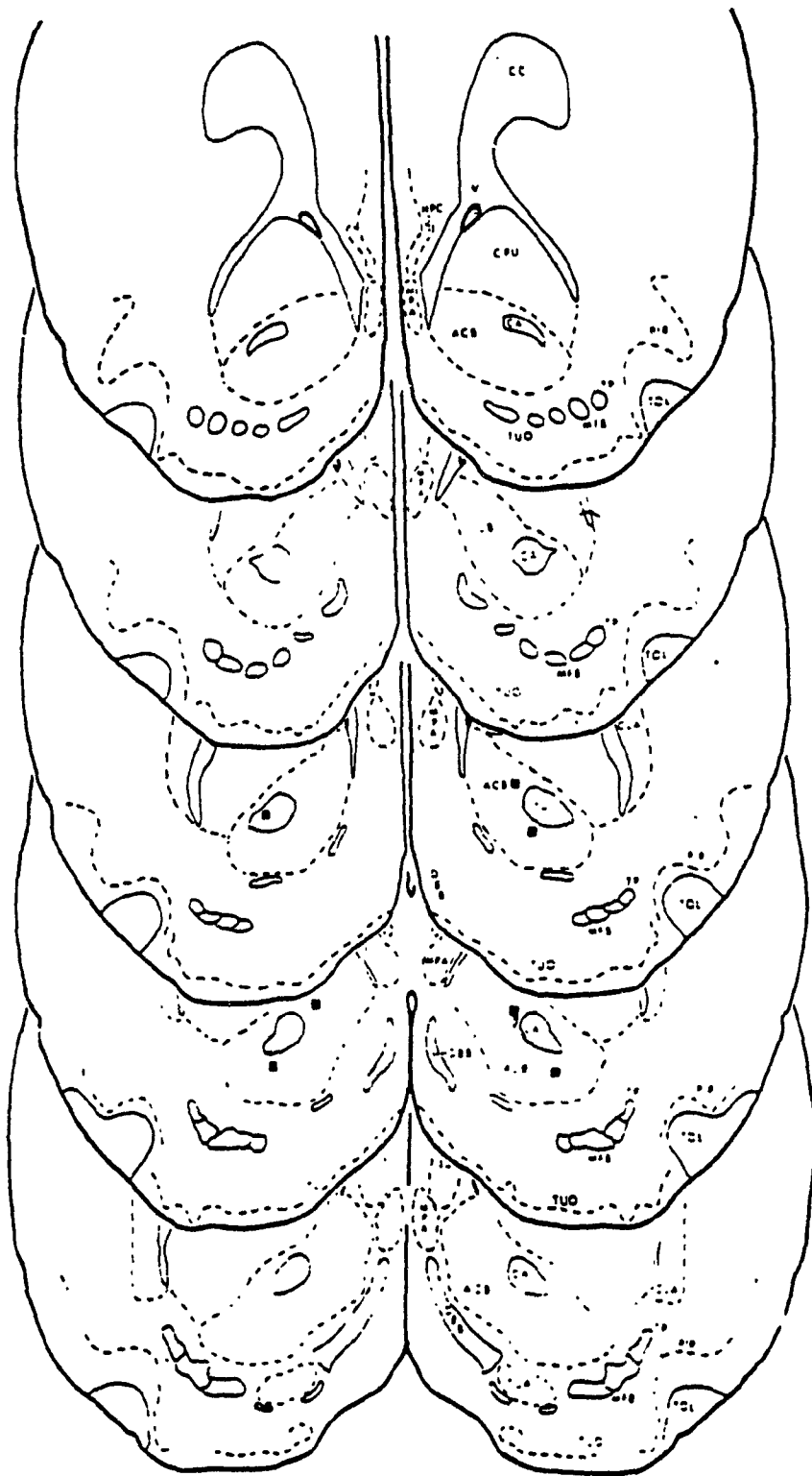


Figure 20 Histological dissection of 30 μ m NAS slices for animals from Experiments 4A, 4B, 5A and 5B. The coronal sections are from the atlas of Pellegrino et al., (1979). Numbers to the left indicate mm from bregm.

4.0



3.2

Figure 21 Histological dissection of 30 μ m mPOA slices for animals from Experiments 4A, 4B, 5A and 5B. The coronal sections are from the atlas of Pellegrino et al., (1979). Numbers to the left indicate mm from bregma.

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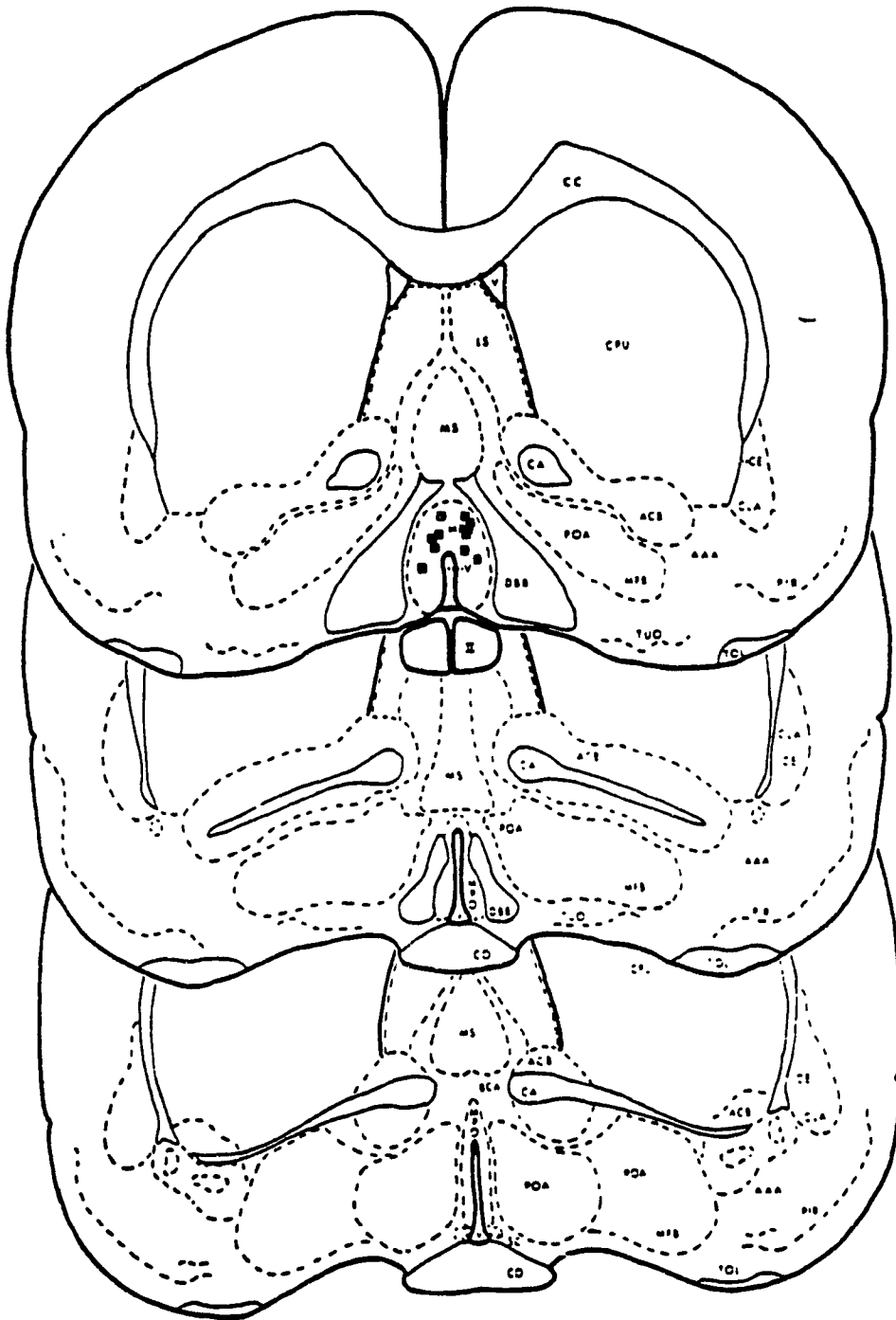


Figure 22 The mean (+ 1 S.E.M.) proportion of VTA (22a), NAS (22b) and mPOA (22c) cannulated males to mount, and the mean (+ 1 S.E.M.) latency for VTA (22d), NAS (22e) and mPOA (22f) to mount following the intra-cranial infusion of either saline, 0.0005, 0.05, 0.5, 5.0 nmol/0.5 μ l/side or 5.0 nmol/0.5 μ l/side plus the systemic injection of 2.0 mg/kg naloxone given i.p. during tests for sexual behaviour in Experiment 5.

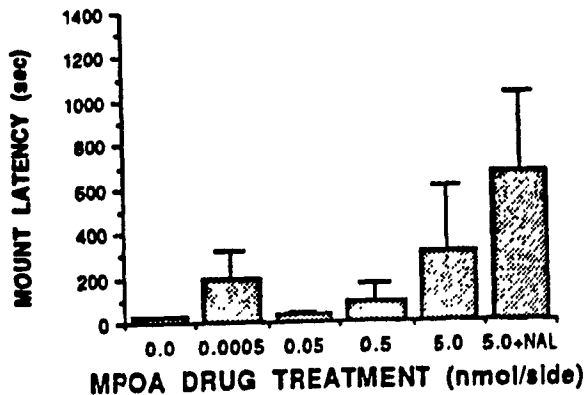
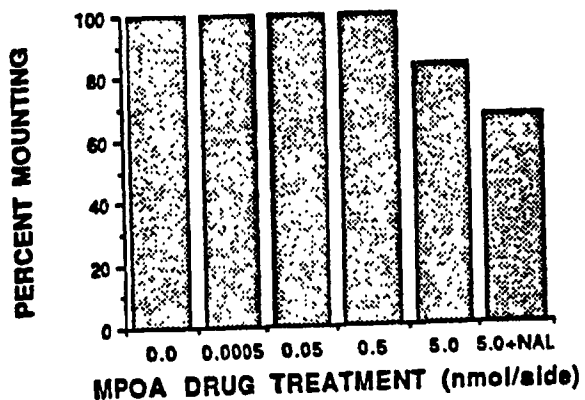
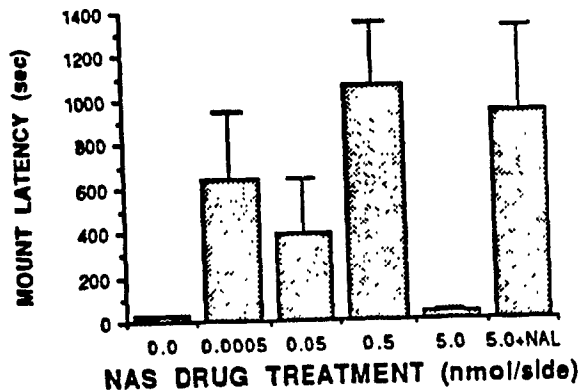
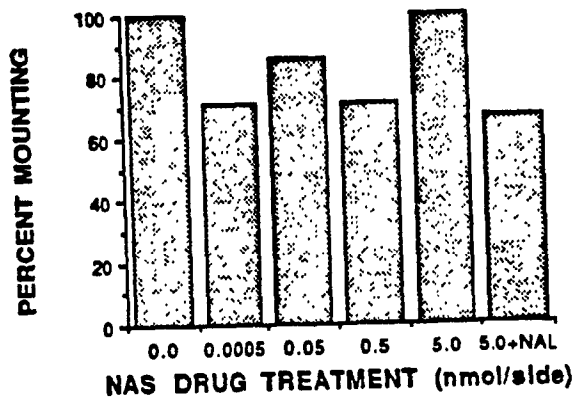
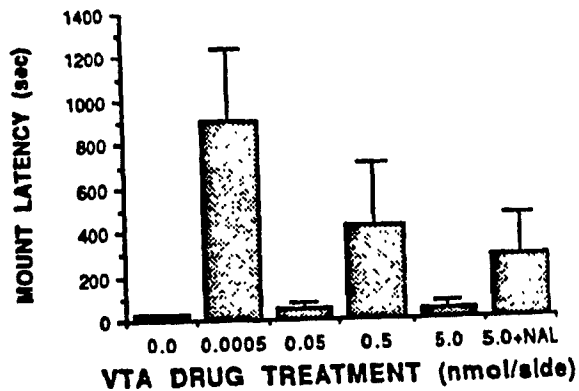
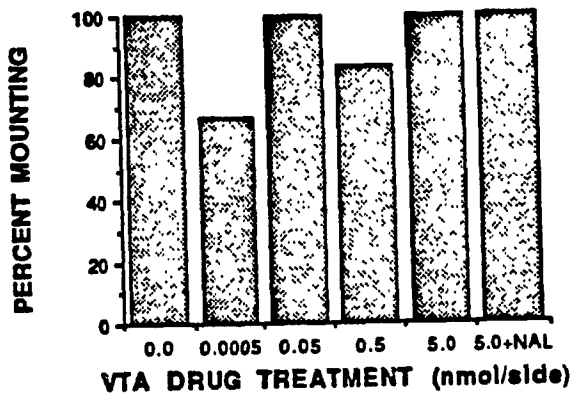


Figure 23 The mean (+ 1 S.E.M.) proportion of VTA (23a), NAS (23b) and mPOA (23c) cannulated males to intromit, and the mean (+ 1 S.E.M.) latency for VTA (23d), NAS (23e) and mPOA (23f) to intromit following the intracranial infusion of either saline, 0.0005, 0.05, 0.5, 5.0 nmol/0.5 μ l/side or 5.0 nmol/0.5 μ l/side plus the systemic injection of 2.0 mg/kg naloxone given i.p. during tests for sexual behaviour in Experiment 5A

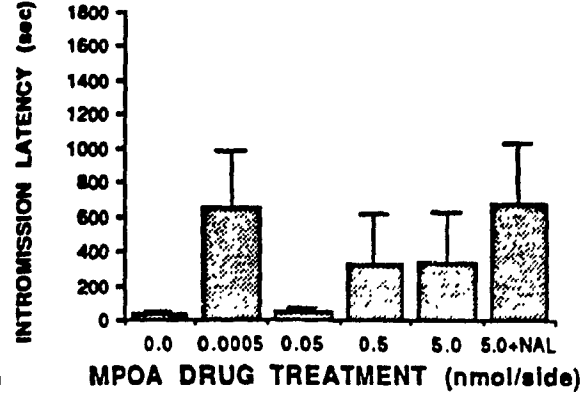
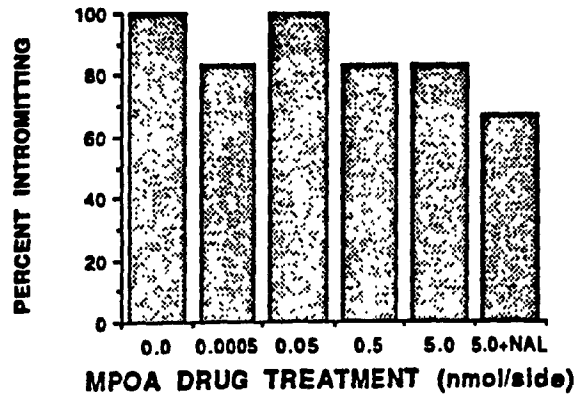
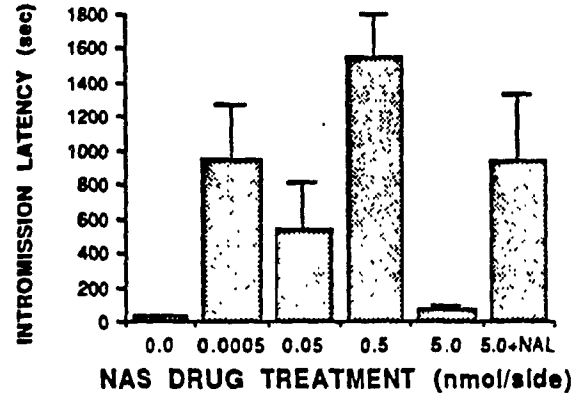
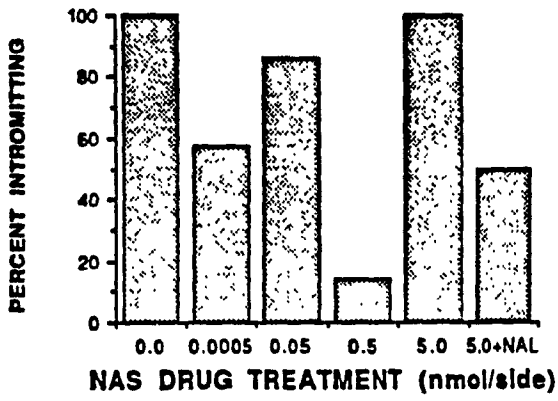
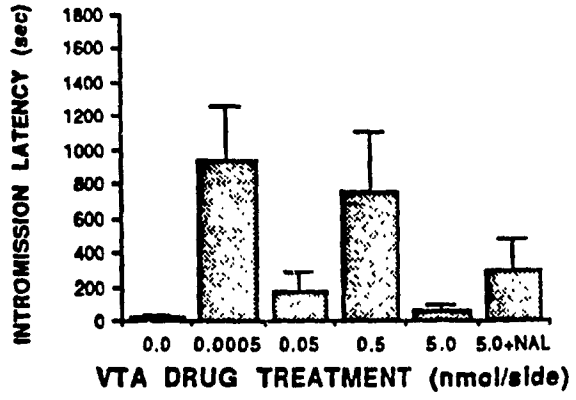
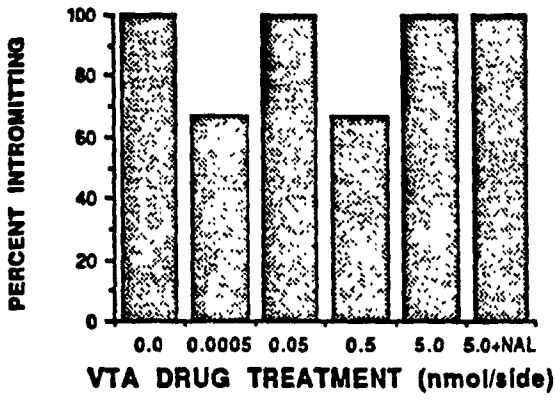


Figure 24 The mean (+ 1 S.E.M.) proportion of VTA (24a), NAS (24b) and mPOA (24c) cannulated males to ejaculate, and the mean (+ 1 S.E.M.) latency for VTA (24d), NAS (24e) and mPOA (24f) to ejaculate following the intracranial infusion of either saline, 0.0005, 0.05, 0.5, 5.0 nmol/0.5 μ l/side or 5.0 nmol/0.5 μ l/side plus the systemic injection of 2.0 mg/kg naloxone given i.p. during tests for sexual behaviour in Experiment 5A.

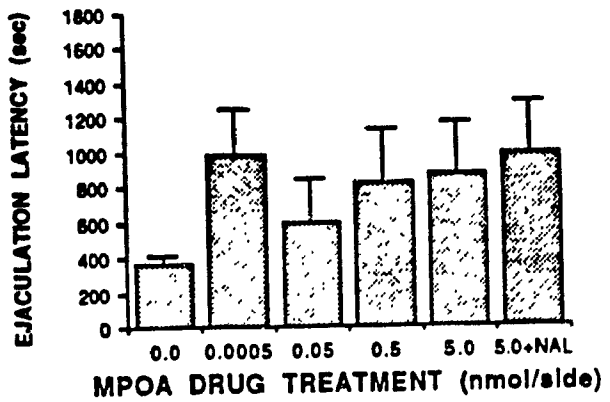
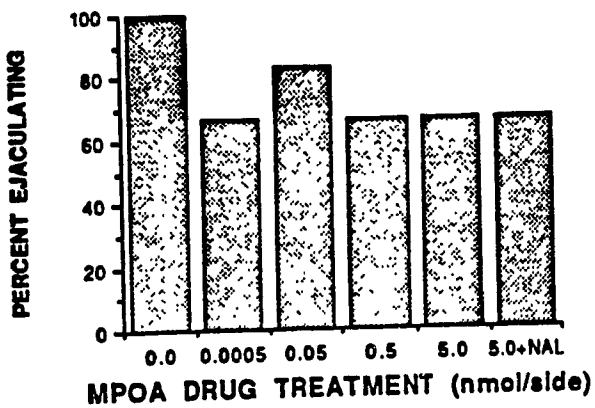
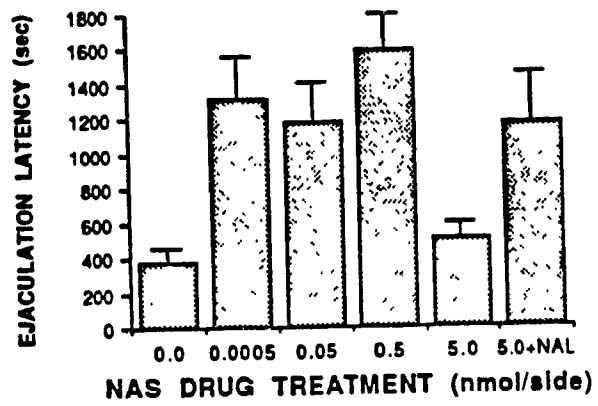
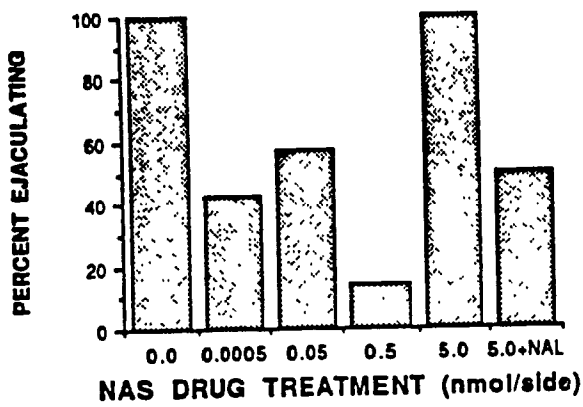
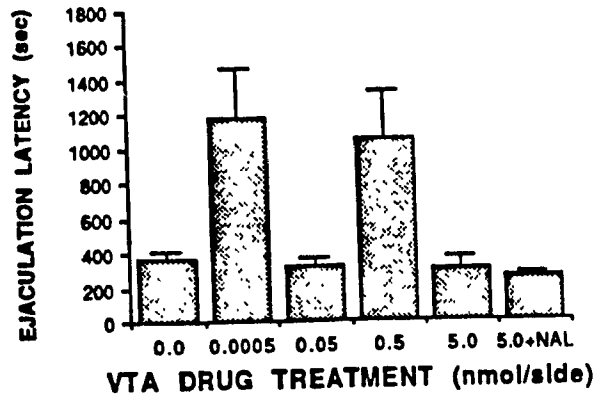
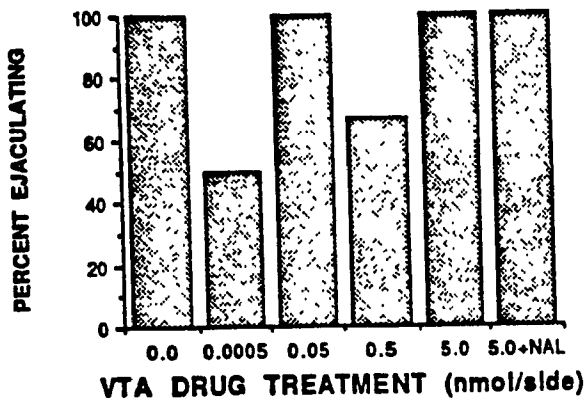


Figure 25 The mean (+ 1 S.E.M.) number of ejaculations by VTA (25a), NAS (25b) and mPOA (25c) cannulated males following the intra-cranial infusion of either saline, 0.0005, 0.05, 0.5, 5.0 nmol/0.5 μ l/side or 5.0 nmol/0.5 μ l/side plus the systemic injection of 2.0 mg/kg naloxone given i.p. during tests for sexual behaviour in Experiment 5A.

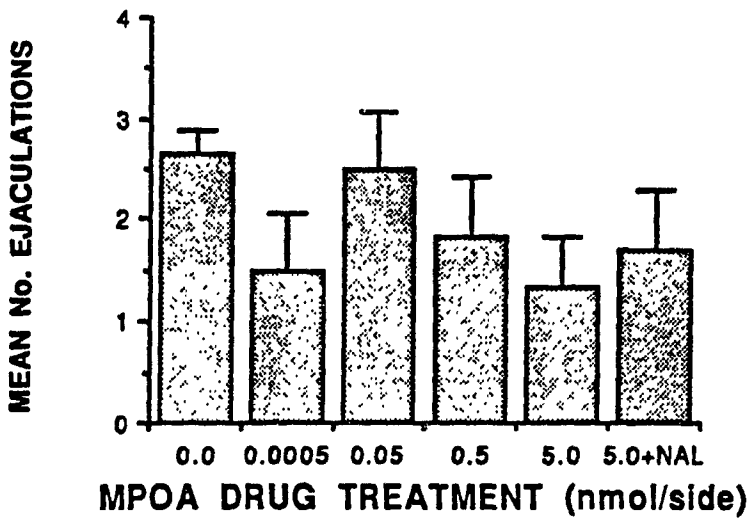
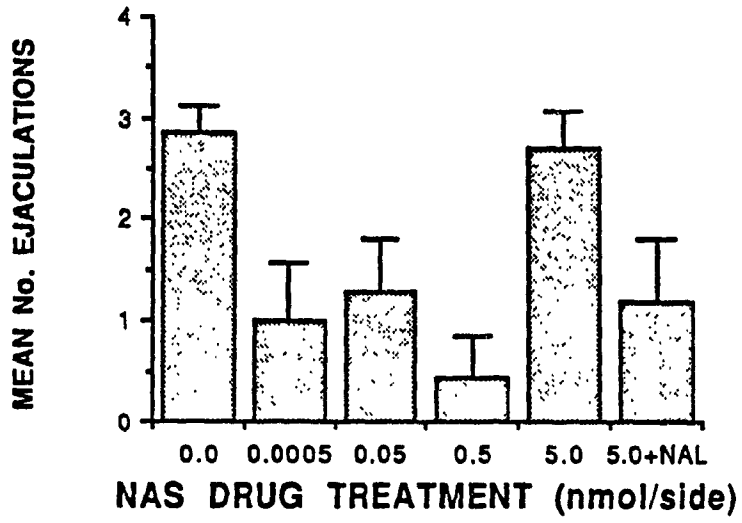
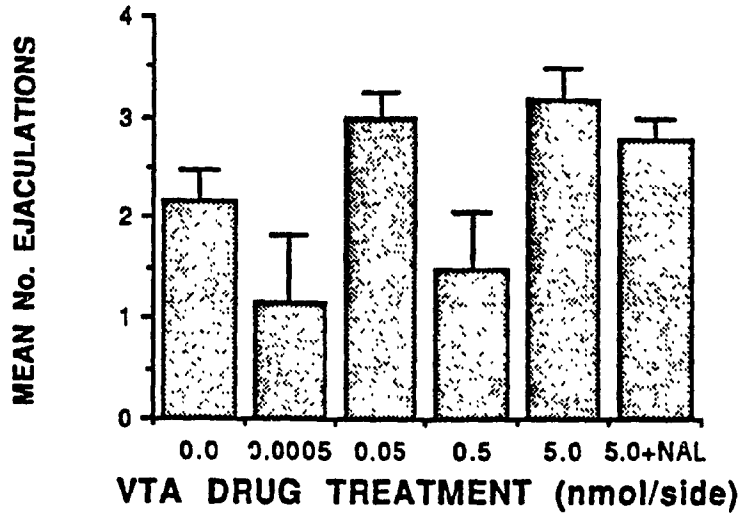
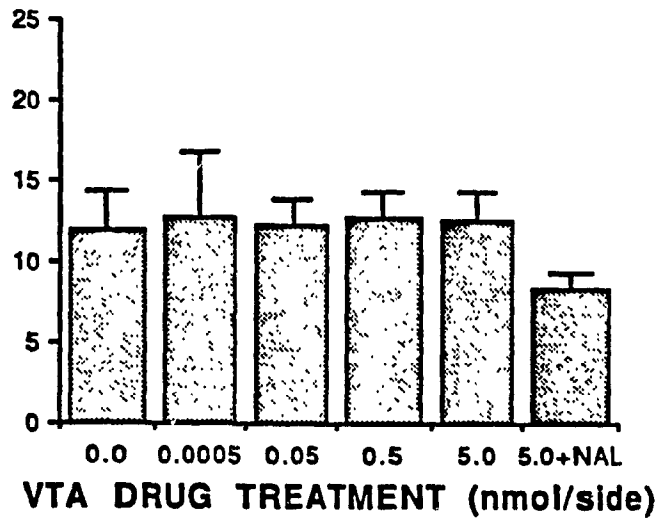
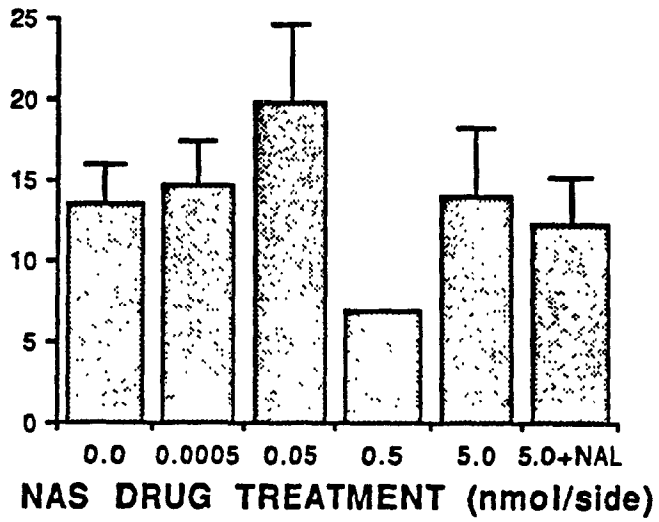


Figure 26 The mean (+ 1 S.E.M.) number of intromissions to precede the first ejaculation among VTA (26a), NAS (26b) and mPOA (26c) cannulated males following the intra-cranial infusion of either saline, 0.0005, 0.05, 0.5, 5.0 nmol/0.5 μ l/side or 5.0 nmol/0.5 μ l/side plus the systemic injection of 2.0 mg/kg naloxone given i.p. during tests for sexual behaviour in Experiment 5A.

INTROMISSIONS TO EJACULATION



INTROMISSIONS TO EJACULATION



INTROMISSIONS TO EJACULATION

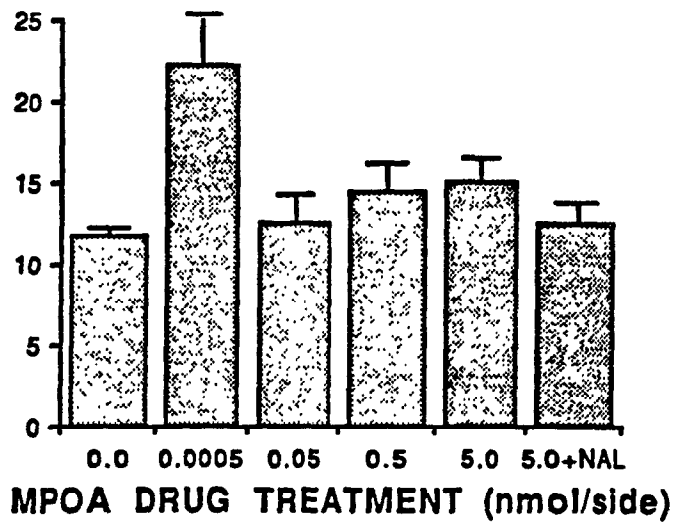


Figure 27 The mean (+ 1 S.E.M.) inter-intromission interval prior to the first ejaculation among VTA (27a), NAS (27b) and mPOA (27c) cannulated males following the intra-cranial infusion of either saline, 0.0005, 0.05, 0.5, 5.0 nmol/0.5 μ l/side or 5.0 nmol/0.5 μ l/side plus the systemic injection of 2.0 mg/kg naloxone given i.p. during tests for sexual behaviour in Experiment 5A.

INTER-INTRISSION INTERVAL (sec)

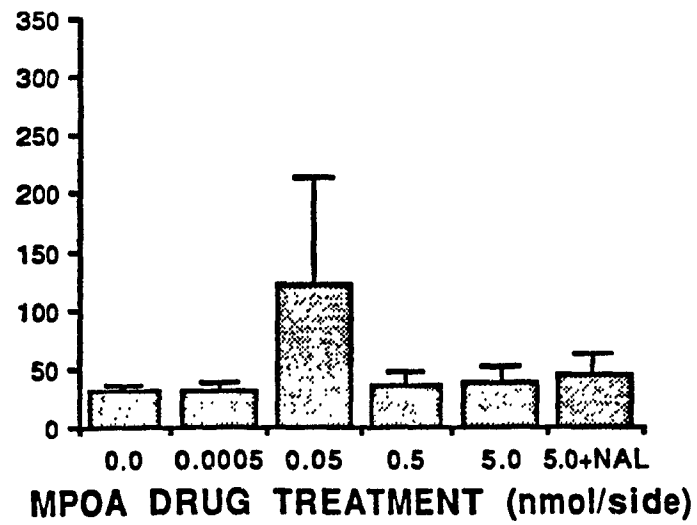
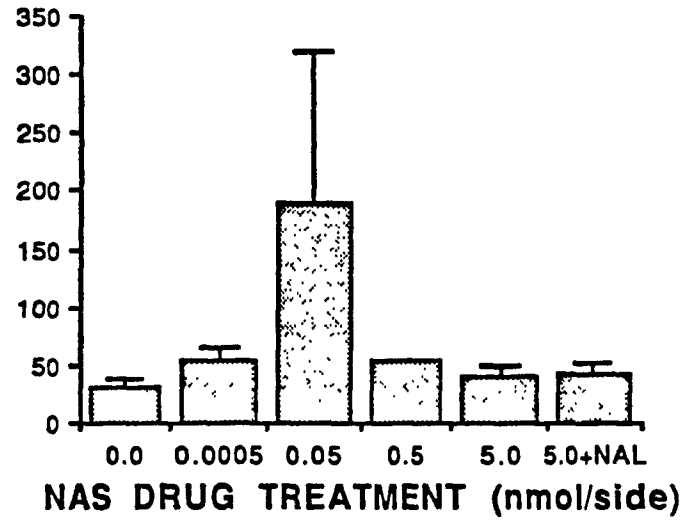
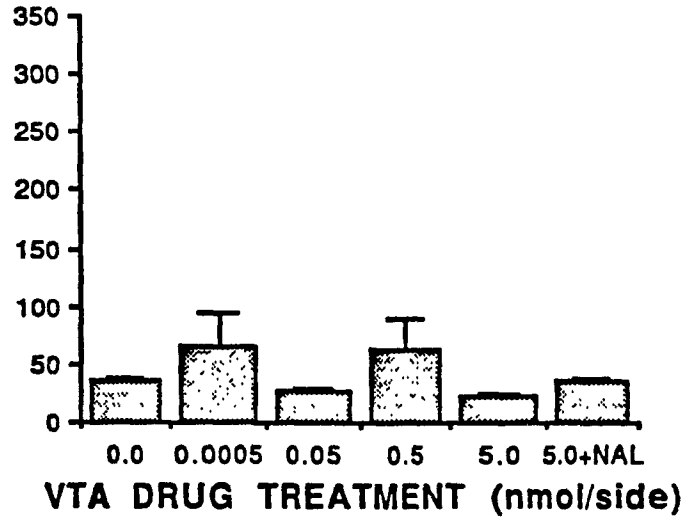


Figure 28 The mean (+ 1 S.E.M.) proportion of observations that non-copulatory behaviours were recorded to occur following the intra-cranial infusion of either saline, 0.0005, 0.05, 0.5, 5.0 nmol/0.5 μ l/side or 5.0 nmol/0.5 μ l/side plus the systemic injection of 2.0 mg/kg naloxone given i.p. during tests for sexual behaviour in Experiment 5A. (FDB: female-directed behaviour; L: laying down; LL: body flattening; Sn: sniffing; Gr: body grooming; GG: genital grooming; W: walking; R: rearing).

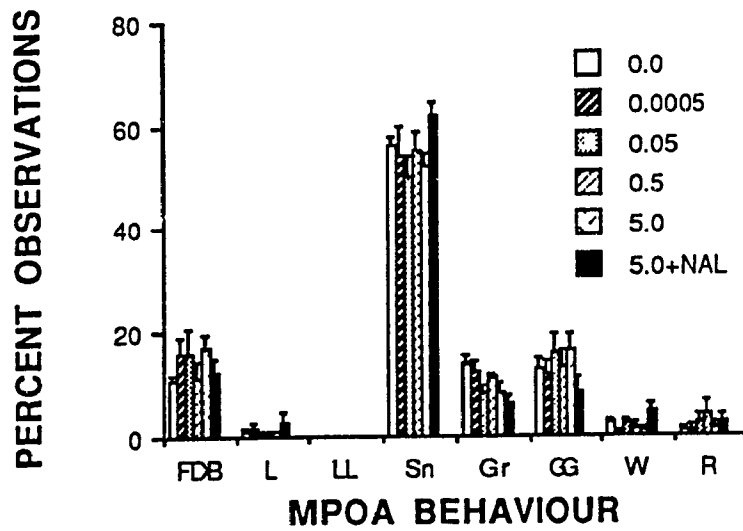
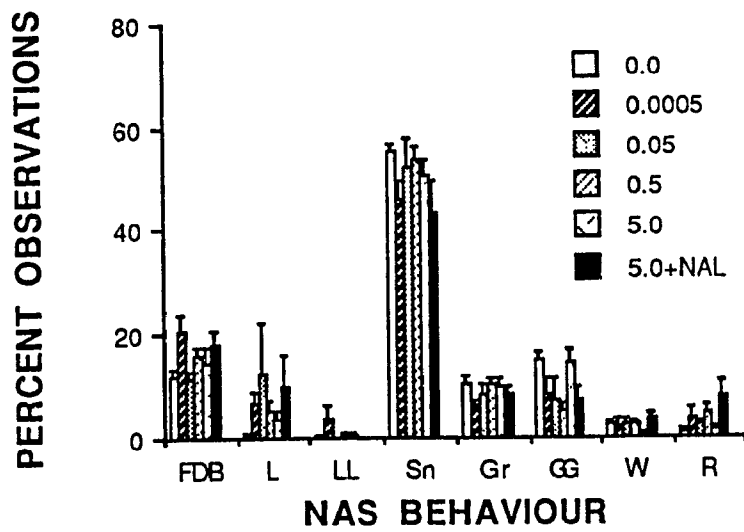
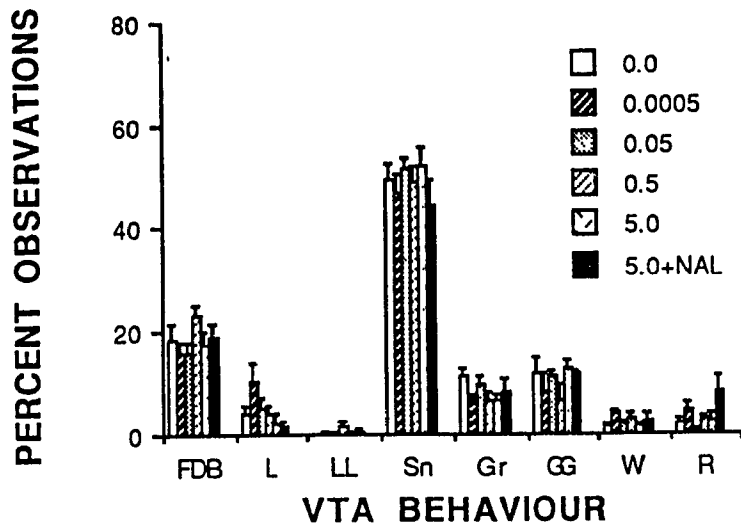


Figure 29 The mean (+ 1 S.E.M.) proportion of observations that female-directed behaviour was recorded to occur during the first and second min following the intra-cranial infusion of either saline, 0.0005, 0.05, 0.5, 5.0 nmol/0.5 μ l/side or 5.0 nmol/0.5 μ l/side plus the systemic injection of 2.0 mg/kg naloxone given i.p. during tests for sexual behaviour in Experiment 5A

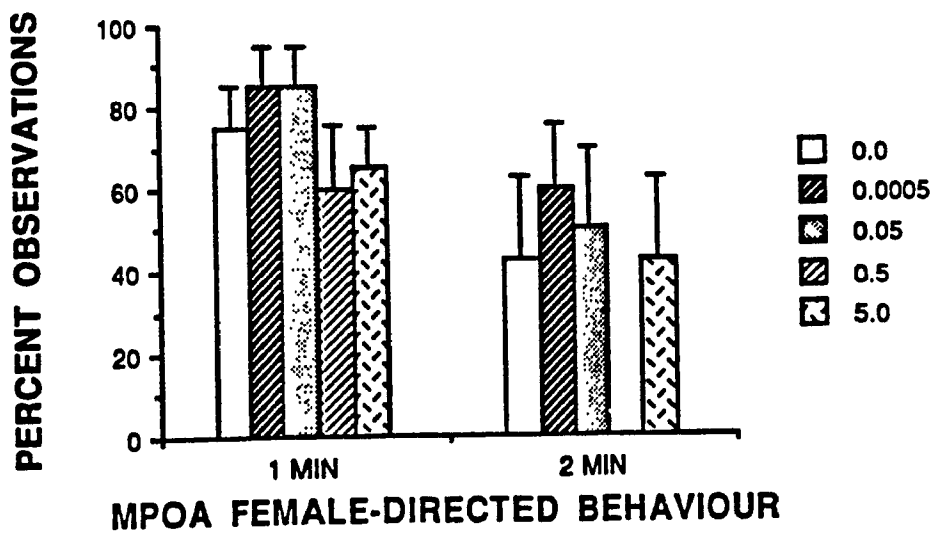
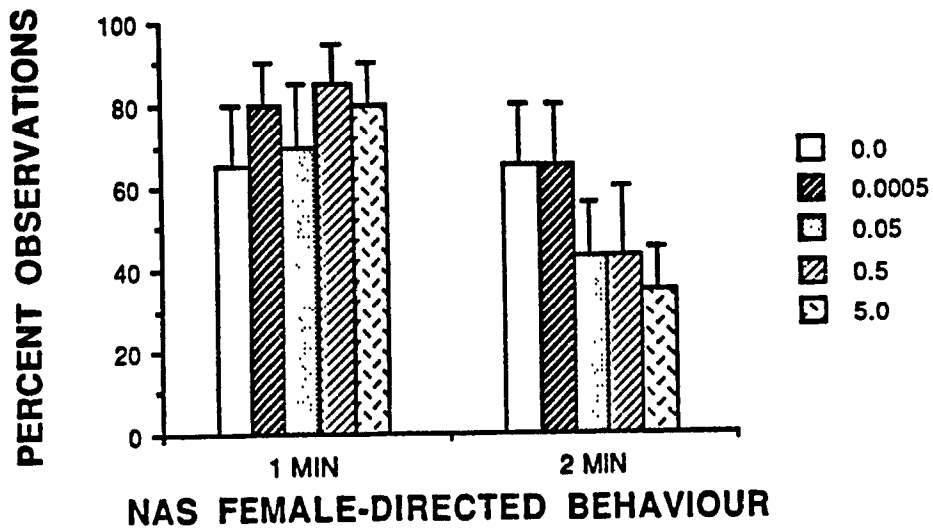
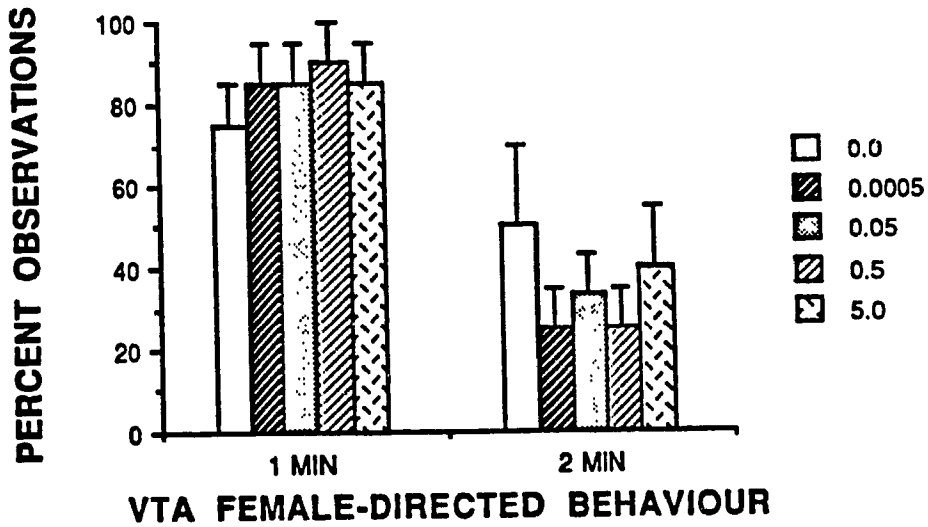
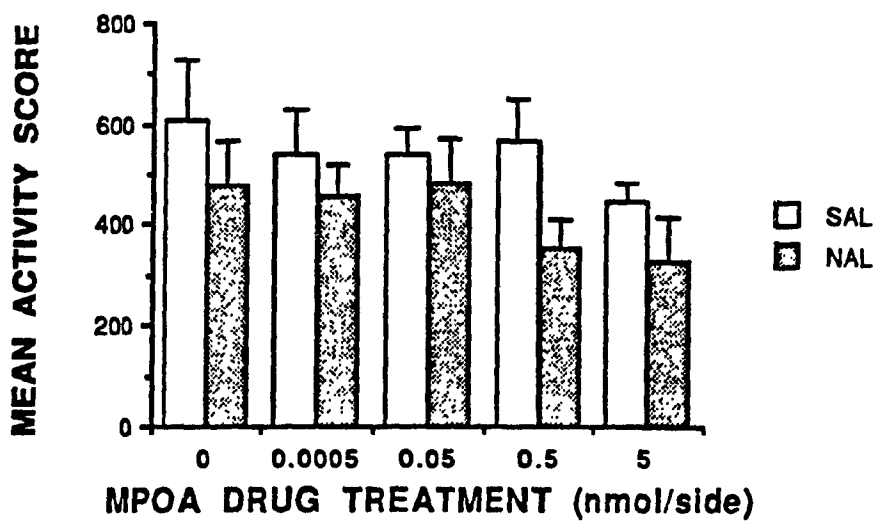
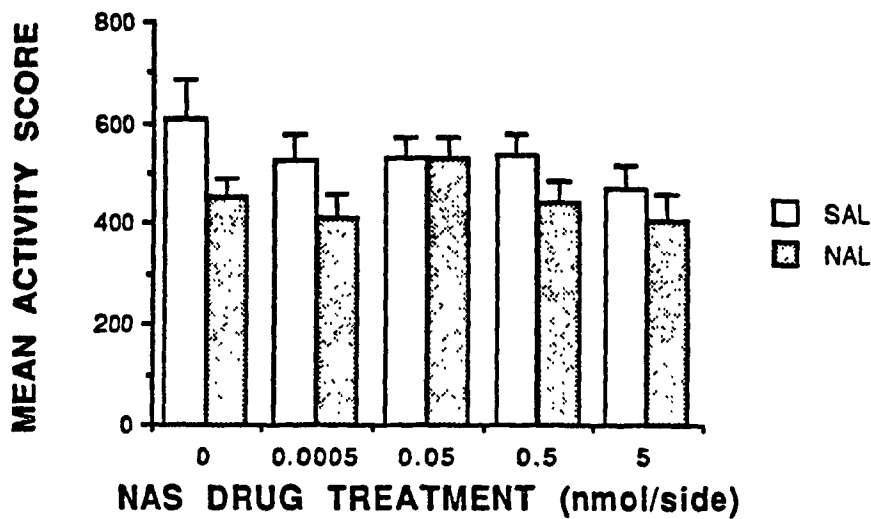
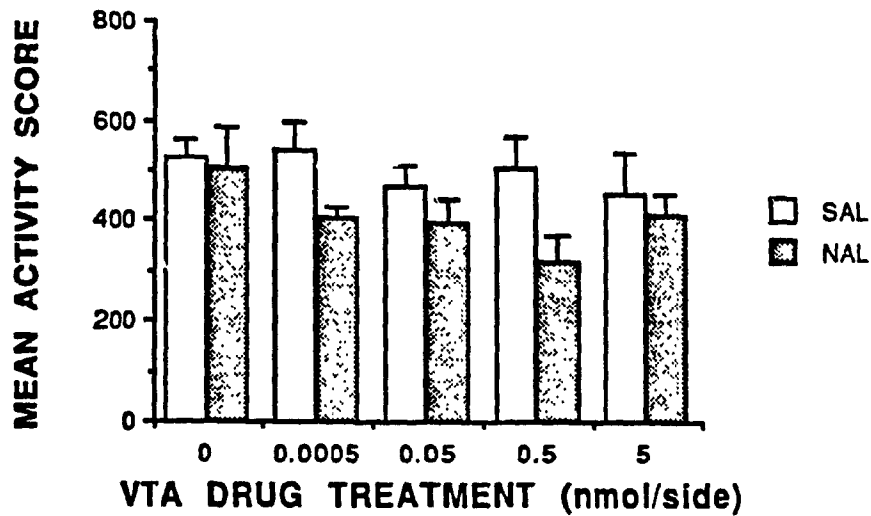


Figure 30 The mean (\pm 1 S.E.M.) 60-min locomotor activity score for VTA (30a), NAS (30b) and mPOA (30c) cannulated males co-administered intra-cranial infusions of saline, 0.0005, 0.05, 0.5 and 5.0 nmol/0.5 μ l/side with systemic injections of either saline or 2 mg/kg naloxone given i.p. in Experiment 5B.



intra-NAS U-50,488H, the mean number of ejaculations during the 30-min test sessions was 1.8 ± 0.8 , $n=4$; 1.8 ± 0.6 , $n=5$; 1.0 ± 1.0 , $n=3$; and 2.7 ± 0.4 , $n=7$, as compared to 2.9 ± 0.3 , $n=7$ following saline. Finally, significantly increased ejaculation latencies [$F(4,24)=8.241$, $p<.0003$] were found following the intra-NAS infusion of all but the highest test dose of U-50,488H [$p<.05$]. These increases were not restricted to the animals that did not ejaculate; among ejaculating males administered intra-NAS infusions of U-50,488H, the first series ejaculation latencies were 687.3 ± 213.1 s, $n=3$; 713.0 ± 113.5 s, $n=4$; 382.0 ± 0.0 s, $n=1$; and 500.6 ± 93.7 s, $n=7$, whereas saline infusions produced ejaculation latencies of 377.8 ± 79.6 s, $n=7$.

The dual treatment of intra-NAS infusions of the highest dose of U-50,488H co-administered with systemic naloxone did not significantly change copulation latencies, but a repeated measures t-test suggested that it did increase the latency to ejaculate [$t(5)=2.92$, $p<.04$].

In contrast to the multiple inhibitory effects of U-50,488H administered into either the VTA or NAS, only one statistically significant effect of intra-mPOA U-50,488H infusions was produced. Intra-mPOA infusions of 0.5 nmol/ 0.5 μ l/side U-50,488H decreased female-directed behaviour [$F(1,5)=27.222$, $p<.004$] (see Fig. 29) during the second [$p<.05$], but not the first min of testing [$p>.05$].

Non-Copulatory Behaviours: Figure 28 depicts the mean percent of observations (out of 60 possible) that various non-copulatory behaviours were recorded during the 30-min sessions. Although few statistically significant effects of intra-cranial U-50,488H were obtained, intra-VTA U-50,488H weakly decreased bodily grooming [$F(4,20)=2.779$, $p<.06$], whereas intra-NAS U-50,488H weakly increased body flattening [$F(4,24)=2.197$, $p<.10$] and significantly decreased genital grooming [$F(4,24)=2.778$, $p<.05$].

Experiment 5B: Tests for Locomotor Activity Following Intra-Cranial Infusions of U-50,488H

On 10 counterbalanced tests for locomotor activity, 17 animals were co-administered either systemic saline plus intra-cranial saline, systemic naloxone (2.0 mg/kg, i.p.) plus intra-cranial saline, systemic saline plus one of four doses of intra-cranial U-50,488H (0.0005-5.0 nmol/0.5 μ l/side) or naloxone plus one of four doses of U-50,488H.

Locomotor Activity: The activity scores from the 60-min tests were subjected to a three-way, between-within-within, ANOVA for Brain Site x U-50,488H Dose x Naloxone, and are presented in Fig. 30. The analysis yielded main effects for U-50,488H Dose [$F(4,52)=5.703$, $p<.0008$] and Naloxone [$F(1,13)=20.338$, $p<.0007$], but not for Brain Site [$F(2,13)=0.195$, $p=.8254$]. Post hoc tests indicated that both 0.5 [$p<.01$] and 5.0 nmol/0.5 μ l/side U-50,488H [$p<.05$] significantly decreased locomotor activity, relative to saline, when collapsed over both Brain Site and Naloxone. Simple main effects analyses indicated that the inhibitory effect of U-50,488H was apparent when it was co-administered with either saline [$F(4,52)=2.943$, $p<.03$] or naloxone [$F(4,52)=4.849$, $p<.003$]. Further, post hoc tests indicated that overall, naloxone also significantly decreased locomotor activity, relative to saline [$p<.01$]. Simple main effects analyses indicated that this effect of systemic naloxone was apparent when it was co-administered with intra-cranial saline [$F(1,13)=5.370$, $p<.04$], 0.0005 nmol U-50,488H [$F(1,13)=19.132$, $p<.002$] or 0.5 nmol/0.5 μ l/side U-50,488H [$F(1,13)=17.070$, $p<.002$].

Discussion

The set of experiments (Experiments 4A, 4B, 5A and 5B) described in this chapter demonstrate that the effects of U-50,488H are, at least in part, mediated by actions at opioid receptors, and that they are due to actions in the central nervous system.

Copulatory Behaviour: Experiment 4A replicated the inhibitory effects of systemic U-50,488H on male sexual behaviour demonstrated in Experiments 1A and 1B. Following U-50,488H injections, fewer males mounted, intromitted or ejaculated. As well, U-50,488H-injected animals displayed less female-directed behaviour (except during the first min), were significantly slower to initiate copulatory behaviour, were slower to ejaculate, and they ejaculated fewer times than saline-injected animals. Most importantly, however, and not previously demonstrated, these inhibitory effects on sexual behaviour were all blocked by systemic injections of naloxone. It would appear that many of U-50,488H's inhibitory effects on both appetitive and consummatory dimensions of male sexual behaviour are mediated through actions on opioid receptors.

Central infusions of U-50,488H induced many of the behaviourally inhibiting effects seen to follow systemic administrations. Intra-VTA U-50,488H decreased the proportion of males that ejaculated, and increased copulation latencies. Interestingly, an unusual and unexpected dose-response to U-50,488H was obtained in the tests for sexual behaviour. Of the four doses tested, only the lowest (0.0005 nmol/0.5 μ l/side) and a middle dose (0.5 nmol/0.5 μ l/side) were effective. The two other test doses (0.05 and 5.0 nmol/0.5 μ l/side) were ineffective. Somewhat unexpectedly, all four doses of U-50,488H given intra-VTA tended to decrease the latency to the second ejaculation and increase the mean number of ejaculations.

Infusions of U-50,488H into the terminal region of the mesolimbic DA pathway also reduced sexual behaviour. With the notable exception of the highest test dose (5.0 nmol/0.5 μ l/side), intra-NAS U-50,488H increased both the mount and intromission latencies as well as decreasing the proportion of males that either intromitted or ejaculated.

The behavioural effects that did occur when U-50,488H was injected into either the VTA or NAS suggest decrements in the appetitive motivation to initiate copulation; copulation latencies were increased to the extent that many animals did not copulate at any time during the 30-min session. Such an interpretation is compatible with substantial

research that identifies the mesolimbic pathway as playing a focal role in the initiation of appetitive behaviours, the approach toward, salient, incentive motivational stimuli (see Everitt, 1990).

Surprisingly, there was no orderly effect of dose of U-50,488H into either mesolimbic region. In the VTA, some doses led to inhibitions of sexual behaviour while other, higher doses had no effect. Intra-VTA infusions of U-50,488H in fact decreased ejaculation latencies in the second ejaculatory series. These findings suggest that U-50,488H might have behaviourally stimulating effects that sometimes mask expected behavioural inhibitions. Opposing behavioural effects of intra-cranial infusions of *k* opioid agonists have been reported previously; unilateral intra-SN administrations of *k* agonists can induce both DA-dependent ipsilateral circling (Herrera-Marschitz et al., 1984) and DA-independent contralateral circling (Herrera-Marschitz et al., 1986; Matsumoto et al., 1988a,b; Tan & Tsou, 1988). Moreover, in tests for male sexual behaviour (Mitchell & Stewart, 1990b) and feeding (Hamilton & Bozarth, 1988; Badiani & Noel, 1991) excitatory effects have been reported following the intra-VTA infusion of *k* agonists. Interestingly, in both behavioural paradigms, only the lower test doses were found to be effective.

The effects that were seen after the administration of U-50,488H intra-VTA and intra-NAS tended to be similar to those following systemic injections. In contrast, intra-mPOA infusions of U-50,488H were largely ineffective. Although weak inhibitory effects were apparent on most measures, infusions of U-50,488H into the mPOA elicited only one statistically significant effect; 0.5 nmol/0.5 μ l/side U-50,488H abolished female-directed behaviour during the second min of testing. However, although mount and intromission latencies, considered measures of sexual arousability or appetitive motivation, were only erratically influenced, ejaculation latencies, considered to be measures of consummatory functioning, were increased in all six males tested following the lowest dose of U-50,488H {362.7 \pm 48.1 s vs 981.8 \pm 273.4 s}. The lack of statistical significance of this effect

probably arises from the small sample size and the extremely variable scores seen following the other doses. Although clearly present, k receptors in the mPOA (e.g., Nock et al., 1988) cannot be ascribed a primary role in the inhibition of male sexual behaviour. Instead, these results suggest possible weak, inhibitory effects of intra-mPOA U-50,488H on both appetitive and consummatory aspects of copulatory behaviour.

Neurochemical Basis of U-50,488H Effects: That at least some doses of U-50,488H infused into the VTA inhibited sexual behaviour was not surprising. Comparable infusions into the more thoroughly studied, but neurochemically similar, SN have been demonstrated to reduce striatal DA release (Christensson-Nylander et al., 1986; Herrera-Marschitz et al., 1986; Reid et al., 1988, 1990; Tan et al., 1988) and DA-dependent behaviours (Herrera-Marschitz et al., 1983, 1984, 1986; Matsumoto et al., 1988a,b; Tan et al., 1988). To the extent that male sexual behaviour is a DA-dependent behaviour (see Everitt, 1990), it was not surprising to find that an opioid treatment believed to decrease DA release should be behaviourally inhibiting. Interestingly, the present results resemble those obtained following the intra-VTA administration of the DA receptor agonist apomorphine (Hull et al., 1990, 1991) which is considered to act at DA autoreceptors to reduce firing in DA neurons. It is possible, therefore, that U-50,488H acts in the VTA by reducing activity in DA neurons.

The absence of effects from the two other doses tested in the VTA is more difficult to explain, but this unusual dose-response is not incompatible with other reports. In several of the experimental paradigms used to test the effects of k agonists in the VTA, opposing excitatory and inhibitory effects have been found. These paradoxical findings are apparent in electrophysiological (e.g., Chavkin et al, 1985; Thompson & Walker, 1989; Alzheimer & Bruggencate, 1990), pharmacological (e.g., Starr, 1985; Spanagel et al., 1990) and behavioural studies (e.g., Herrera-Marschitz et al., 1986; Bechara & van der Kooy, 1987; Hamilton & Bozarth, 1988; Tan & Tsou, 1988; Mitchell & Stewart, 1990b; Badiani & Noel, 1991). Therefore, it seems plausible to suggest that there may be

behavioural tests where both excitatory and inhibitory actions are activated in such a way as to nullify the detectable effect of the other. The neural substrates for these effects cannot be determined from the present study, but U-50,488H might induce behaviourally excitatory effects from any of the following five mechanisms: GABAergic inhibition (Mogenson, Wu & Manchanda, 1979; Mogenson, Wu & Jones, 1980; Ågmo & Paredes, 1985; Ågmo, Paredes & Fernández, 1987; Tan & Tsou, 1988; Gillham, Jennes & Deutch, 1990; Thompson & Walker, 1990); μ opioid receptor activation (e.g., Lahti et al., 1982); inhibited mesocortical DA neurons that disinhibit descending cortico-striatal (Lee & Deutch, 1990; Sesack & Pickel, 1990) and cortico-mesencephalic (Christie, Bridge, James & Beart, 1985; Sesack & Pickel, 1990) excitatory glutamatergic and/or aspartamatergic pathways to induce the non-impulse-dependent release of mesolimbic DA (Scatton, Worms, Lloyd & Bartholini, 1982; Pulvrienti, Sung & Koob, 1989; Swerdlow, Pulvrienti & Koob, 1989; Deutch, Clark & Roth, 1990) - perhaps such a mechanism might also explain the paradoxical decreases in post-ejaculatory refractory periods that some investigators have reported to follow electrolytic lesions of the VTA (Barfield et al., 1975; Clark et al., 1975); the differential activation of k opioid receptor subtypes (Clark et al., 1989; Rothman et al., 1990); and the induced release of excitatory tachykinins - neuropeptides that are co-localized in DYN neurons projecting to the VTA/SN (Besson et al., 1986; Anderson & Rainer, 1990) or mPOA (Noel & Newman, 1991) - that are known to facilitate both locomotor activity (Kelley, Stinus & Iversen, 1979; Pinnock, Woodruff & Turnbull, 1983; Kalivas & Miller, 1984; Deutch, Maggio, Bannon, Kalivas, Tam, Goldstein & Roth, 1985; Kalivas, Deutch, Maggio, Mantyh & Roth, 1985; Kelley, Cador & Stinus, 1985) and male sexual behaviour (Dorman & Malsbury, 1989; Leyton & Stewart, unpublished data).

The present study does not allow one to choose from these various, possibly not unrelated mechanisms. Their further exploration would be of interest, however, so as to perhaps explain the striking disparity between the clearly dominant inhibitory effects of

intra-VTA U-50,488H observed here and the excitatory effects reported to follow VTA infusions of *k* opioid agonists in studies of both spontaneous (Hamilton & Bozarth, 1988, Badiani & Noel, 1991) and stimulation-induced feeding (Wise et al., 1986; Jenck et al., 1987) as well as male sexual behaviour (Mitchell & Stewart, 1990b).

The inhibitory effects of intra-NAS injections of U-50,488H are most probably mediated by decreases in NAS DA release; NAS applications of *k* agonists have been demonstrated to decrease both DA release (e.g., Heijna et al., 1989, 1990a,b) and DA neural firing (e.g., Walker et al., 1987) suggesting inhibitory mechanisms on both presynaptic DA terminals and postsynaptic descending inhibitory feedback pathways. Intra-NAS infusions of haloperidol also decrease measures of appetitive motivation to copulate while leaving consummatory sexual functioning intact (Pfaus, 1990; Pfaus & Phillips, 1991). This parallel between intra-NAS infusions of U-50,488H - a treatment believed to reduce the terminal release of DA - and haloperidol - a dopamine receptor antagonist - suggests that reduced DA neurotransmission underlies the attenuated sexual motivation seen following NAS infusions of both drugs.

The absence of an orderly effect of doses of U-50,488H in the NAS might indicate the presence of U-50,488H-induced excitatory effects. This possibility is suggested by the absence of sexual behaviour deficits following the highest tested U-50,488H dose (5.0 nmol/0.5 μ l/side). In further support of this idea is the finding that when systemic naloxone was given at the same time as the high dose of intra- NAS U-50,488H, significant effects on sexual behaviour were found, similar to those produced by the lower U-50,488H doses (see Figs 22-25). This result suggests that at high doses U-50,488H might have opioid receptor-mediated excitatory effects in addition to its depressant effect. The substrate for these hypothesized excitatory effects is not known, but could include any one of the five mechanisms discussed above with respect to the VTA.

To the extent that weak inhibitory effects of intra-mPOA U-50,488H were seen, it seems possible that they too were produced by decreases in DA release from the A14

incerto-hypothalamic DA pathway. For, although the effects are much greater, intra-mPOA infusions of neuroleptics duplicate the multiple inhibitory effects on sexual behaviour that are seen to follow systemic administrations (Pfaus, 1990; Pfaus & Phillips, 1991). Alternatively, these weak effects from intra-mPOA injections of U-50,488H might indicate actions from the drug diffusing elsewhere. The absence of anatomical controls strictly demarcating the anatomical borders of the effects that were observed precludes any unequivocal conclusion that these behavioural inhibitions resulted from the stimulation of *k* opioid receptors restricted to the mPOA, but the similarity of these data with those obtained following intra-mPOA injections of neuroleptics at least suggests that this interpretation is possible.

In summary, the behavioural deficits produced by at least some doses of U-50,488H into either the VTA or NAS, and to a lesser extent the mPOA, were similar to, but markedly less than those seen following systemic U-50,488H, allowing for the possibility that *k* receptor stimulation in these regions is disruptive. These actions are compatible with the known anatomy of *k* receptors (Quirion et al., 1983; Lynch et al., 1985; Mansour et al., 1987; Nock et al., 1988), the known pharmacology of *k* receptor activation in these regions, the known role of the mesolimbic DA pathway in appetitive behaviours (see Everitt, 1990), and the mPOA's role in sexual behaviour (see Sachs & Meisel, 1988).

Non-Copulatory Behaviour: Compatible with deficits in appetitive dimensions of sexual behaviour, the inhibitory effects of systemic U-50,488H on locomotor activity observed in Experiments 2A and 2B were replicated in Experiment 4B. Unexpectedly, systemic naloxone did not prevent this decrease. Both previous research (Leander, 1983) and data collected in the current thesis (Experiment 4A) indicate that 2.0 mg/kg is sufficient to serve as a *k* receptor antagonist, but naloxone itself is known to decrease locomotor activity (Amir, Solomon & Amit, 1979; Walker, Berntson, Paulucci & Champney, 1981; Ukai & Kameyama, 1985; Experiment 5B), and it may be that this inhibitory action makes it an unsuitable choice to antagonize *k* opioid agonist-induced depressant effects on

locomotion. Reports from previous investigators are compatible with this idea. *K* agonist-induced decreases in rat locomotor activity are blocked by the mixed μ/k opioid antagonist Mr2266 (Ukai & Kameyama, 1985), but not by naloxone (Ruhland & Zeugner, 1983). Moreover, Mr2266 itself does not decrease locomotor activity (Ukai & Kameyama, 1985). Perhaps Mr2266's more ready *k* receptor antagonism allows it to prevent *k* agonist-induced decreases in locomotor activity without it swamping other opioid receptors so thoroughly that spontaneous locomotor activity is reduced.

When these same animals were tested in Experiment 5B, significant decreases in locomotor activity from both intra-cranial U-50,488H and systemic naloxone were found. As had been seen in Experiment 4B, the U-50,488H-induced decreases in locomotor activity were not attenuated by systemic naloxone, and moreover, naloxone potentiated these decreases. The most probable explanation is that naloxone itself decreases motor activity (Amir et al., 1979; Walker et al., 1981; Ukai & Kameyama, 1985; Experiment 5B), making it a poor candidate to antagonize this inhibitory effect of U-50,488H.

Intra-cranial infusions of U-50,488H into all three tested brain sites decreased locomotor activity. The inhibitory effect of infusions of U-50,488H into the mesolimbic sites was not unexpected; *k* agonists administered into either DA cell body (Reid et al., 1988a,b, 1990) or terminal regions (Mulder et al., 1984, 1989; Clow & Jhamandas, 1987; Schoffelmeer et al., 1988; Werling et al., 1988; Heijna et al., 1989, 1990a,b) are known to induce decreased DA release associated with decreased locomotor activity (e.g., Di Chiara & Imperato, 1988) and aversive states (e.g., Bals-Kubik et al., 1990). In contrast, the mPOA is not commonly considered to be involved in locomotor activity, although Mogenson has presented evidence that it is an important nucleus for integrating external sensory input and internal physiological states to determine appropriate behavioural responses (Mogenson, Jones & Yim, 1980; Swanson, Mogenson, Gerfen & Robinson, 1984). Possibly, intra-mPOA U-50,488H induces aversive states that discourage the animal from interacting with its environment.

Also replicating earlier findings (Experiments 1A, 1B, 2A and 2B), U-50,488H altered the occurrence of numerous non-copulatory behaviours recorded during the tests for sexual behaviour. Not surprisingly, naloxone prevented the U-50,488H-induced decrease in genital grooming. This finding is compatible with the earlier suggestion that U-50,488H decreased copulation-bound genital grooming simply because the males were copulating less. Thus, when the co-administration of naloxone prevented this copulatory inhibition, genital grooming also resumed.

The systemic U-50,488H-induced inhibition of bodily grooming was not significantly prevented by naloxone. This was unexpected given the reports indicating both that U-50,488H decreases synaptic levels of DA and that bodily grooming can be decreased by reducing D1 receptor stimulation (Greidanus et al., 1989). Possibly naloxone decreases bodily grooming; it would not be surprising to learn that naloxone decreases DA release. Unfortunately, this latter possibility has not yet been tested. Interestingly, bodily grooming was also decreased by intra-VTA infusions of U-50,488H, suggesting that the critical D1 receptors might be situated in either the A10 cell body region or in a mesolimbic terminal region other than the NAS.

Naloxone also failed to prevent the body flattening that was elicited by U-50,488H. Naloxone did, however, attenuate U-50,488H-induced increases in the less severe lying down activity, thereby substantiating the distinction to be made between these two behaviours. It seems likely that the increases in phenomenologically normal lying down reflect general, non-specific behavioural decrements that would be expected if κ opioid receptor stimulation decreases DA release. Conversely, the elicitation of body flattening might result from actions at some non-opioid mechanism. This seems unlikely, however, as the selective κ antagonist NBNI does prevent U-50,488H-induced flattening in guinea pigs (Brent et al., 1990). An alternative explanation compatible with both the present findings and the Brent et al. report, is that systemic naloxone might mimic U-50,488H-induced flattening making it an inadequate choice to prevent the behaviour. The neurons on

which these opioid receptors lie remain to be determined, but as discussed previously, the similarity between U-50,488H-induced and serotonergic-mediated body flattening indicates that the relevant k receptors might be interacting with 5-HT neurons. Interestingly, the intra-NAS infusion of U-50,488H weakly [$p < .10$] increased body flattening reminiscent of body flattening induced by intra-NAS infusions of 5-HT_{1a} agonists (Hillegaart, 1991).

In conclusion, both the systemic and intra-cranial administrations of U-50,488H can induce a wide range of behavioural changes. Either systemic injections of U-50,488H or intra-cranial infusions to the VTA, NAS and mPOA would seem to induce decrements in the appetitive motivation to approach incentive stimuli. These inhibitions were indicated by decreased locomotor activity, decreased female-directed behaviour, and delays to initiate copulatory behaviour with an estrous female. Systemic U-50,488H injections also inhibited consummatory dimensions of sexual behaviour, but these attenuations were only weakly replicated by intra-cranial U-50,488H infusions. The effects of systemic U-50,488H on male sexual behaviour were prevented by systemic naloxone indicating that these behavioural inhibitions were induced by actions at opioid receptors. Further, given both the known inhibitions on DA neurotransmission induced by k agonists and the similarities between U-50,488H and neuroleptic-induced sexual behaviour inhibitions, it seems likely that many of the observed behavioural inhibitions result from k opioid receptor stimulation-induced decreases in DA cell firing and release.

Decreases in DA release were also suggested by the systemic and intra-VTA U-50,488H-induced attenuated bodily grooming, a displacement activity associated with D1 receptor stimulation (Greidanus et al., 1989) and thwarted behavioural motivation (Hansen et al., 1982; Hansen & Drake af Hagensrum, 1984).

Both systemic and intra-NAS U-50,488H administration increased body flattening, a behaviour also seen following inhibitions of 5-HT neurotransmission (Hillegaart, 1990). Similarly, inhibited 5-HT activity also decreases the number of mounts and intromissions to precede ejaculation (Hillegaart, 1990), and increases copulation latencies (Mendelson &

Gorzalka, 1985), two behavioural alterations also seen to follow either systemic or intranasal U-50,488H.

These findings suggest that much of the behavioural syndrome induced by the administration of U-50,488H might result from inhibitions of both DA and 5-HT neurotransmission.

Another approach to examining the anatomical localization of the behavioural effects induced by systemic injections of U-50,488H is to challenge systemic administrations with central injections of a κ opioid receptor antagonist. This was done in the experiments to be described in Chapter four.

CHAPTER FOUR

The Effects of Central Applications of a Specific *Kappa* Opioid Receptor Antagonist on U-50,488H-Induced Inhibitory Effects

The preceding experiments indicate that U-50,488H inhibits male sexual behaviour, that most of the effects are opioid mediated, and that some of them can be obtained by actions of U-50,488H in the central nervous system. Experiments 6A, 6B and 6C were designed to investigate whether inhibitory effects of U-50,488H seen after systemic injection could be blocked or attenuated by the central application of a specific *k* opioid receptor antagonist.

In Experiment 6A, male rats were tested following the systemic administration of the dose of U-50,488H that had been shown to have inhibitory effects on male sexual behaviour that were naloxone reversible. These animals were then challenged with infusions of the specific *k* receptor antagonist, NBNI, into either the VTA, NAS or mPOA, and were compared to animals administered either systemic U-50,488H plus intra-cranial saline, or saline plus intra-cranial NBNI or both systemic and intra-cranial injections of saline. This experiment was designed to address two related questions: what is the effect of preventing endogenous *k* agonists from stimulating central *k* receptors in either the VTA, NAS or mPOA? and; what is the effect of blocking *k* receptor stimulation at one site when systemically administered U-50,488H stimulates *k* receptors at other sites? In Experiments 6A, 6B and 6C, animals were tested for both sexual behaviour and locomotor activity.

In the course of working with NBNI, evidence of long-lasting effects were found. To further explore this possibility, animals from Experiment 6A, that had previously been administered intra-cranial NBNI, were tested for sexual behaviour a second time following systemic injections of U-50,488H (Experiment 6B). The long-lasting effect of NBNI was assessed a final time when these animals were tested for locomotor activity following U-50,488H injection (Experiment 6C).

Methods

Subjects

Male Wistar rats weighing 260-280 g upon arrival were obtained from Charles River Canada Inc. (USA) and housed individually as in Experiment 1. Eighty-nine of these males were then selected following a screen to ensure that the males displayed consistent levels of copulatory behaviour.

Surgery

After having been selected from the larger population, all animals were stereotaxically implanted with chronic angled bilateral guide cannulae (26-gauge stainless steel tubing) aimed at the VTA (A/P -3.6, L \pm 0.6, D/V -8.9), NAS (A/P +3.4 \pm 2.8, L, D/V -7.4), or mPOA (A/P +2.4, L \pm 0.2, D/V -6.7), positioned 1.0 mm above the final injection site. The incisor bar was set at 5.0 mm superior to the interaural line. The animals were then given one week to 10 days to recover prior to beginning testing.

Histology

Following the experiment all animals were perfused transcardially with saline and 10% formalin solution under deep anaesthesia. Brains were stored in a 10% formalin solution for at least five days. Histological verification of cannulae tip placement was subsequently made on 30 μ m thionin-stained coronal sections.

Apparatus

The apparatus were as described in Experiment 1.

Procedure

In Experiment 6A, a between groups design study, 89 male rats were co-administered a systemic injection of either saline or 5.0 mg/kg U-50,488H given s.c. along with a bilateral intracranial infusion of either saline or 3.0 μ g/0.5 μ l/side nor-binaltorphimine (NBNI) (17,17'*bis* (cyclopropylmethyl) 6,6',7,7'-tetrahydro-4,5 α :4',5 α -diepoxy-6,6'-(methylimino)[7,7'-bomorphinan]-3,3',14,14'-tetrol)

(Research Biochemicals, Inc.) (Portoghese, Lipkowski & Takemori, 1987a,b; Portoghese, Nagase, Lipowski, Larson & Takemori, 1988; Takemori, Begonia, Ho, Naeseth & Portoghese, 1988; Smith, Medzihradsky, Hollingsworth, DeCosta, Rice & Woods, 1989; Song, Barbas, Portoghese & Takemori, 1989). The systemic injections were immediately followed by the intracranial infusions. The males were then placed together in a carrying bucket where they waited for 15 min prior to being taken to the mating boxes. Five min later an estrous female was paired with the male for 30 min. All males were tested only once.

In Experiment 6B, 53 of the male rats from Experiment 6A that had had intracranial injections of NBNI were tested for sexual behaviour a second time for possible long-term effects of NBNI. All were given with intra-cranial infusions of saline and systemic injections of either saline or 5.0 mg/kg U-50,488H given s.c., and then paired with an estrous female. The results were compared to those from NBNI naive males administered systemic injections of either saline or U-50,488H in Experiment 6A. Twenty-six days after the last central NBNI infusion, six mPOA cannulated males were tested again for sexual behaviour following the administration of 8.0-10.0 mg/kg U-50,488H given s.c.

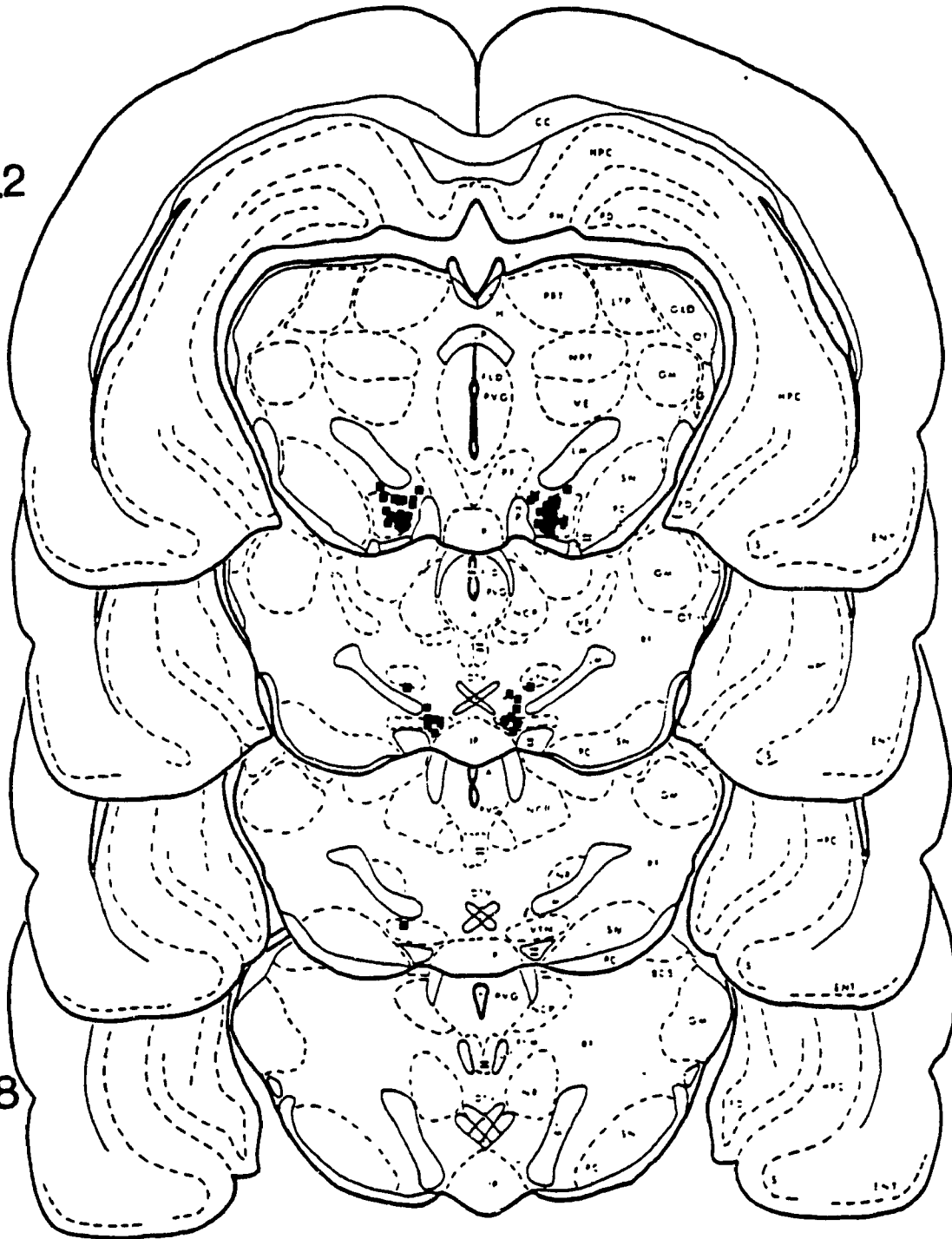
In Experiment 6C, 34 of the males from Experiments 6A that had been previously administered NBNI two to six days earlier were tested for locomotor activity in the photocell boxes for 90 min. On two counterbalanced tests, separated by a drug-free day, all animals were co-administered 5.0 mg/kg U-50,488H given s.c. with intra-cranial infusions of either saline or 3.0 µg/0.5µl/side NBNI.

Statistical Analyses

For the purpose of analyses in Experiment 6A, animals that had received injections of saline only, both systemically and intra-cranially, were collapsed into a single group irrespective of their cannulation site. Similarly, animals that had received systemic U-50,488H plus intracranial saline were collapsed to form a second group irrespective of

Figure 31 Histological dissection of 30 μ m VTA slices for animals from Experiments 6A, 6B and 6C. The coronal sections are from the atlas of Pellegrino et al., (1979). Numbers to the left indicate mm from bregma.

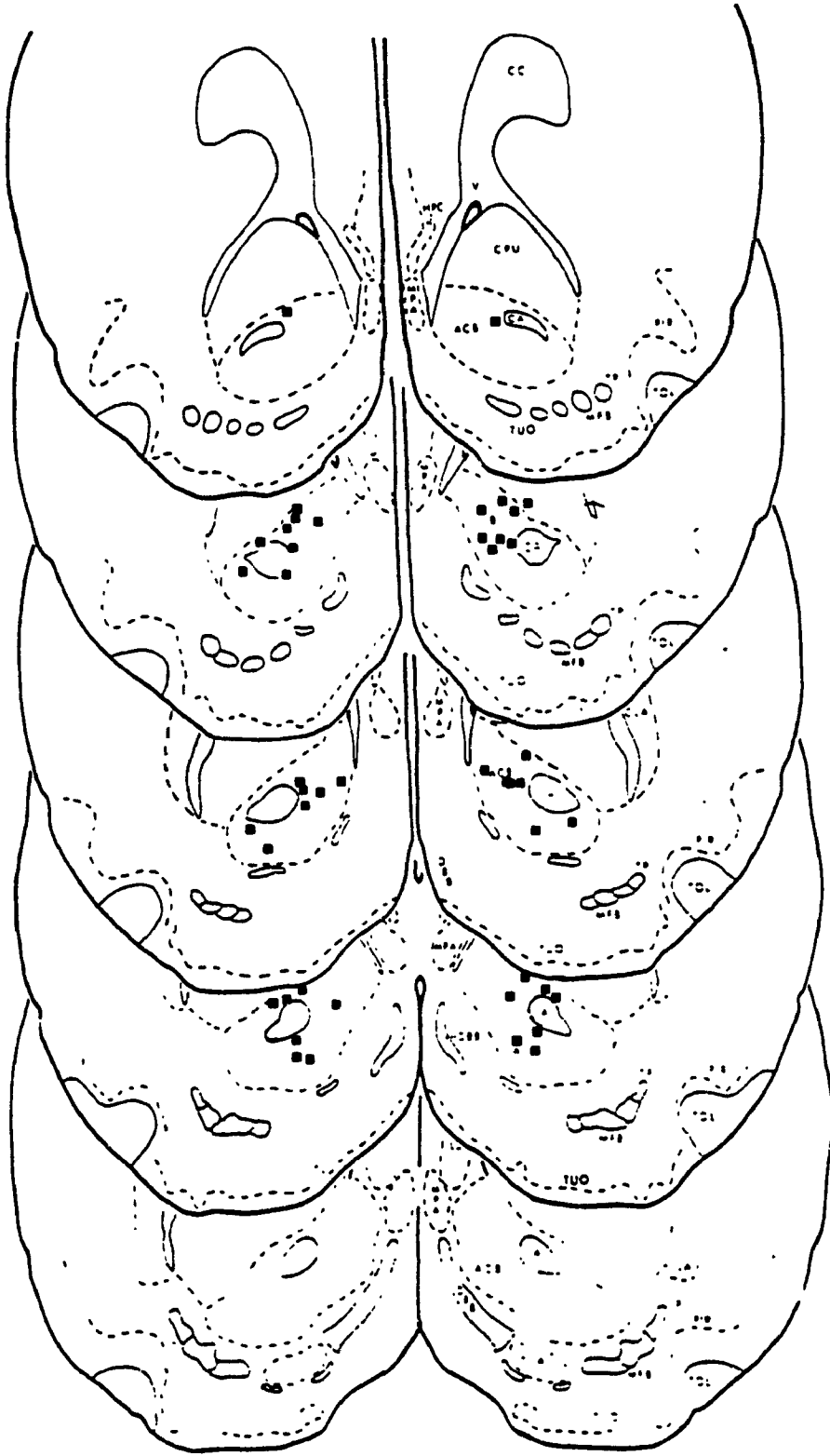
-3.2



-3.8

Figure 32 Histological dissection of 30 μ m NAS slices for animals from Experiments 6A, 6B and 6C. The coronal sections are from the atlas of Pellegrino et al., (1979). Numbers to the left indicate mm from bregma.

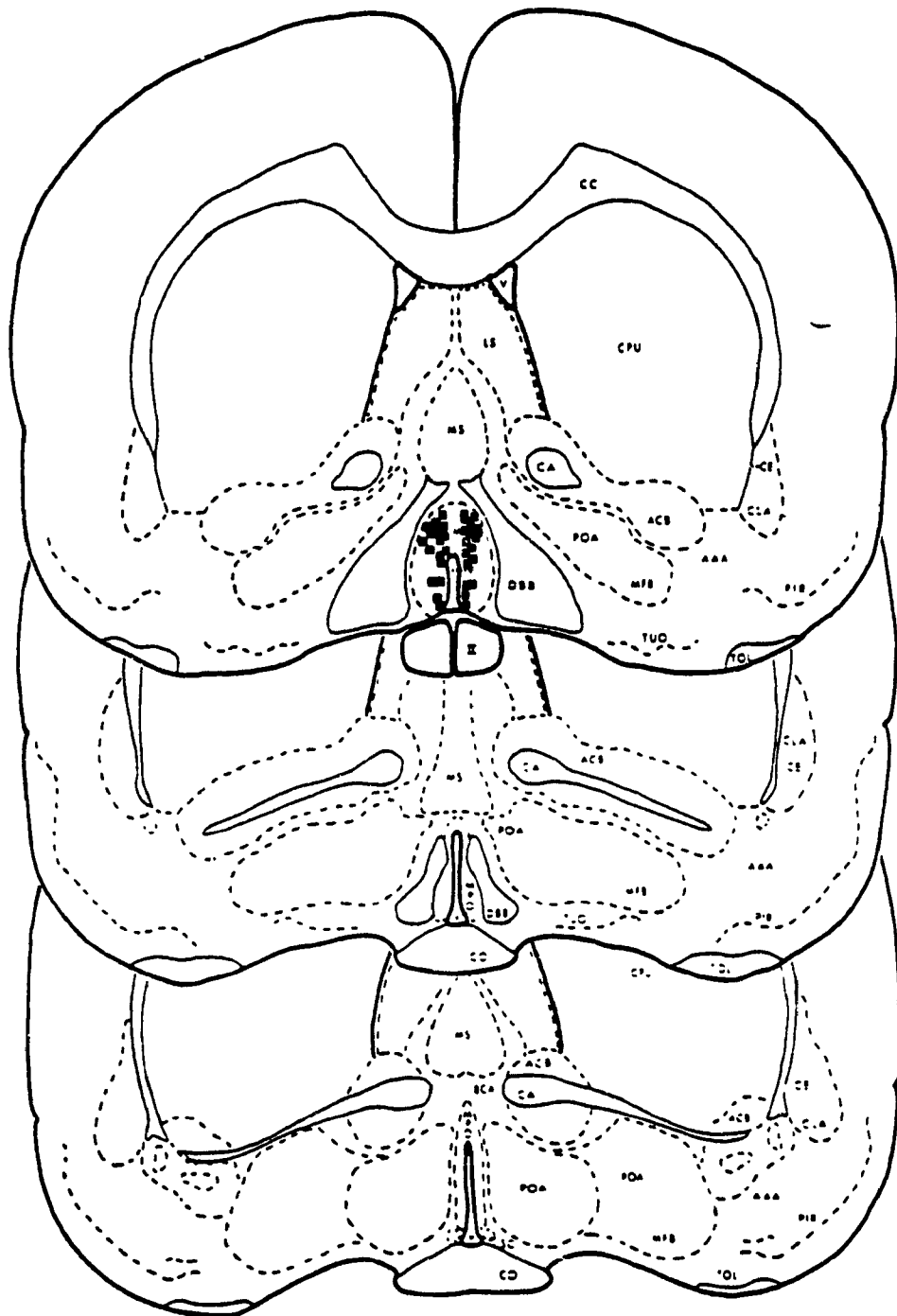
4.0



3.2

Figure 33 Histological dissection of 30 μm mPOA slices for animals from Experiments 6A, 6B and 6C. The coronal sections are from the atlas of Pellegrino et al., (1979). Numbers to the left indicate mm from bregma.

2.4



cannulation site. The remaining animals were grouped on the basis of both intra-cranial site of injection and systemic drug treatment.

The proportion of animals that mounted, intromitted or ejaculated was analysed by chi square with Yates' Correction for Continuity. The latency of animals to mount, intromit and ejaculate, the refractory period, the mean number of ejaculations and intromissions to the first ejaculation, and the III were each analysed by three separate between groups measures ANOVAs for each brain site. The correlation between the III and the post-ejaculatory refractory period was analysed by the Pearson product-moment correlation coefficient. Only those animals that did mount, intromit, ejaculate or begin a second ejaculatory series were assigned scores for these latencies. Animals that mounted, but did not ejaculate, were assigned a score of zero for number of ejaculations; animals that did not mount at all were excluded from the analyses. Post hoc comparisons, when performed, were with Newman-Keuls tests.

The data for Experiment 6B were analysed as in Experiment 6A. The proportion of animals that mounted, intromitted or ejaculated were analysed by chi square. The parametric data were analysed by separate between-groups ANOVAs for each brain site. Post hoc comparisons were performed as above.

The 30-min locomotor activity scores from the 90-min test in Experiment 6C were analysed by a three-way between-within ANOVA for Brain Site x NBNI Administration x Time. The data from these animals on the saline test were then compared to the data collected in Experiment 2B, and analysed by a between-within groups ANOVA for Drug Treatments x Time.

Results

Experiment 6

In contrast to the previous within groups design experiments where, for statistical purposes, animals were assigned copulation latencies of 1800 s if they did not

copulate, the between groups design used in the present experiment did not require that all subjects be assigned a score. Thus for example, animals that did not mount, were not included in the mount latency analyses of Experiments 6A and 6B.

On the basis of histological examination of cannulae placements, three animals were removed from the analysis of data. Figures 31-33 illustrate the cannulae tip localizations of 70 recovered brains.

Experiment 6A: Tests for Male Sexual Behaviour Following the Co-Administration of Systemic U-50,488H plus Intra-Cranial NBNI

On one test for male sexual behaviour, 89 rats were co-administered either systemic saline plus intra-cranial saline, systemic saline plus intra-cranial NBNI (3.0 $\mu\text{g}/0.5$ $\mu\text{l}/\text{side}$), systemic U-50,488H (5.0 mg/kg, s.c.) plus intra-cranial saline or U-50,488H plus NBNI.

Copulatory Behaviour: The inhibitory effects of systemic U-50,488H on male sexual behaviour seen in Experiments 1A, 1B and 4A were replicated in Experiment 6A. The most important new finding, however, was that selective aspects of U-50,488H's inhibitory effects were blocked by infusions of the *k* opioid receptor antagonist NBNI into discrete brain regions. Among at least some animals, intra-VTA infusions of NBNI prevented U-50,488H-induced increases in both copulation latencies and IIIs as well as decreases in the number of ejaculations. Similarly, among animals administered intra-NAS infusions of NBNI, this putative *k* opioid receptor antagonist prevented systemic U-50,488H-induced increases in both copulation latencies and the III, and attenuated the U-50,488H-induced decrease in the number of intromissions to precede ejaculation. Interestingly, inhibitory effects of NBNI were apparent in some animals when it was infused into either the VTA or NAS. This bimodal effect of NBNI was explored by dividing group data at their 50th percentile and comparing the two sub-groups both to each other and to the appropriate drug control group.

No inhibitory effects of NBNI were observed following its injection into the mPOA, but intra-mPOA NBNI did prevent many of U-50,488H's effects on sexual behaviour. Intra-mPOA infusions of NBNI prevented the U-50,488H-induced decrease in the proportion of animals that initiated copulation or ejaculated during the test session, as well as attenuating U-50,488H-induced increases in copulation latencies, ejaculation latencies and the III, as well as U-50,488H-induced decreases in both the mean number of ejaculations and number of intromissions to precede ejaculation.

The following results section, with the accompanying Figs. 34-37, describes in more detail the changes to copulatory behaviour that were observed following systemic injections of U-50,488H and intra-cranial infusions of NBNI.

Among animals administered intra-VTA drug infusions, NBNI both prevented U-50,488H-induced sexual behaviour deficits and had some inhibitory effects of its own. Half of the animals treated with intra-VTA NBNI displayed increased mount (458.4 ± 274.7 s vs 29.0 ± 6.2 s) and intromission (110.8 ± 32.4 s vs 38.8 ± 10.9 s) latencies as well as decreases in the mean number of ejaculations (1.4 ± 0.7 vs 2.9 ± 0.3), relative to saline treated animals [$p < .05$]. Other animals administered intra-VTA NBNI did not differ from the saline control group [$p > .05$]. Intra-VTA NBNI also perturbed the significant correlation between the III and the post-ejaculatory refractory period [$r = -0.198$, $p > .20$] that was seen in saline [$r = +0.804$, $p < .02$], but not in U-50,488H [$r = -0.306$, $p > .02$] nor U-50,488H plus intra-VTA NBNI dual treated animals [$r = -0.284$, $p > .20$]. In contrast to these inhibitory effects seen in half of the animals not co-administered U-50,488H, intra-VTA NBNI prevented the U-50,488H-induced increases in both mount (55.8 ± 23.0 s vs 671.1 ± 207.4 s) and intromission latencies (57.2 ± 24.2 s vs 692.8 ± 209.2 s) in half of the animals co-administered both drugs, relative to the U-50,488H-injected group [$p < .05$]. Other animals co-administered both U-50,488H and intra-VTA NBNI did not differ from U-50,488H-injected animals [$p > .05$] on these measures. Finally, among all of the animals co-administered U-50,488H plus intra-VTA NBNI, NBNI prevented both the U-50,488H-

induced decrease in the mean number of ejaculations that were recorded during the test sessions [$p < .05$] and the U-50,488H-induced increase in the III [$p < .01$].

Among animals administered intra-NAS drug infusions, NBNI also had some inhibitory effects that contrasted with its efficacy to prevent some of the inhibitory effects of systemic U-50,488H. Half of the animals that were administered intra-NAS infusions of NBNI displayed significantly increased mount { 644.4 ± 309.3 s vs 29.0 ± 6.2 s} and intromission { 561.5 ± 383.3 s vs 38.8 ± 10.9 s} latencies, as well as decreased numbers of ejaculations (0.4 ± 0.2 vs 2.9 ± 0.3), relative to saline treated animals [$p < .05$]. Other animals administered intra-NAS NBNI did not differ from the saline control group [$p > .05$]. Intra-NAS NBNI also perturbed the significant correlation between the III and the post-ejaculatory refractory period [$r = +0.563$, $p > .10$] and did not re-establish this relation in U-50,488H-injected animals [$r = -0.412$, $p > .20$]. In contrast to these inhibitory effects of intra-NAS NBNI seen in half of the tested animals not co-administered U-50,488H, intra-NAS NBNI significantly prevented systemic U-50,488H-induced increases in both mount [$p < .05$] and intromission [$p < .05$] latencies as well as U-50,488H-induced decreases in the III [$p < .01$] among all tested animals. Finally, the U-50,488H-induced reduction in the number of intromissions to precede ejaculation, relative to saline [$p < .02$], was prevented in animals co-administered intra-NAS NBNI [$p > .05$].

Among animals administered intra-mPOA infusions of NBNI, no inhibitory effects of the receptor antagonist were found, although this treatment did disrupt the correlation between the III and the post-ejaculatory refractory period [$r = +0.536$, $p > .20$], and failed to prevent this same effect among U-50,488H-injected animals [$r = +0.156$, $p > .20$]. Intra-mPOA infusions of NBNI did, however, significantly attenuate U-50,488H-induced increases in mount [$p < .01$], intromission [$p < .01$] and ejaculation [$p < .05$] latencies, and the III [$p < .01$], as well as prevented U-50,488H-induced decreases in the proportion of animals that mounted {8/14 vs 9/9} [$\chi^2(3) = 9.47$, $p < .05$], intromitted {8/14 vs 8/9} [$\chi^2(3) = 6.92$, $p < .10$] or ejaculated {5/14 vs 8/9} [$\chi^2(3) = 14.76$, $p < .01$]. Finally, the U-

Figure 34 The mean (+ 1 S.E.M.) proportion of cannulated males to mount (34a), intromit (34b) and ejaculate (34c) as well as the mean (+ 1 S.E.M.) latency for animals to mount (34d), intromit (34e) and ejaculate (34f), and the mean (+ 1 S.E.M.) number of recorded ejaculations (34g) following the intracranial infusion of either saline or NBNI (3.0 μ g/0.5 μ l/side) co-administered with systemic injections of either saline or U50 (5.0 mg/kg, s.c.) during tests for sexual behaviour in Experiment 6A.

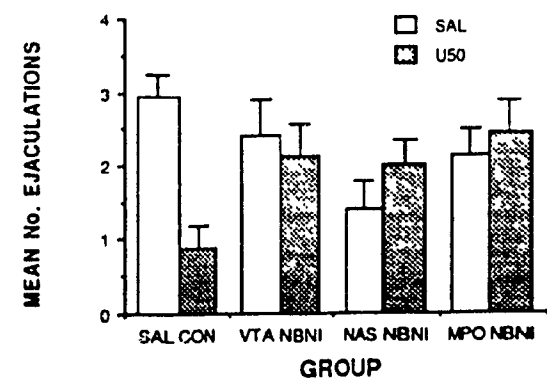
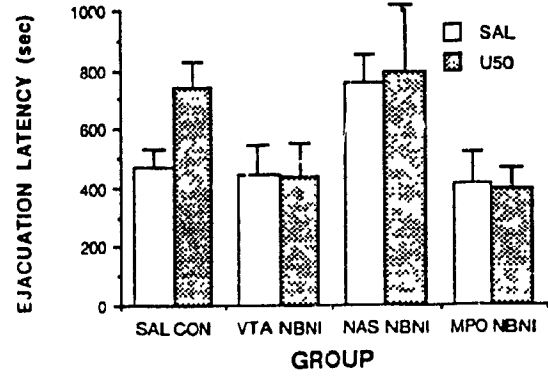
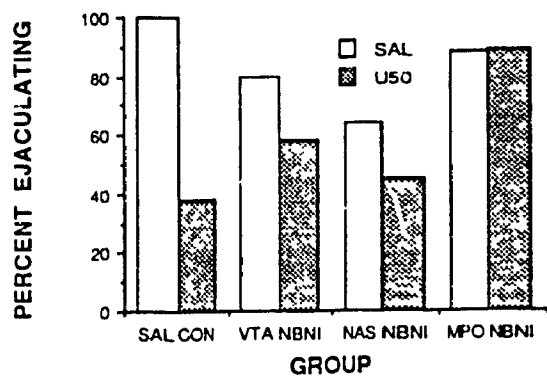
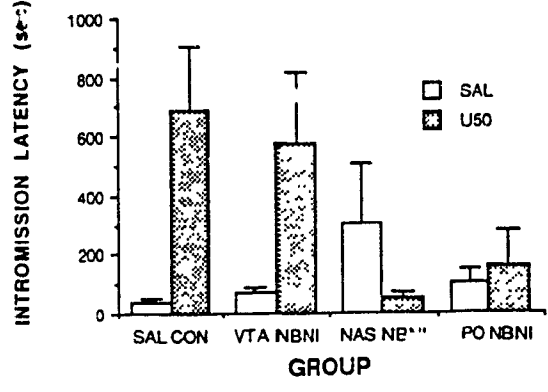
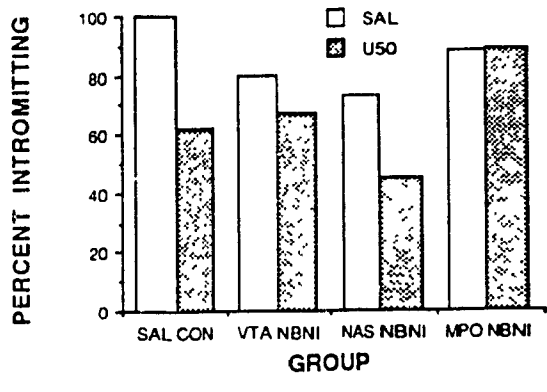
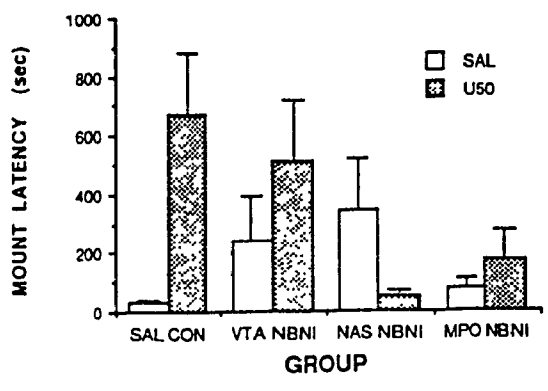
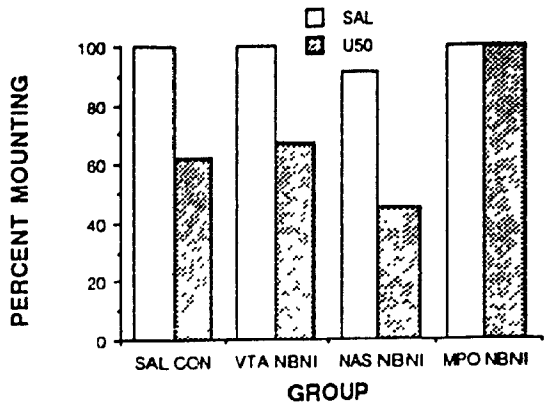


Figure 35 The mean (+ 1 S.E.M.) post-ejaculatory refractory period following the intra-cranial infusion of either saline or NBNI (3.0 μ g/0.5 μ l/side) co-administered with systemic injections of either saline or U50 (5.0 mg/kg, s.c.) during tests for sexual behaviour in Experiment 6A.

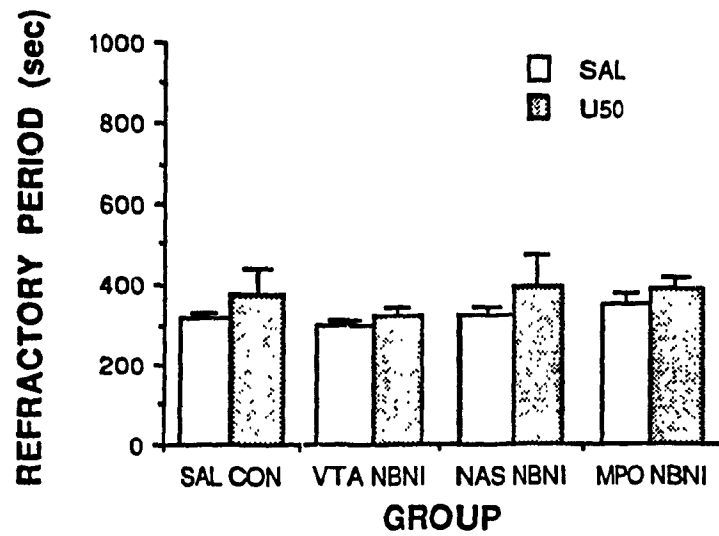


Figure 36 The mean (+ 1 S.E.M.) number of intromissions to precede the first ejaculation following the intra-cranial infusion of either saline or NBNI (3.0 μ g/0.5 μ l/side) co-administered with systemic injections of either saline or U50 (5.0 mg/kg, s.c.) during tests for sexual behaviour in Experiment 6A.

INTROMISSIONS TO EJACULATE

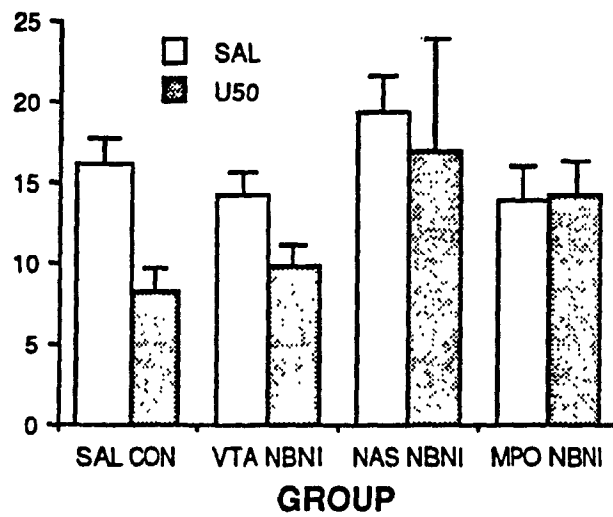


Figure 37 The mean (+ 1 S.E.M.) inter-intromission interval prior to the first ejaculation following the intra-cranial infusion of either saline or NBNI (3.0 $\mu\text{g}/0.5 \mu\text{l}/\text{side}$) co-administered with systemic injections of either saline or U50 (5.0 mg/kg, s.c.) during tests for sexual behaviour in Experiment 6A.

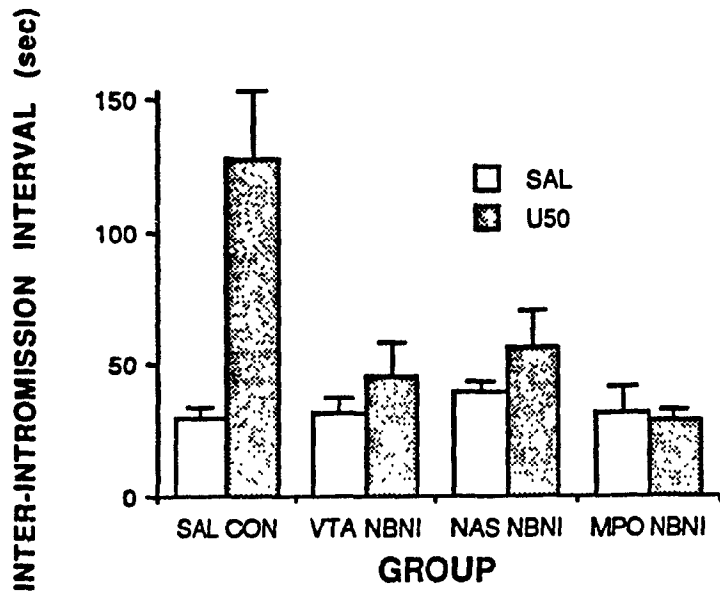


Figure 38 The mean (+ 1 S.E.M.) proportion of observations that non-copulatory behaviours were recorded to occur following the intra-cranial infusion of either saline or NBNI (3.0 μ g/0.5 μ l/side) co-administered with systemic injections of either saline or U50 (5.0 mg/kg, s.c.) during tests for sexual behaviour in Experiment 6A. (FDB: female-directed behaviour; L: laying down; LL: body flattening; Sn: sniffing; Gr: body grooming; GG: genital grooming; W: walking; R: rearing).

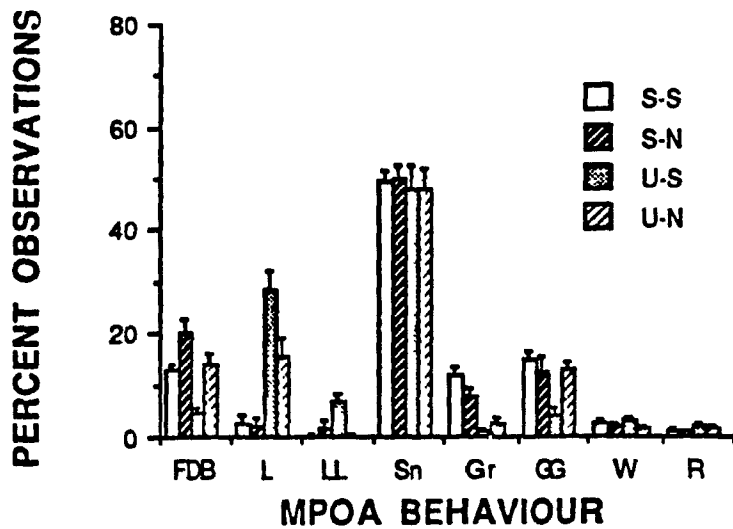
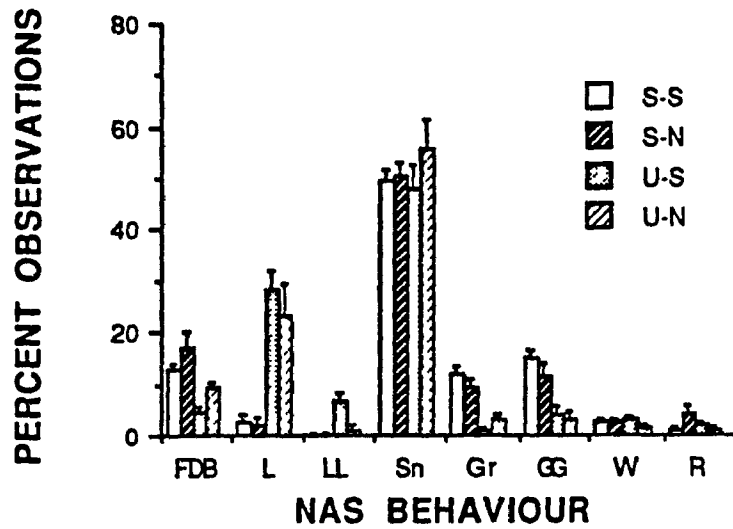
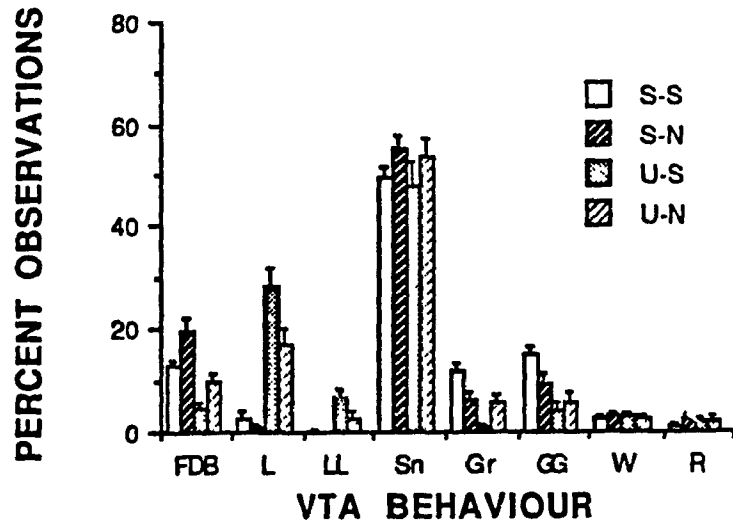
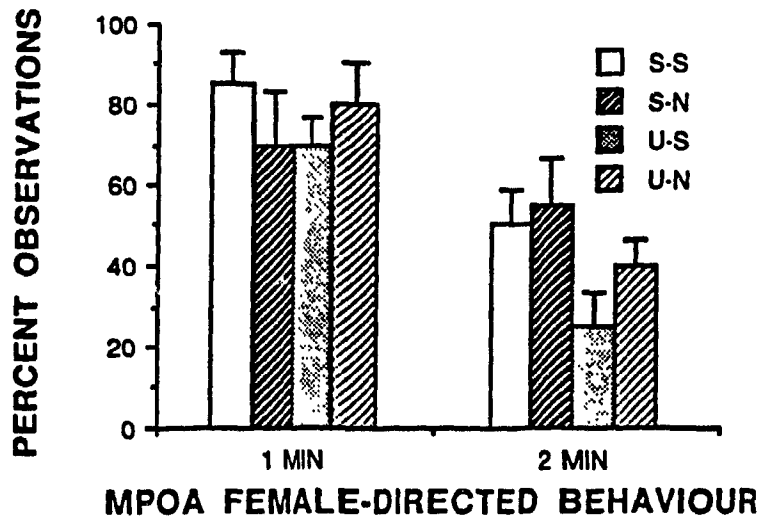
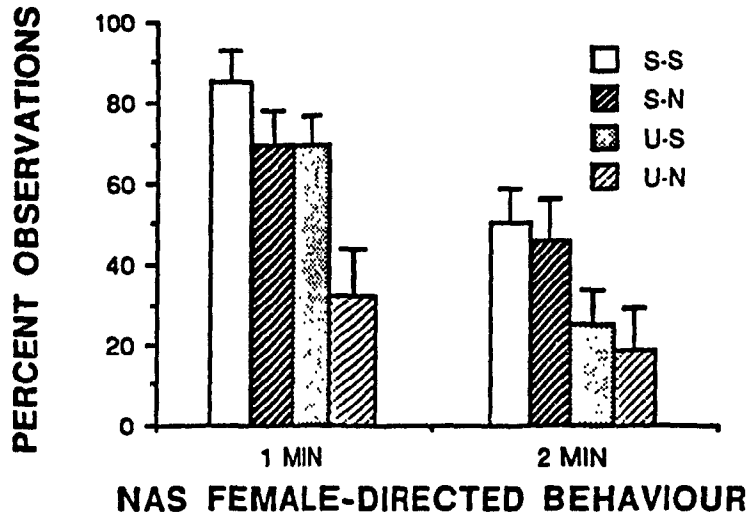
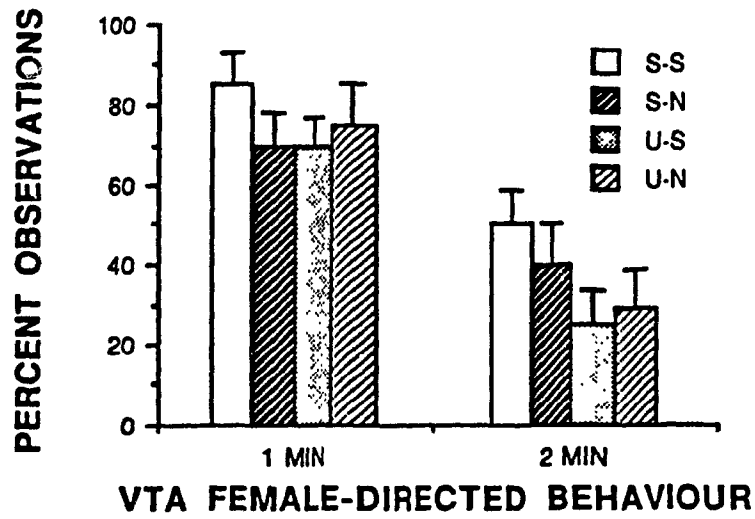


Figure 39 The mean (+ 1 S.E.M.) proportion of observations that female-directed behaviour was recorded to occur during the first and second min following the intra-cranial infusion of either saline or NBNI (3.0 μ g/0.5 μ l/side) co-administered with systemic injections of either saline or U50 (5.0 mg/kg, s.c.) during tests for sexual behaviour in Experiment 6A.



50,488H-induced reduction in the number of intromissions to precede ejaculation was not seen in animals co-administered intra-mPOA NBNI [$p > .05$].

Non-Copulatory Behaviours: The mean percent of observations (out of 60 possible) that various non-copulatory behaviours occurred during the tests for sexual behaviour is shown in Fig. 38a-c. As can be seen, U-50,488H significantly decreased female-directed behaviour [$p < .01$], bodily grooming [$p < .01$] and genital grooming [$p < .01$] while significantly increasing both lying down behaviour [$p < .01$] and body flattening [$p < .01$].

Non-Copulatory Behaviour: Effects of Intra-VTA NBNI Infusions: Intra-VTA infusions of NBNI significantly increased female-directed behaviour [$p < .01$] and significantly decreased both bodily grooming [$p < .01$] and genital grooming [$p < .01$]. When co-administered with systemic U-50,488H, intra-VTA NBNI significantly prevented the U-50,488H-induced decreases in both female-directed behaviour [$p < .01$] and bodily grooming [$p < .05$], while also preventing U-50,488H-induced increases in both lying down behaviour [$p < .01$] and body flattening [$p < .01$].

Non-Copulatory Behaviours: Effects of Intra-NAS NBNI Infusions: Intra-NAS infusions of NBNI significantly increased female-directed behaviour [$p < .01$], but did not influence other non-copulatory behaviours when administered alone. When co-administered with systemic U-50,488H, intra-NAS NBNI significantly prevented the U-50,488H-induced decreases in female-directed behaviour [$p < .01$] and the U-50,488H-induced increases in body flattening [$p < .01$].

Non-Copulatory Behaviours: Effects of Intra-mPOA NBNI Infusions: Intra-mPOA infusions of NBNI significantly increased female-directed behaviour [$p < .01$], but did not significantly alter other non-copulatory measures. When co-administered with systemic U-50,488H, intra-mPOA NBNI prevented the U-50,488H-induced decreases in both female-directed behaviour [$p < .01$] and genital grooming [$p < .01$] as well as U-50,488H-induced increases in lying down behaviour [$p < .01$].

Figure 39a-c depicts the changing occurrence of female-directed behaviour observed during the first two min of the 30-min test session. When the data from U-50,488H-injected males were compared with those from saline treated animals in a two-way, between-within ANOVA, the analysis yielded significant main effects for both Drug [$F(1,26)=7.200$, $p<.02$] and Min [$F(1,26)=21.847$, $p<.0002$], reflecting the decreased occurrence of female-directed behaviour in the second min relative to the first, and the decreases seen to follow U-50,488H injection. Simple main effects analyses confirmed that, over time, there were significant decreases in female-directed behaviours following either saline [$F(1,26)=9.028$, $p<.007$] or U-50,488H injection [$F(1,26)=13.000$, $p<.002$]. Moreover, U-50,488H was seen to significantly reduce female-directed behaviour during the second min [$F(1,52)=4.650$, $p<.04$], but not during the first [$F(1,52)=2.372$, $p=.130$], relative to saline.

When these data from saline and U-50,488H injected animals were analysed with animals administered intra-VTA NBNI, a significant main effect of Min was again found [$F(1,46)=14.618$, $p<.00001$], reflecting the overall trend for female-directed behaviours to occur more often during the first than during the second min of testing. A significant effect of Drug, however, was not obtained [$F(3,46)=2.116$, $p=.1111$]. Simple main effects analyses indicated that female-directed behaviour significantly decreased over the two min among animals from all four treatment groups [$p<.04$]. However, the different Drug treatments did not induce significantly different effects at either the first [$p=.493$] or second tested min [$p=.193$].

When the data from animals administered intra-NAS NBNI were analysed, the omnibus ANOVA yielded significant main effects of both Drug [$F(3,46)=10.089$, $p<.00001$] and Min [$F(1,46)=17.788$, $p<.0002$]. Simple main effects analyses confirmed that female-directed behaviour significantly decreased from the first to the second min following the administration of both saline [$p<.02$] and U-50,488H [$p<.003$], but not intra-NAS NBNI [$p=.075$], or the co-administration of both U-50,488H and intra-NAS

NBNI [$p=.368$]. Significant effects of Drug were seen at both the first [$p<.002$] and second min [$p<.05$], reflecting the tendency for both U-50,488H and U-50,488H plus NBNI treated animals to display less female-directed behaviour.

From the ANOVA analysing female-directed behaviour among animals administered intra-mPOA NBNI, significant main effects of Min [$F(1,42)=23.302$, $p<.00001$], but not Drug [$F(3,42)=1.963$, $p=.1342$], were obtained. Simple main effects analyses indicated that female-directed behaviour significantly decreased over time following every drug treatment [$p<.009$] except intra-mPOA NBNI plus systemic saline [$p=.434$]. Significant effects of Drug were not seen at either the first [$p=.465$] or the second min [$p=.106$] of testing.

Experiment 6B: Tests for Male Sexual Behaviour Following the Injection of U-50,488H Administered 3-4 Days Following Intra-Cranial NBNI

On one test for male sexual behaviour, 53 of the animals from Experiment 6A that had been administered intra-cranial NBNI were re-tested for sexual behaviour following the systemic injection of either saline or 5.0 mg/kg U-50,488H given s.c. At a later date, 26 days following their last intra-mPOA infusion of NBNI, six males were re-tested for sexual behaviour following the systemic administration of either 8.0 or 10.0 mg/kg U-50,488H given s.c.

Copulatory Behaviour: When animals were tested 3-4 days following intra-cranial infusions of NBNI the anatomical specificity of the receptor antagonist's effects were no longer seen as they had been when animals were tested 20 min following intra-cranial NBNI infusions in Experiment 6A. As can be seen in Figs. 40-46, previous intra-cranial NBNI infusions had no inhibitory effects among saline-injected animals, but site indiscriminately prevented systemic U-50,488H-induced decreases in the proportion of animals that mounted [$p<.05$], intromitted [$p<.05$] or ejaculated [$p<.01$] as well as the mean number of ejaculations executed during the 30-min test session [$p<.05$]. Similarly,

Figure 40 The mean (+ 1 S.E.M.) proportion of cannulated males to mount (40a), intromit (40b) and ejaculate (40c) as well as the mean (+ 1 S.E.M.) latency for animals to mount (40d), intromit (40e) and ejaculate (40f), and the mean (+ 1 S.E.M.) number of recorded ejaculations (40g) following the systemic administration of either saline or U50 (5.0 mg/kg, s.c.) among animals administered intra-cranial infusions of either saline or NBNI (3.0 µg/0.5 µl/side) 3-4 days previously during tests for sexual behaviour. Experiment 6B.

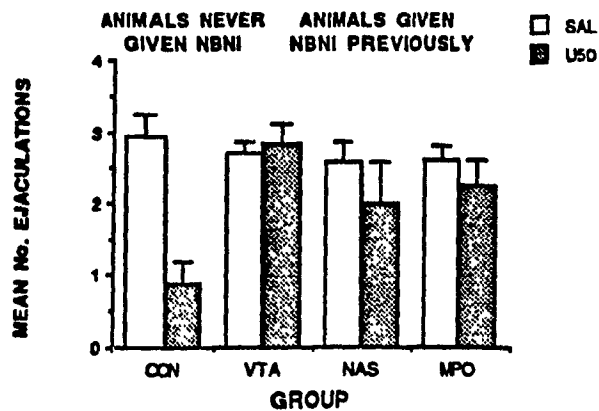
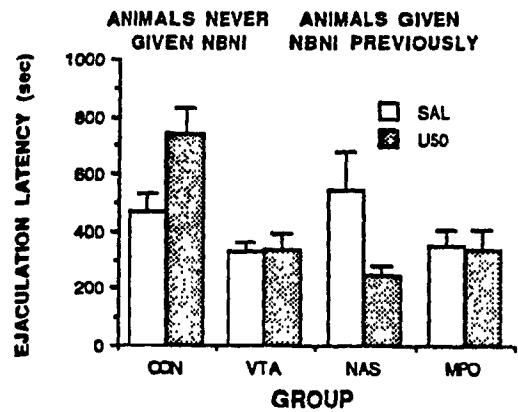
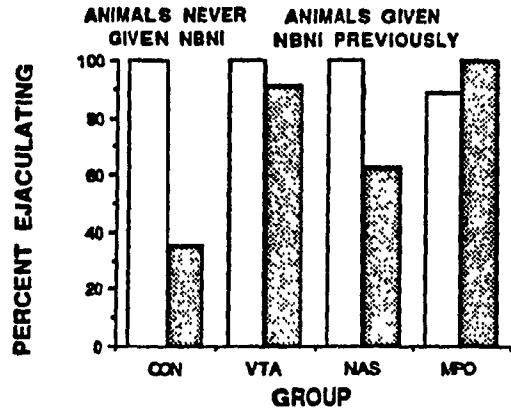
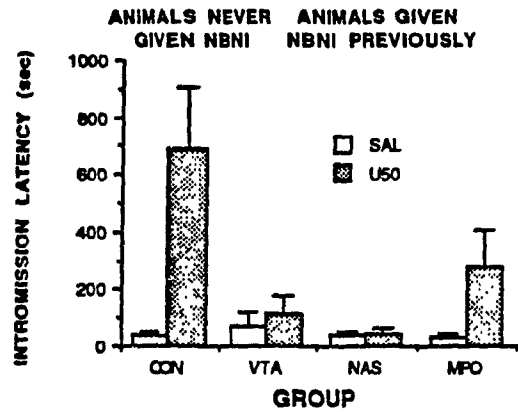
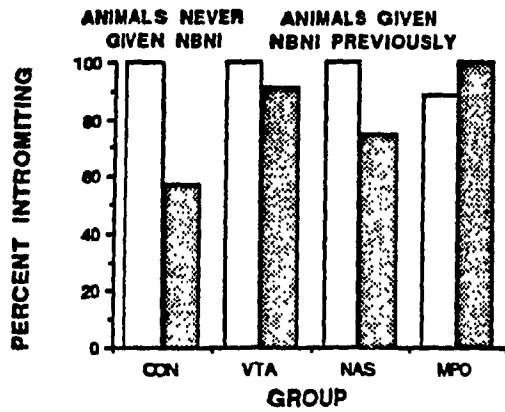
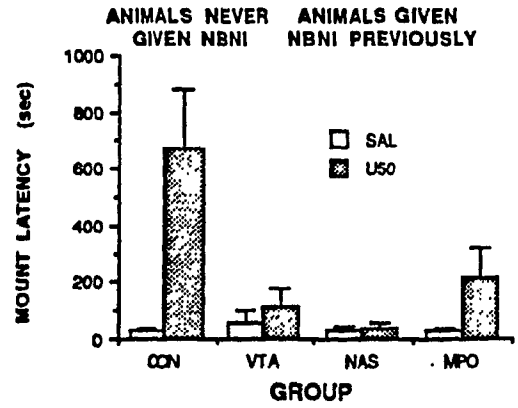
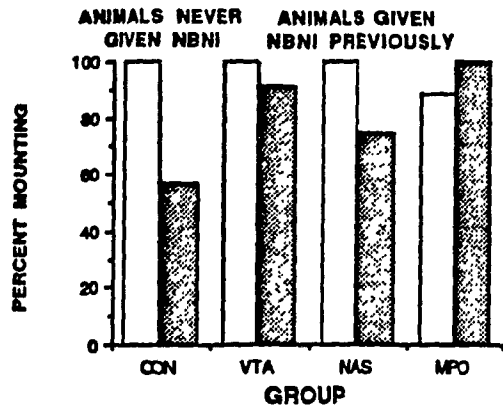


Figure 41 The mean (+ 1 S.E.M.) post-ejaculatory refractory period following the systemic administration of either saline or U50 (5.0 mg/kg, s.c.) among animals administered intra-cranial infusions of either saline or NBNI (3.0 μ g/0.5 μ l/side) 3-4 days previously during tests for sexual behaviour. Experiment 6B.

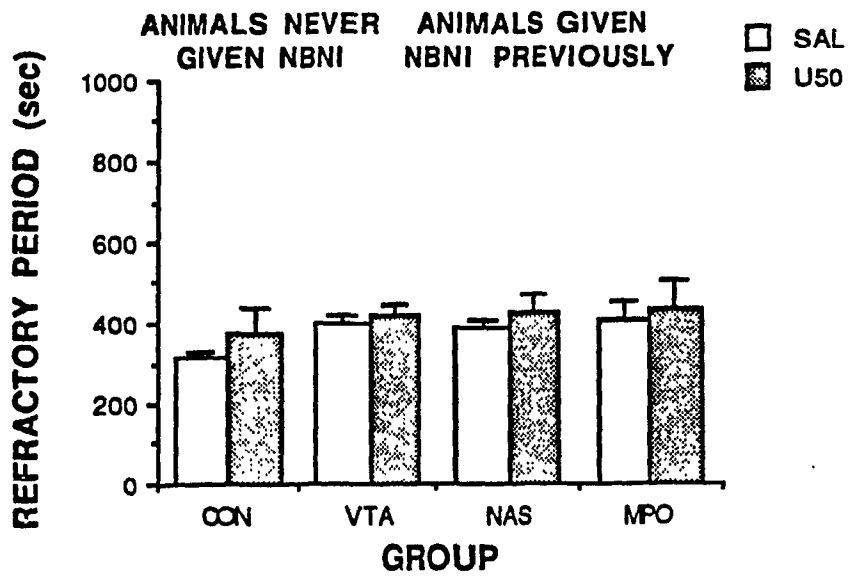


Figure 42 The mean (+ 1 S.E.M.) number of intromissions to precede the first ejaculation following the systemic administration of either saline or U50 (5.0 mg/kg, s.c.) among animals administered intra-cranial infusions of either saline or NBNI (3.0 µg/0.5 µl/side) 3-4 days previously during tests for sexual behaviour. Experiment 6B.

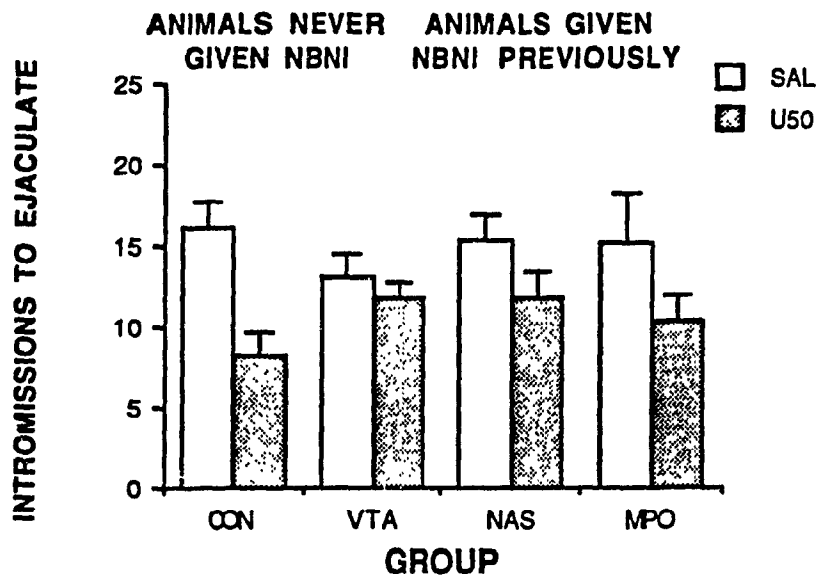


Figure 43 The mean (+ 1 S.E.M.) inter-intromission interval prior to the first ejaculation following the systemic administration of either saline or U50 (5.0 mg/kg s.c.) among animals administered intra-cranial infusions of either saline or NBNI (3.0 μ g/0.5 μ l/side) 3-4 days previously during tests for sexual behaviour. Experiment 6B.

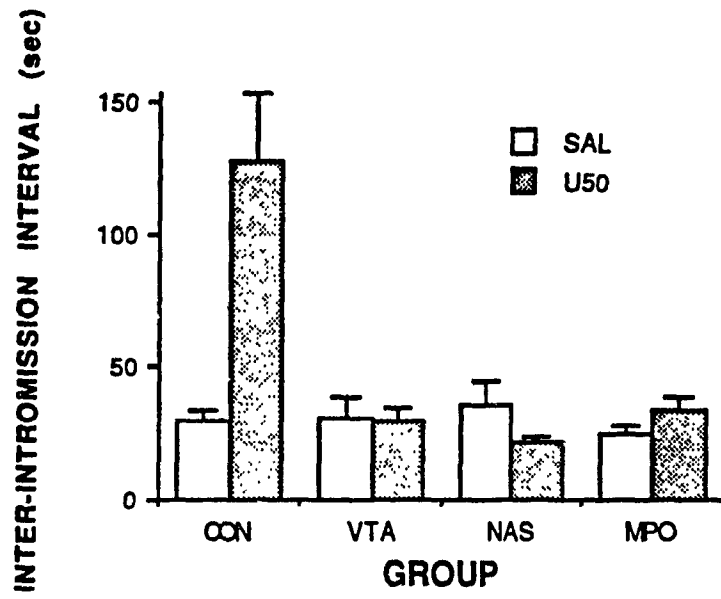


Figure 44 The mean (+ 1 S.E.M.) proportion of observations that non-copulatory behaviours were recorded to occur following the systemic administration of either saline or U50 (5.0 mg/kg s.c.) among animals administered intracranial infusions of either saline or NBNI 3-4 days previously during tests for sexual behaviour. Experiment 6B. (FDB: female-directed behaviour; L: laying down; LL: body flattening; Sn: sniffing; Gr: body grooming; GG: genital grooming; W: walking; R: rearing.

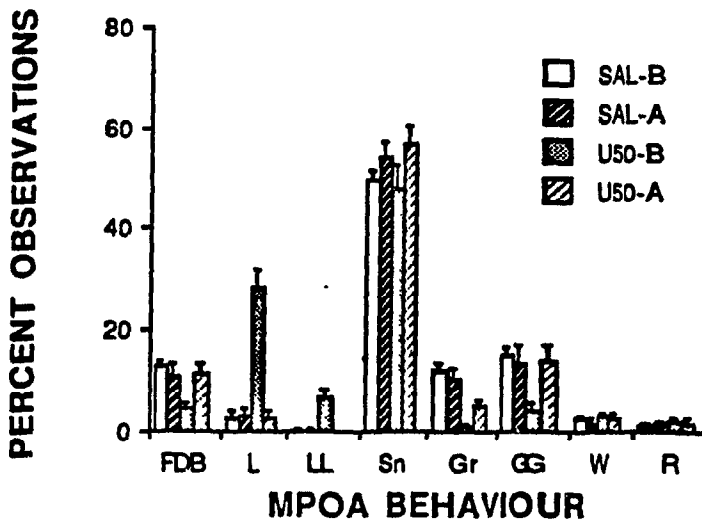
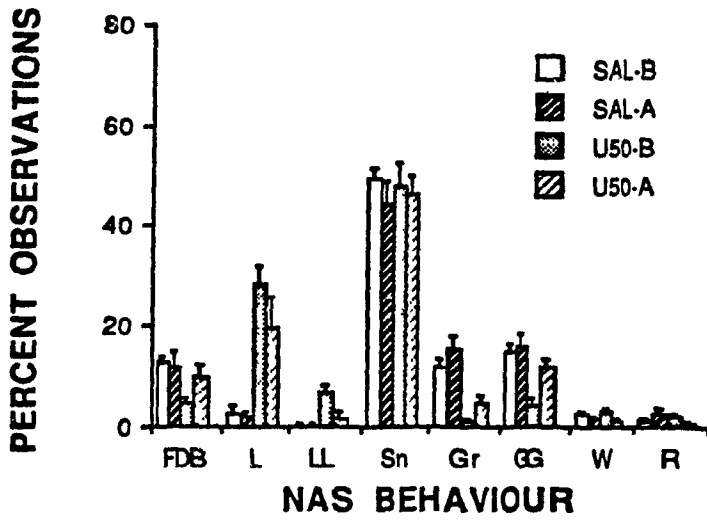
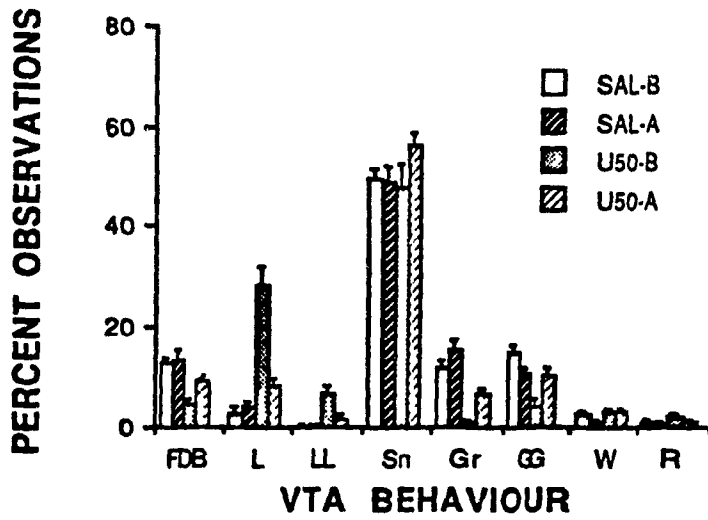
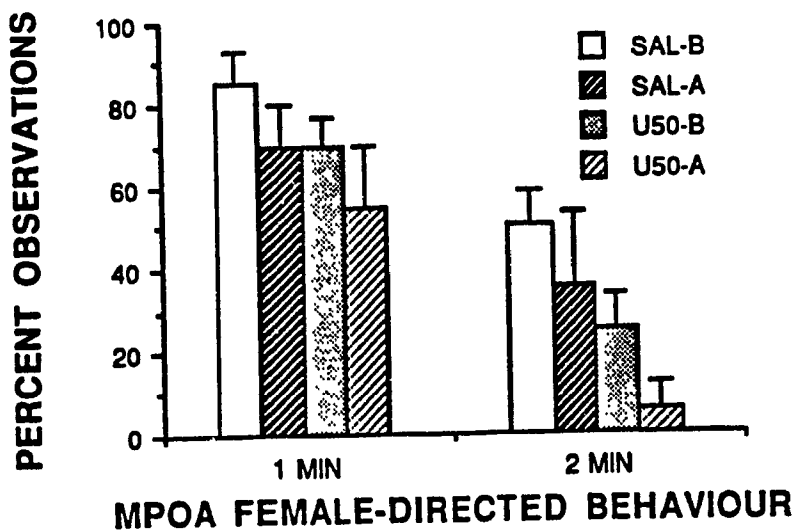
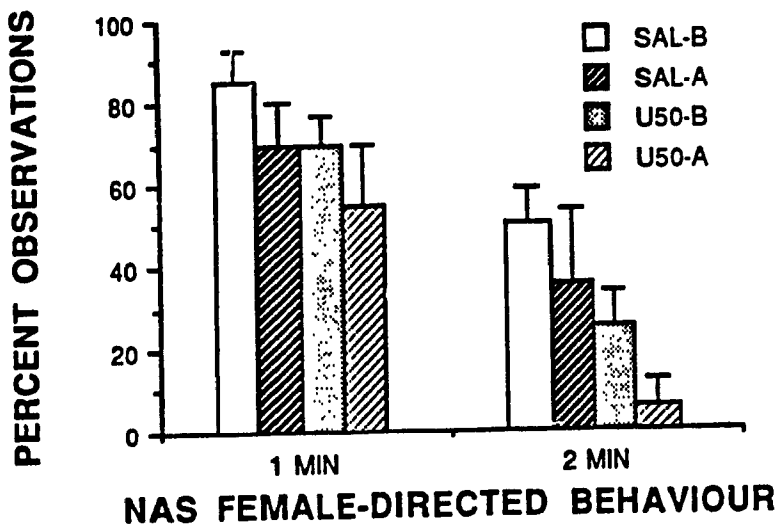
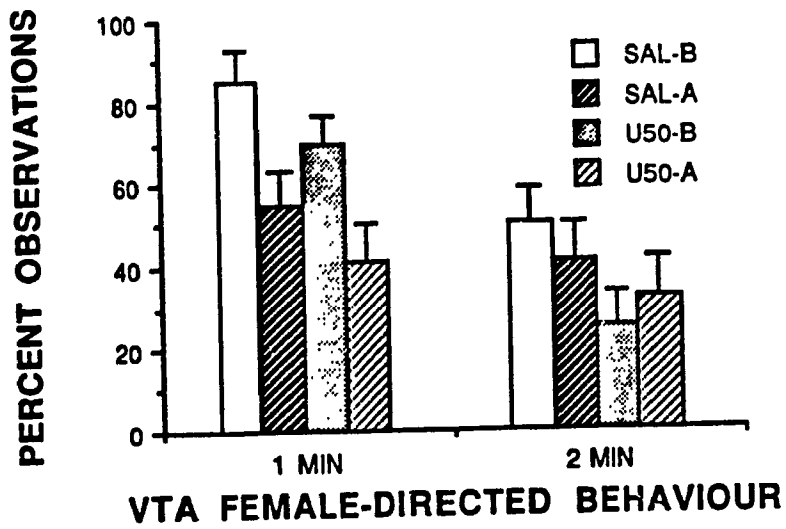


Figure 45 The mean (+ 1 S.E.M.) proportion of observations that female-directed behaviour was recorded to occur during the first and second min following the systemic administration of either saline or U50 (5.0 mg/kg s.c.) among animals administered intra-cranial infusions of either saline or NBNI (3.0 μ g/0.5 μ l/side) 3-4 days previously during tests for sexual behaviour. Experiment 6B.



previous intra-cranial infusions of NBNI into any of the three tested sites prevented U-50,488H-induced increases in mount [$p < .01$], intromission [$p < .01$] and ejaculation latencies [$p < .01$] as well as in the III [$p < .01$].

As had been seen in animals acutely treated with intracranial NBNI in Experiment 6A, the correlation between the III and the post-ejaculatory refractory period remained perturbed in animals had been previously administered either intra-VTA [$r = -0.130$, $p > .20$] or intra-NAS [$r = +0.547$, $p > .20$] infusions of NBNI. Interestingly, animals that had been previously administered intra-mPOA infusions of NBNI displayed some recovery of this relation [$r = +0.677$, $p < .05$, 1 tail test; $p > .05$, 2 tail test]. In contrast, previous NBNI treatment remained ineffective at re-establishing this relation in all three groups of U-50,488H-injected males [VTA: $r = +0.473$, $p > .10$, NAS: $r = -0.0478$, $p > .20$; mPOA: $r = -0.165$, $p > .20$].

Copulatory Behaviour: 26 Days Following Intra-mPOA NBNI: The apparent long-lasting ability of NBNI to antagonize many disruptive influences of U-50,488H was further explored in six males previously administered intra-mPOA NBNI. All of these animals had been previously administered NBNI on three separate occasions, the most recent of which had been 26 days earlier. At this time, these males were tested for sexual behaviour following the injection of either 8.0 or 10.0 mg/kg U-50,488H given s.c., a dose range that had been previously seen in Experiments 1A and 2B to severely diminish all behavioural activity, and all but completely abolish copulatory activity. Not surprisingly, one of the males failed to display a single mount throughout the entire 30-min test session. More extraordinary, however, was that the remaining five males mounted in 56.6 ± 19.3 s, intromitted in 58.6 ± 19.9 s, ejaculated in 348.6 ± 57.4 s, and displayed exactly 3.0 ± 0.0 ejaculations each. Remarkably, these figures compare quite favourably to saline treated control males, none are significantly different [$p > .05$].

Non-Copulatory Behaviours: VTA Treated Males: Figure 44a-c illustrates the mean percent of observations (out of 60 possible) that various non-copulatory behaviours

occurred during the tests for sexual behaviour. As can be seen in Fig. 44a, previous infusions of intra-VTA NBNI significantly decreased genital grooming [$p < .05$], relative to animals that had not been previously administered NBNI, but other non-copulatory behaviours were unchanged by this treatment. When systemic U-50,488H was administered to animals previously administered intra-VTA infusions of NBNI, the receptor antagonist significantly prevented the U-50,488H-induced decreases in female-directed behaviour [$p < .05$], bodily grooming [$p < .01$] and genital grooming [$p < .05$] as well as U-50,488H-induced increases in both lying down [$p < .01$] and body flattening [$p < .01$].

Non-Copulatory Behaviours: NAS Treated Males: Figure 44b depicts the mean percent of observations (out of 60 possible) that various non-copulatory behaviours occurred among animals administered intra-NAS drug infusions. As can be seen, previous intra-NAS infusions of NBNI did not alter any of the recorded non-copulatory behaviours among saline-injected animals. Conversely, among U-50,488H-injected animals, previous infusions of intra-NAS NBNI significantly prevented the U-50,488H-induced decreases in both female-directed behaviour [$p < .05$] and genital grooming [$p < .01$] as well as blocking the U-50,488H-induced increases in body flattening [$p < .01$].

Non-Copulatory Behaviours: mPOA Treated Males: Figure 44c shows the mean percent of observations (out of 50 possible) that various non-copulatory behaviours occurred among animals administered intra-mPOA NBNI. As can be seen, previous intra-mPOA infusions of NBNI to saline-injected animals did not significantly alter any of the non-copulatory behaviours. Among U-50,488H-injected animals however, previous intra-mPOA NBNI significantly attenuated the U-50,488H-induced decreases in female-directed behaviour [$p < .05$], bodily grooming [$p < .05$] and genital grooming [$p < .01$] as well as the U-50,488H-induced increases in both lying down behaviour [$p < .01$] and body flattening [$p < .01$].

Figure 45a-c depicts the changing occurrence of female-directed behaviour during the first two min of testing. The omnibus ANOVA analysing female-directed behaviour

Figure 46 The mean (± 1 S.E.M.) 30-min locomotor activity scores among animals intra-cranially infused with NBNI (3.0 μ g/0.5 μ l/side) 3-10 days previously tested following acute U50 injections (5.0 mg/kg, s.c.) co-administered either with intra-cranial saline (46a) or NBNI (3.0 μ g/0.5 μ l/side) (46b) in Experiment 6C.

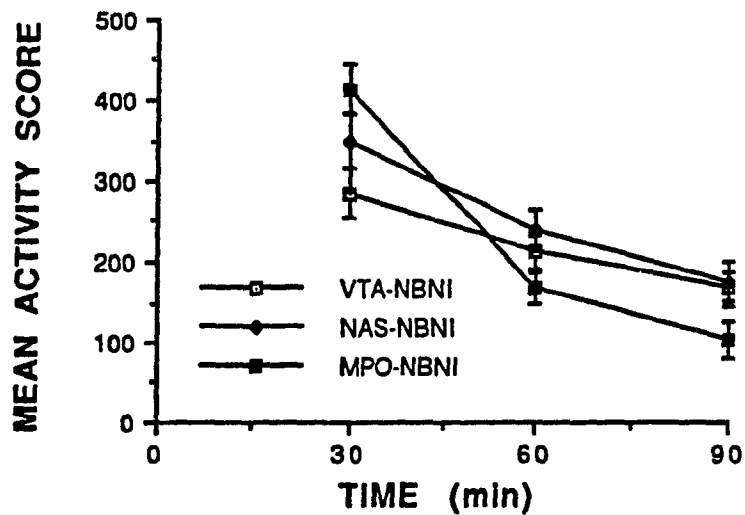
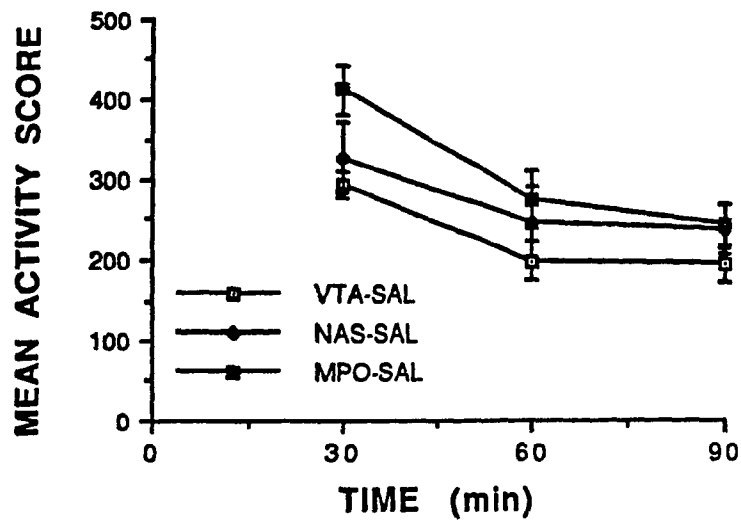
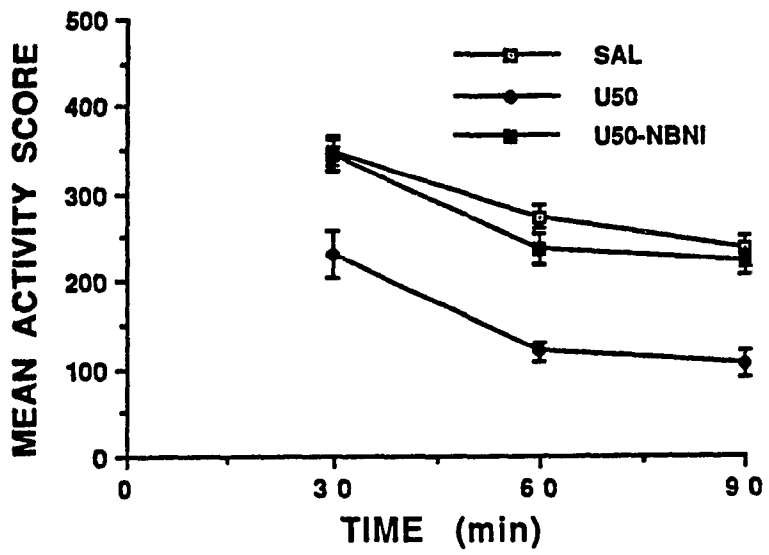


Figure 47 The mean (± 1 S.E.M.) 30-min locomotor activity scores among animals systemically injected with either saline or U50 (5.0 mg/kg, s.c.) following previous intra-cranial infusions of either saline or NBNI (3.0 μ g/0.5 μ l/side) 3-10 days previously. Experiment 6C.



among males previously administered intra-VTA NBNI yielded significant main effects of both Drug [$F(3,46)=4.613$, $p<.007$] and Min [$F(1,46)=16.910$, $p<.0003$]. Simple main effects analyses indicated that female-directed behaviour significantly decreased over time following either saline [$p<.004$] or U-50,488H administration [$p<.002$]. In contrast, previous intra-VTA infusions of NBNI decreased the female-directed behaviour that was seen during the first min such that after either saline [$p=.301$] or U-50,488H injections [$p=.489$] there were no overall changes during the first two min. Further reflecting the inhibitory effects of NBNI, there were significant effects of Drug during the first [$p<.005$], but not the second min [$p=.203$].

Among animals previously administered intra-NAS NBNI, significant effects of both Drug [$F(3,39)=4.557$, $p<.008$] and Min [$F(1,39)=30.151$, $p<.00001$] were seen. Simple main effects analyses indicated that these differences reflected significant decreases over time in all four drug treatment groups [$p\leq.05$]. Moreover, Drug treatments were seen to alter the occurrence of female-directed behaviours during the second [$p<.03$], but not the first min [$p=.255$], reflecting inhibitory effects of U-50,488H that were not attenuated by previous NBNI.

The omnibus ANOVA examining the data from animals previously administered intra-mPOA NBNI yielded a main effect for Min [$F(1,41)=22.224$, $p<.00001$], but not for Drug [$F(3,41)=1.867$, $p=.1503$]. Simple main effects analyses indicated that female-directed behaviour occurred significantly less often during the second min following every drug treatment [$p<.04$] except intra-mPOA NBNI plus systemic saline [$p=.100$]. There was not a significant effect of Drug at either the first [$p=.360$] or the second min [$p=.391$].

Experiment 6C: Tests for Locomotor Activity Following the Injection of U-50,488H Administered 2-6 Days Following Intra-Cranial NBNI

In two counterbalanced tests for locomotor activity, 34 of the males from Experiment 6A that had been administered intra-cranial NBNI were monitored in photocell

boxes following the co-administration of 5.0 mg/kg U-50,488H given s.c. plus intracranial infusions of either saline or NBNI (3.0 μ g/0.5 μ l/side).

Locomotor Activity: The statistical analysis of the 30-min activity scores indicated that, overall there were no significant differences between the three Brain Sites [F(2,31)=1.284, p=.2912]. Conversely, clear Brain Site x Time [F(4,62)=10.298, p<.0001] and Brain Site x Drug x Time [F(4,62)=3.574, p<.02] interactions were obtained. As illustrated in Fig. 46a,b, these interactions reflected the tendency for saline treated animals previously administered intra-mPOA NBNI to be more active than other animals throughout the entire 90-min session, but for animals acutely administered intra-mPOA NBNI to be less active during the final two 30-min intervals, relative to animals administered intra-VTA and intra-NAS injections [p<.01].

Also apparent was a weak effect of Drug Treatment [F(1,31)=3.757, p=.0617] reflecting a non-significant tendency for animals administered intra-cranial NBNI to be less active than animals administered intra-cranial saline. As can be seen in Fig. 46a,b, this weak main effect of Drug resulted from a significant Drug x Time interaction [F(2,62)=11.964, p<.0001], and reflected the greater activity of intra-cranial saline animals during the final two 30-min intervals of testing, relative to intra-cranial NBNI treated animals [p<.05]. It would appear that U-50,488H-injected animals previously administered intra-cranial NBNI are more active if they are not simultaneously co-administered an additional acute NBNI treatment.

The omnibus ANOVA also yielded a significant main effect for Time [F(2,62)=152.181, p<.00001], reflecting the general trend for all animals to become less active from the beginning to the end of the 90-min session.

The possibility of a long-lasting *k* receptor antagonism by NBNI suggested in Experiment 6B became apparent again when the activity scores from Experiment's 6C's U-50,488H-injected animals acutely receiving intra-cranial saline, but previously administered NBNI, were compared to the U-50,488H-injected animals from Experiment 2B that had

..ever been treated with NBNI. As can be seen in Fig. 47, the previous intra-cranial administration of NBNI antagonized the locomotor activity inhibitory effects of systemic U-50,488H injection. As indicated by the statistical analysis, the significant effect of Drug Treatment [$F(2,63)=17.287, p<.00009$] reflected an inhibitory effect of U-50,488H, relative to either saline treated control animals [$p<.01$] or U-50,488H-injected animals previously administered intra-cranial NBNI [$p<.01$]. These findings corroborate the results of Experiment 6B indicating that previously administered intra-cranial NBNI remains capable of antagonizing the inhibitory effects of U-50,488H for at least four days.

Discussion

Experiments 6A, 6B and 6C demonstrate that the effects of systemic U-50,488H are mediated by central *k* opioid receptors. As previously seen in Experiments 1A, 1B, 2A, 2B, 4A and 4B, and replicated in Experiment 6A and 6C, the systemic administration of the *k* agonist U-50,488H diminished both locomotor activity and a broad spectrum of measures commonly recorded to reflect both appetitive and consummatory dimensions of male sexual behaviour. More importantly, however, selective aspects of these U-50,488H-induced attenuations could be prevented by the application of the *k* opioid receptor antagonist NBNI to mesolimbic and incertohypothalamic brain sites.

Copulatory Behaviour: The mesolimbic DA pathway is widely accepted to play a focal role in appetitive dimensions of various behaviours (see Everitt, 1990). The present experiments raise the possibility that *k* opioid receptors situated at both the cell body and terminal regions of this mesolimbic pathway also influence appetitive motivation. In tests for sexual behaviour, acute infusions of NBNI into the NAS - a mesolimbic terminal field containing *k* receptors that when stimulated, are known to inhibit DA release, and hence, when blocked, may facilitate stimulated DA release - both increased the frequency of spontaneous female-directed behaviour - a behaviour considered indicative of sexual interest in the female - and prevented the U-50,488H-induced abbreviation of its display.

Further compatible with central *k* opioid receptors having a regulatory role in appetitive behaviour, intra-NAS NBNI also prevented the systemic U-50,488H-induced prolongation of mount and intromission latencies. These findings suggest that endogenous NAS *k* receptor ligands tonically suppress the central motivational states associated with the initiation of sexual behaviour. This *k* agonist-induced suppression might be important for regulating the pacing of sexual activity. In contrast, consummatory dimensions of male sexual behaviour seem not to be determined in this brain region; intra-NAS NBNI did not prevent either the U-50,488H-induced reductions in the proportion of males that mounted, intromitted and ejaculated, nor the U-50,488H-induced increases in ejaculation latencies.

As had been suggested from small numbers of animals in Experiments 1A, 1B and 4A, and was seen more clearly in the larger sample in Experiment 6A, systemic U-50,488H both decreased the number of intromissions to precede ejaculation and increased the III. More importantly, intra-NAS infusions of NBNI antagonized these effects of U-50,488H. To the extent that the III is believed to reflect the re-arousability of a male following an intromission, these findings suggest that *k* receptor stimulation might alter arousability components induced by intromissions. Interestingly, U-50,488H was also seen to perturb the positive correlation between the III and the post-ejaculatory refractory period that is typically seen in control animals. Co-administration of intra-NAS NBNI treatment did not restore the correlation, and NBNI alone was equally disruptive. These findings might reflect the importance of *k* receptors and their endogenous ligands for regulating the duration of refractory periods that follow intromissions and ejaculations. The mechanisms through which U-50,488H might be inducing these changes in numbers of intromissions, and the III, cannot be unequivocally determined from the present experiments, but the remarkable similarity between the behaviours produced by U-50,488H agonists and both 5-HT_{1a} autoreceptor agonists (Hillegaart, 1991) and 5-HT₂ postsynaptic antagonism (Mendelson & Gorzalka, 1985), suggests that a *k* opioid, 5-HT interaction might be relevant. Both treatments decrease the number of mounts and

intromissions to precede ejaculation, both decrease body temperature, both decrease motor activity, and both induce body flattening.

Surprisingly, when NBNI was administered to NAS cannulated males not receiving accompanying U-50,488H injections, significant increases in copulation latencies became apparent in half of the tested animals. Moreover, in half of the animals co-administered both the putative *k* receptor agonist and antagonist, intra-NAS NBNI did not attenuate the U-50,488H-induced decreased mean number of ejaculations.

The most likely explanation for this paradoxical NBNI-induced behavioural inhibition comes from recent suggestions that the putative *k* antagonist also has some transitory - less than four hour - antagonist properties at μ receptors (Birch, Hayes, Sheehan & Tyers, 1987; Magnan & Tiberi, 1989; Endoh, Koike, Matsuura, & Nagase, 1990; Horan, Taylor, Yamamura, & Porreca, 1991). The systemic administration of μ receptor antagonists is known to decrease locomotor activity (Experiment 5B, this thesis; Amir et al., 1979; Walker et al., 1981; Ukei & Kameyama, 1985), to produce conditioned place aversions (Bals-Kubik et al., 1989), and to inhibit male sexual behaviour (Szechtman et al., 1979, 1981; Meller et al., 1980; McConnell et al., 1981; Sachs et al., 1981; Abbott et al., 1984; Lieblich et al., 1985a,b; Miller & Baum, 1987; Peters et al., 1988; Arcila et al., 1990; Leyton & Stewart, 1990). These effects from systemically administered μ antagonists most probably result from actions at more than one site, but the NAS is one likely region. The discrete infusion of μ agonists into the NAS is known to increase locomotor activity (Pert & Sivit, 1977; Kalivas, Widerlov, Stanely, Breese & Prange, 1983; Vezina, Kalivas & Stewart, 1986), to produce conditioned place preferences (Van der Kooy, Mucha, O'Shaughnessy & Bucenieks, 1983), and to increase male sexual behaviour (Band & Hull, 1988, 1990). Given the apparent stimulatory and rewarding consequences of NAS μ opioid receptor activation, it seems plausible to suggest that the present inhibitory effects of NAS NBNI result from antagonist actions on local μ receptors.

The sexual behaviour data from all animals administered intra-VTA NBNI suggested that this treatment had comparatively few influences on male sexual activity. Intra-VTA NBNI alone increased spontaneous female-directed behaviour and perturbed the correlation between the III and the post-ejaculatory refractory period. When co-administered with U-50,488H, intra-VTA NBNI antagonized the k agonist's decrease in both female-directed behaviour and the mean number of ejaculations, as well as prevented the U-50,488H-induced increase in III. However, similar to that seen in the NAS, mixed effects of intra-VTA NBNI on the measured motivation of males to copulate with estrous females were also identified. Among half of these males, intra-VTA NBNI alone had no detectable effect. Among the other half, however, NBNI increased the latencies to mount and intromit. Similarly, the U-50,488H-induced increases in mount and intromission latencies were antagonized in only half of the animals, and therefore this expected effect was masked when the data from all VTA treated animals were analysed together.

The increased copulation latencies in some intra-VTA NBNI treated animals are compatible with the suggested μ antagonist actions of the nominal k receptor antagonist. Similar to that reported following μ receptor agonist infusions to the NAS - a mesolimbic terminal field - administrations of μ agonists to the VTA - the mesolimbic cell body region - increase locomotor activity (Kelley, Stinus & Iversen, 1980; Vezina & Stewart, 1984; Kalivas, 1985; Vezina et al., 1986), induce conditioned place preferences (Bozarth & Wise, 1983; Bozarth, 1987; Vezina & Stewart, 1987) and increase male sexual behaviour (Mitchell & Stewart, 1987; 1990a), whereas μ antagonist VTA infusions decrease feeding (Segall & Margules, 1989). These excitatory, appetitive, and rewarding effects of μ agonists, and inhibitory effects of μ antagonists in the VTA suggest that VTA μ receptor antagonism from NBNI might prevent the endogenous ligands for these receptors from inducing their activating influences on sexual behaviour.

The reason why some animals were more responsive to the μ , and others to the k receptor actions of NBNI, is not clear. Histological examinations did not reveal identifiable

cannulae site placement differences. An alternative explanation refers to the currently undetermined half-life of the hypothesized μ receptor antagonism of NBNI. Since animals were tested 20 min after their central infusion of NBNI, it seems plausible to suggest that behaviourally inhibitory effects on copulation latencies of NBNI reflect initial antagonist actions at μ receptors, whereas later excitatory effects on ejaculation latencies reflect both degraded affinity for μ receptors and accompanying preferential affinity for k receptors. A critical evaluation of this suggestion must await the full characterization of NBNI's temporal changes in receptor affinity.

Among animals administered intra-mPOA drug infusions, the hypothesized μ receptor-mediated inhibitory effect of NBNI were not seen - this difference between the mPOA and mesolimbic sites might reflect the relatively sparse levels of μ receptors in the mPOA compared to higher levels in both the VTA and NAS (Mansour et al., 1987). Intra-mPOA infusions of NBNI did, however, attenuate many of the putative k receptor-mediated effects of systemic U-50,488H injections. In contrast to the reversal of only U-50,488H-induced appetitive decrements seen in animals administered NBNI to either the VTA or the NAS, intra-mPOA NBNI antagonized U-50,488H-induced effects considered to reflect both appetitive and consummatory dimensions of male sexual behaviour. Unlike that seen following NBNI infusions into the mesolimbic regions, intra-mPOA NBNI prevented the systemic U-50,488H-induced decrease in the proportion of males that mounted, intromitted and ejaculated, and also antagonized the U-50,488H-induced increase in ejaculation latencies. More similar to infusions of NBNI into the mesolimbic sites, intra-mPOA NBNI partially attenuated the U-50,488H-induced increases in copulation latencies, fully prevented the U-50,488H-induced abbreviations of female-directed behaviour, and when administered alone, also increased female-directed behaviour. Finally, and also seen following infusions of NBNI into both mesolimbic sites, intra-mPOA NBNI antagonized the U-50,488H-induced decrease in the number of intromissions that precede the first

ejaculation, and perturbed the correlation between the III and the post-ejaculatory refractory period.

The striking contrast between the unexpectedly minor role of the mPOA in male sexual behaviour suggested in Experiment 5A and the clearly major role, both more commonly ascribed to it and seen in Experiment 6A, was surprising. These findings would suggest that *k* receptor stimulation in the mPOA does not work pronounced behavioural changes in isolation from other brain regions. On the contrary, they suggest that the mPOA mechanisms engaged by *k* agonists work in concert with mesolimbic systems to regulate sexual behaviour in the male rat. It would appear that a male rat having *k* receptors stimulated in the mPOA plus either in the VTA or the NAS, is severely hampered in its expression of copulatory behaviour. Conversely, a male rat pharmacologically protected from *k* agonist activity in the mPOA while having *k* receptor stimulation in the mesolimbic system is slow to initiate copulatory behaviour, but shows minimal disturbances should the copulatory threshold be crossed. Such an interpretation is compatible with Experiment 5A where animals were administered U-50,488H intracranially.

These results suggest that the inhibitory effects induced by mPOA *k* receptor stimulation interact with limbic structures to control both the initiation and pacing of sexual behaviour in the male rat. The neuropharmacology of these inter-connecting pathways is not well understood (Simerly, Gorski & Swanson, 1986), but anatomical connections to the MPN - the specific mPOA subnucleus involved in regulating male sexual behaviour - from both the VTA and NAS (Simerly & Swanson, 1986), as well as efferents from the mPOA to the VTA (Conrad & Pfaff, 1976), are established, indicating that communication between these systems during sexual behaviour is certainly possible. Moreover, Mogenson and his colleagues suggest that the mPOA communicates with the limbic system to integrate sensory input and internal physiological states to coordinate appropriate behavioural responses (Mogenson et al., 1980a; Swanson et al., 1984).

Interestingly, in addition to transitory μ antagonist activity, NBNI has unusually long-lasting potency at k receptors; four days after systemic administration (Endoh et al., 1990) and 28 days after i.c.v. infusion (Horan et al., 1991), NBNI continues to antagonize k opioid agonist-induced analgesia. It seems likely that this characteristic of NBNI accounts for the unexpected long-lasting - up to 26 days - influences of the opioid antagonist observed in the present study (Experiments 6B and 6C).

In Experiment 6B, animals were tested for sexual behaviour three or four days following their central NBNI infusion. At this time, the males were systemically injected with either saline or U-50,488H. Saline-injected animals previously administered NBNI did not differ from saline treated control animals on any measure of sexual behaviour, supporting the suggestion that the inhibitory effects of NBNI observed in Experiment 6A reflected transitory μ receptor antagonist activity. In contrast, when animals previously administered NBNI were tested 20 min following injection of U-50,488H, significant antagonism of the k agonist's inhibitory effects was obtained, undistinguished by the site of previous central infusions of NBNI. In VTA, NAS and mPOA treated animals, previous infusions of NBNI prevented the U-50,488H-induced decrease in the proportion of males that mounted, intromitted or ejaculated. Similarly, in animals previously administered NBNI, U-50,488H neither increased latencies to either copulation or ejaculation, nor decreased either the mean number of ejaculations during the test session or the number of intromissions to precede the first ejaculation. In short, animals previously administered intra-cranial infusions of NBNI displayed a sexual behaviour profile that was not different from control males, and moreover, systemic U-50,488H failed to alter this activity. Interestingly, the one exception to this apparently benign, and U-50,488H agonist-protective, activity of NBNI, was the continued perturbed correlation between the III and the post-ejaculatory refractory period. Neither saline nor U-50,488H-injected animals previously treated with NBNI displayed significant correlations between these two variables. The continued disruption of this relation by either agonist or antagonist activity

at *k* opioid receptors further suggests the importance of these receptors, and their endogenous ligands, in the regulation of refractory periods.

The long-lasting ability of NBNI to antagonize the inhibitory effects of U-50,488H on male sexual behaviour was further explored in six mPOA cannulated males 26 days after infusions of NBNI. Remarkably, in five of these animals, the previous infusion of NBNI continued to exert a complete antagonism of U-50,488H's inhibitory effects. Moreover, these animals were tested with high doses of U-50,488H, either 8.0 or 10.0 mg/kg given s.c., that were found in Experiments 1B and 2B to severely decrease all behavioural activity. These findings suggest that over an astonishing period of time, NBNI maintains strikingly potent antagonist properties at *k* opioid receptors.

As described above, when animals were tested 20 min following central infusions of NBNI, site-dependent effects of NBNI were seen. In contrast, the behavioural demarcation based on original infusion site was lost when animals were tested either three to four or 26 days after NBNI treatment. The current experiments do not explicitly address why this might be so, but given the opioid antagonist's apparent resistance to metabolic degradation - as indicated by its long-lasting effects - it is conceivable that unbound NBNI might, over time, diffuse throughout the brain to bind to *k* receptors distant from the original site of infusion. Plausibly, three days might be sufficient time for NBNI to spread to, among other regions, the VTA, NAS and mPOA regardless of its site of origin. These new regions might include the peripheral nervous system, however, the high doses of systemic NBNI that are required to elicit centrally mediated effects suggests that NBNI does not readily cross the blood-brain barrier (P. S. Portoghese, personal communication).

Non-Copulatory Behaviours: Central infusions of NBNI were also observed to antagonize various non-copulatory effects of U-50,488H. When tested either 20 min or three to four days following central infusions, intra-VTA NBNI antagonized both U-50,488H-induced increases in lying and body flattening and U-50,488H-induced decreases in bodily grooming. The latter finding contrasts with the failure of systemic naloxone, in

Experiment 4A, to antagonize U-50,488H-induced decreases in bodily grooming, and supports the earlier suggestion that *k* opioid receptors are indeed the substrate on which U-50,488H acts to decrease bodily grooming. Moreover, it is compatible with previous suggestions that decreased bodily grooming results from decreased D1 receptor stimulation (Greidanus et al., 1989). Perhaps undetermined characteristics of naloxone might make it an inadequate antagonist to prevent this effect of U-50,488H. Notably, 20 min, but not three to four days following intra-VTA infusions, NBNI itself decreased bodily grooming. This NBNI-induced decreased grooming suggests that VTA μ receptor blockade, obtained from either naloxone or this transient property of NBNI, is incompatible with antagonizing U-50,488H-induced decreases in bodily grooming. Possibly, VTA μ receptor blockade itself might decrease bodily grooming.

Intra-NAS infusions of NBNI at both test times also antagonized U-50,488H-induced increases in body flattening. In contrast, intra-NAS infusions of NBNI prevented U-50,488H-induced decreases in genital grooming only when NBNI infusions administered three to four days earlier.

Intra-mPOA infusions of NBNI at either test time prevented both U-50,488H-induced increases in either lying down or body flattening and U-50,488H-induced decreases in genital grooming. Conversely, intra-mPOA infusions of NBNI prevented U-50,488H-induced bodily grooming only when administered three to four days earlier.

Both long-lasting antagonism of *k* opioid receptors and transient antagonism of μ opioid receptors by intra-cranial NBNI were suggested again by the locomotor activity data from Experiment 6C. U-50,488H-injected males tested two to six days after the central infusion of NBNI were more active than U-50,488H-injected animals that had not been previously administered NBNI. Moreover, U-50,488H-injected animals tested immediately following either intra-cranial NBNI were less active than U-50,488H-injected males tested two to six days after NBNI treatment. These findings suggest that U-50,488H-induced decreases in locomotor activity result from actions at *k* opioid receptors.

Conversely, the transient inhibition of locomotor activity elicited by acute central infusions of NBNI most likely reflects the transient antagonism of μ opioid receptors. As was observed in Experiment 5B, and has been reported elsewhere (Amir et al., 1979; Walker et al., 1981; Ueki & Kameyama, 1985), locomotor activity is decreased by μ receptor opioid antagonists.

In summary, NBNI would appear to have both transient μ receptor antagonist and strikingly long-lasting k receptor antagonist properties. These characteristics of NBNI have been exploited in Experiments 6A, 6B and 6C to indicate that both inhibitions of μ opioid receptors and stimulation of k opioid receptors in either the VTA or NAS can decrease both appetitive dimensions of male sexual behaviour and locomotor activity. Conversely, the construed NBNI-induced μ receptor antagonism in the mPOA would appear to decrease locomotor activity but not male sexual behaviour; the latter finding is most probably attributable to the very low levels of μ opioid receptors present in the mPOA (Mansour et al., 1987); the former is not understood at this time. On the other hand, mPOA k receptor stimulation would appear to diminish both appetitive and consummatory components of sexual behaviour in the male rat. The post- k receptor mechanisms mediating these behavioural effects have not been explicitly examined, but the evoked behavioural profile resembles the effects that might be expected from inhibitions of both DA and 5-HT neurotransmission.

CHAPTER FIVE

GENERAL DISCUSSION

The present thesis explored the potential role of *k* opioid receptors - and by implication their endogenous ligands - in the regulation of male rat sexual behaviour. Considered together, the experimental results suggest that stimulated *k* receptors near or in either the VTA or NAS inhibit appetitive components whereas stimulated *k* receptors near or in the mPOA function in conjunction with the mesolimbic pathway to inhibit both appetitive and consummatory dimensions of male sexual behaviour. The possibility that *k* receptors play such a role was suggested by a number of findings in the literature. First, both the systemic (see Pfaus & Gorzalka, 1987a) and i.c.v. (e.g., Meyerson & Terenius, 1977; McIntosh et al., 1980; Meyerson, 1981; Imura et al., 1985) administration of opioids are known to alter male sexual behaviour. Secondly, *k* opioid agonists are known to inhibit both DA cell firing (e.g., Walker et al., 1987) and DA release (e.g., Di Chiara & Imperato 1988; Heijna et al., 1989, 1990a,b) thereby decreasing DA mediated behaviours (e.g., Herrera-Marschitz et al., 1986; Di Chiara & Imperato, 1988; Matsumoto et al., 1988a,b; Ohno et al., 1989). Thirdly, midbrain DA systems are involved in male sexual behaviour (e.g., Ahlenius et al., 1987; Mitchell & Stewart, 1989; Pfaus et al., 1989; Pleim et al., 1990; Pfaus, 1990; Eaton et al., 1991; Pfaus & Phillips, 1989, 1991). Finally, the VTA and NAS - respectively, the cell body and terminal regions of the mesolimbic DA system - and the mPOA - terminal region of the incertohypothalamic DA system - have been specifically identified to play critical roles in appetitive (Everitt et al., 1989; Everitt, 1990; Band & Hull, 1990a, Mitchell & Stewart, 1990a; Warner et al., 1991; Pfaus & Phillips, 1991) and consummatory dimensions (see Sachs & Meisel, 1988) of male sexual behaviour. Therefore, to the extent that *k* opioid receptors might participate in inhibitory negative feedback to regulate DA pathways (e.g., Trujillo et al., 1990), it was hypothesized that their stimulation and antagonism would alter male sexual behaviour.

U-50,488H Inhibits Both Appetitive and Consummatory Dimensions of Male Sexual Behaviour

In Experiments 1A, 1B, 4A, and 6A, the systemic administration of the *k* agonist U-50,488H was found to dose-dependently inhibit multiple measures commonly agreed to mirror both appetitive and consummatory dimensions of male sexual behaviour. These U-50,488H-induced inhibitions included decreased female-directed behaviour, and increased latencies both to initiate copulation and to ejaculate. Intriguingly, the decrease in female-directed behaviour was not apparent in the first min of testing; males that had been administered U-50,488H initially approached females with a frequency undifferentiated from saline controls, and only later displayed decreases. These latter observations are reminiscent of neuroleptic treated animals during feeding studies (Wise & Colle, 1984; Wise & Raptis, 1986; Koechling et al., 1988), and indicate that the behavioural inhibitions might reflect a diminished ability to maintain interest in incentive stimuli rather than motor incapacitation. Compatibly, the inhibitory effects of systemic U-50,488H on both copulatory and ejaculatory latencies were similar to those seen to follow systemic injections of neuroleptics (e.g., Pfaus & Phillips, 1989, 1991); this parallel further suggests that the U-50,488H-induced deficits in sexual behaviour were attributable to *k* opioid receptor agonist-induced decreases in DA neurotransmission. An alternative explanation was suggested by the fact that many of the U-50,488H-induced behavioural changes - increased copulation latencies, decreased mounts and intromissions prior to ejaculation, increased body flattening, decreased body temperature - also resembled those produced by both 5-HT_{1a} receptor agonists (Ahlenius et al., 1980; Stefanick et al., 1982; Clark et al., 1983; Ahlenius & Larsson, 1984b; Clark & Smith, 1986; Schnur et al., 1988; Ågmo et al., 1989; Hillegaart et al., 1989; Hillegaart, 1991) and 5-HT₂ receptor antagonists (Mendelson & Gorzalka, 1985). These experimental findings raise the possibility that the U-50,488H-induced decreases in mounts and intromissions, accompanied by increased body flattening and decreased body temperature are attributable to decreased 5-HT neurotransmission,

whereas the U-50,488H-induced decreases in female-directed behaviour result from decreases in DA release. Finally, the U-50,488H-induced increases in both copulation and ejaculation latencies could result from decreases in either DA or 5-HT neurotransmission. The involvement of midbrain DA systems in both appetitive and consummatory aspects of behaviour is well established, whereas the involvement of 5-HT pathways is much less so. More needs to be known both about the involvement of 5-HT in regulating behaviour and the interaction between *k* opioid receptor agonists and serotonergic systems before more detailed descriptions are possible (For a recent review of work examining the role of 5-HT in sexual behaviour, see Gorzalka, Mendelson, & Watson, 1990).

In Experiment 4A, the U-50,488H-induced sexual behaviour inhibitions were antagonized by systemic naloxone, indicating that U-50,488H elicited these effects through opioid receptors. In Experiment 5A, intra-cranial infusions of U-50,488H revealed that the copulatory deficits produced by systemic U-50,488H could also be produced by discrete administrations to either the VTA, NAS or to a lesser degree the mPOA. However, the ineffectiveness of some doses - most notably when infused into the VTA - suggested that U-50,488H might compose multiple, and at times competitively opposed, effects on male sexual behaviour. Supporting this speculation, previously published behavioural studies of intra-VTA infusions of *k* agonists have reported either excitations (Wise et al., 1986; Hamilton & Bozarth, 1986, 1987, 1988; Jenck et al., 1987; Mitchell & Stewart, 1987, 1990b; Badiani & Noel, 1991) or no effect of this treatment (Jenck, Gratton & Wise, 1987; Jenck, Bozarth & Wise, 1988). Perhaps U-50,488H can induce both excitatory and inhibitory effects.

The most important finding from this study was that the inhibitions of male sexual behaviour by systemic injections of U-50,488H could be prevented by central infusions of the *k* opioid receptor antagonist NBNI. Infusions of NBNI into either the cell body or terminal region of the mesolimbic DA pathway increased female-directed behaviour, and prevented both systemic U-50,488H-induced decreases in female-directed behaviour and

increases in copulation latencies. Occasionally, however, mesolimbic infusions of NBNI were seen to decrease sexual behaviour in some animals. These inhibitions were attributed to transient antagonist properties at μ receptors (Endoh et al., 1990; Horan et al., 1991). Such mixed effects of NBNI were not seen in the mPOA, a site with few μ receptors (Mansour et al., 1987). Intra-mPOA NBNI both increased female-directed behaviour and blocked systemic U-50,488H-induced inhibitions of appetitive and consummatory measures of male sexual behaviour. A suggested role for the mPOA in maintaining appetitive interest in sexual stimuli is not without precedent, and might reflect both the influence of penile reflexes and other input systems (e.g., Pfaus & Phillips, 1991; Warner et al., 1991). Alternatively, changes in appetitive motivation observed following injections directed at the mPOA might have resulted from drug diffusions to distal sites. Of particular concern is the possibility that injected drug might have risen up the angled cannulae reaching the posterior edges of the NAS through which they passed. Unfortunately, the current experiments do not allow one to dismiss this possibility, but it is of interest to note that similar behavioural changes have been induced from both intra-mPOA administrations of neuroleptics through vertical cannulae that do not pass through the NAS (e.g., Pfaus & Phillips, 1991), and anatomically restricted electrolytic lesions of the mPOA (Edwards & Einhorn, 1986). In both the present study and the Pfaus and Phillips research cannulae tips were a minimum of 1.0 mm from even the most posterior edge of the NAS suggesting that only small volumes of the drug were likely to diffuse between these two sites, but in the absence of controls delimiting the absolute anatomical boundaries of the drug-induced effects, it is best to interpret the present findings cautiously.

As discussed earlier, the contrast between the few inhibitory effects of mPOA k receptor stimulation observed in Experiment 5A and the multiple inhibitions observed in Experiment 6A might reflect the difference between stimulating mPOA k receptors in isolation versus in conjunction with mesolimbic regions. Compatible with the idea that k receptor stimulation in the two regions might normally be coordinated in the drug-free rat,

the mPOA is anatomically connected to interact with the limbic system (Mogenson, et al., 1980; Swanson et al., 1984; Simerly & Swanson, 1986).

Both these anatomical observations and accumulated behavioural data have suggested to Mogenson and his colleagues (Mogenson, et al., 1980; Mogenson, 1984; Swanson et al., 1984) that a neural model describing the selection and execution of motivated behaviours might include sensory input to the mPOA - which itself receives input from the amygdala, olfactory bulbs and hypothalamus - that is relayed to the VTA and in turn, to the NAS where motivational states are integrated in the formation of motor output. Hence, this triad of central nuclei - the mPOA, the VTA, and the NAS - may serve to relay motivational states and coordinate the appropriate instrumental response. Moreover, this function would seem not to be peculiar to sexual behaviour, but instead, might apply to the expression of all motivated behaviours.

Experiment 6B revealed that the *k* antagonist effects of NBNI are extraordinarily long-lasting. In a large sample of animals, NBNI prevented the behavioural inhibitions induced by U-50,488H for three to four days after its initial administration. Moreover, in a smaller group of animals, the same potent antagonist properties were observed 26 days after the last of three intra-cranial infusions of NBNI. This long-lasting antagonist effect might reflect continued receptor blockade (Endoh et al., 1990; Horan et al., 1991). Interestingly, in Experiment 6B, the site specificity of intra-cranial NBNI was no longer apparent as it had been in Experiment 6A. The most probable explanation for this change is that, over time, the metabolically resistant NBNI diffuses throughout the brain to bind with available *k* receptors in multiple regions.

U-50,488H Decreases Locomotor Activity

Parallel to these tests for male sexual behaviour, tests for locomotor activity were also conducted. Experiments 2A, 2B and 4B confirmed previous reports (e.g., Di Chiara & Imperato, 1988) that, like sexual behaviour, locomotor activity is also decreased by

systemic U-50,488H. Intra-cranial infusions of U-50,488H, in Experiment 5B, suggested that U-50,488H-induced decrements in locomotor activity were mediated centrally, although, the absence of site specific effects between mesolimbic or mPOA brain sites was unexpected. The VTA and NAS have well established roles in regulating behavioural excitation and accompanying locomotor activity; it was not surprising to observe that treatments believed to inhibit mesolimbic DAergic neurotransmission would decrease locomotor activity. In contrast, the mPOA is not usually considered to exert controlling influences on general activity, although Mogenson and colleagues suggest that it is an important site for integrating sensory input and internal state feedback to be relayed to descending spinal motor systems (Mogenson, et al., 1980; Swanson et al., 1984). Alternatively, though not incompatibly, mPOA *k* receptor stimulation might be aversive, producing non-specific decrements in behavioural excitement; *k* receptor stimulation produces both conditioned place aversion (Shippenberg & Herz, 1987, 1991; Bals-Kubik et al., 1989, 1990) and decreased locomotor activity (Experiment 4B and 5B; Di Chiara & Imperato, 1988).

In Experiment 6C, previous intra-cranial infusions of NBNI to all three tested sites were observed to fully antagonize systemic U-50,488H-induced locomotor decreases further indicating that these decrements were mediated at central *k* opioid receptors. Interestingly, the additional acute infusion of NBNI to these same animals decreased locomotor activity, suggesting again that NBNI transiently antagonizes μ opioid receptors.

U-50,488H Decreases Body Temperature and Increases Body Flattening

Replicating reports from other investigators (see Adler et al., 1988), systemic U-50,488H decreased body temperature in Experiment 3. This finding suggested that the body flattening induced by both systemic (Experiments 1A, 1B, 4A and 6A) and intra-NAS U-50,488H (Experiment 5A), and prevented by intra-cranial NBNI (Experiments 6A and 6B), was not a response to elevated peripheral body temperature. Possibly, the flattening

is related to the slow-wave EEG that is seen in both U-50,488H-injected animals (Young, 1989) and copulating males during the post-ejaculatory refractory period (Barfield & Geyer, 1975). On the other hand, the body flattening that is seen to follow both the administration of U-50,488H and ejaculation might result from increased brain temperatures that are not detected by intra-peritoneal heat sensors. This idea remains to be tested.

Interestingly, both increases in body flattening and decreases in body temperature are also observed following infusions of either 5-HT or 8-OH-DPAT into either the dorsal raphe or the NAS (Hillegaart et al., 1989; Hillegaart, 1991), suggesting that U-50,488H might be inducing these effects through interactions with 5-HT systems.

U-50,488H Decreases Bodily Grooming

In Experiments 1A, 1B, 4A and 6A, systemic U-50,488H was also observed to decrease bodily grooming, and in the latter experiment, this inhibition was antagonized by intra-VTA infusions of NBNI. Moreover, intra-VTA infusions of U-50,488H decreased bodily grooming (Experiment 5A). To the extent that *k* receptor stimulation is known to decrease DA release, these findings are compatible with previous suggestions that bodily grooming covaries with the amount of D1 receptor stimulation (Greidanus et al., 1989). They also suggest that the relevant changes in DAergic activity might occur in the VTA. One can speculate that this U-50,488H-induced decrease in body grooming might be related to the decreases in sexual behaviour. Bodily grooming is a displacement activity that increases among non-copulating mPOA lesioned males placed with an estrous female. This increased bodily grooming has been interpreted to indicate the continued motivation - perhaps mediated by the intact mesolimbic DA system - to copulate (Hansen et al., 1982; Hansen & Drake af Hagelsrum, 1984). Conversely, lesioning mesolimbic DA terminals decreases displacement activity (Robbins & Koob, 1980). These previous reports coupled with the present findings suggest that U-50,488H's actions in the VTA to depress

motivational states (Experiments 5A, 5B and 6A) might explain the reduction in displacement activity. If displacement activity represents the re-direction of motivation, then one would predict that unmotivated animals would not display displacement behaviour. A problem with this interpretation is that although intra-NAS infusions of *k* receptor antagonists and agonists increased and decreased male sexual behaviour respectively (Experiments 5A and 6A), they did not significantly change bodily grooming. Perhaps the D1 receptors critical for mediating body grooming are located in either the VTA or a mesolimbic terminal region other than the NAS.

Functional Significance of U-50,488H-Induced Sexual Inhibitions

In the present study, *k* opioid receptor stimulation diminished the ability of the male to maintain interest in, and execute the appropriate motor response to an estrous female. The animal appeared to be less inclined to either investigate, interact with or engage the female in sexual activity. The increased temporal spacing of intromissions, the decreased number of mounts and intromissions, and the increased ejaculation latencies that was seen when copulatory activity did begin, might reflect the diminished capacity for genital stimulation to evoke continued interest in the female. The hypothesized presence of endogenous mechanisms that might act to suppress sexual behaviour could be of functional value. Possibly they are the mechanisms that mediate the refractory periods that are seen to follow either intromissions or ejaculations. Alternatively, they may serve to regulate the temporal pattern of sexual behaviour. A third possibility is that they may tonically suppress sexual activity in the absence of arousing stimuli; sexual stimuli may, in turn, disinhibit these arousability mechanisms; the central motivational state associated with the pursuit of sexual consummation likely arises from both the presentation of sexual stimuli and internal neuroendocrinological states (Bindra, 1974; Toates, 1986).

The first suggested possibility has some appeal. Certainly U-50,488H-injected males resemble males in the midst of a refractory period. These U-50,488H treated males

are slow to begin copulating; they spend an otherwise inordinate amount of time lying flat on the floor, a typical post-ejaculatory behaviour; and relatively few mounts and intromissions precede the ejaculations that do occur. In these respects, U-50,488H-injected males behave very much like animals that have recently ejaculated.

Particularly intriguing for this consideration is the possibility that endogenous DYN release functions as inhibitory feedback to DA neurons (e.g., Heijna et al., 1989, 1990a,b; Reid et al., 1988, 1990a; Trujillo et al., 1990). As discussed earlier, DYN neurons are well positioned to release DYN onto *k* receptors located on both cell body and terminal regions of the mesolimbic and incertohypothalamic DA pathways. Moreover, it is known that stimulating the release of DA induces the release of DYN, whereas *k* receptor stimulation decreases DA release. These findings are particularly provocative in view of the fact that ejaculation is accompanied by a sharp rise followed by a precipitous drop in synaptic DA levels (Pfaus et al., 1989; Eaton et al., 1991). Plausibly, the abrupt rise in DA triggers a release of DYN that, in turn, potently inhibits further release. Perhaps a transient DYN-induced inhibition of DA release corresponds to the post-ejaculatory refractory period. Similarly, the briefer refractoriness seen to follow intromissions might also reflect DYN-mediated inhibitory feedback.

One observation that appears incompatible with this suggestion was that U-50,488H did not extend the refractory period when an ejaculation did occur. This is perhaps contrary to what one might predict if it were presumed that exogenous *k* agonists were summing with endogenous *k* receptor ligands. On the other hand, the presence of exogenous *k* agonists might inhibit the release of endogenous *k* agonists. DYN release is induced by DA (e.g., Trujillo et al., 1990). Therefore, if U-50,488H administration decreases DA release, then less DYN might be released. Hence, U-50,488H might substitute for, rather than summate with DYN with the final overall result being an unperturbed refractory period. This possibility would allow for an initial drug-induced "refractory" period - measured as increased mount and intromission latencies - that does

appear to be present, without demanding that the post-ejaculatory refractory period be unusually extended.

A further intriguing possibility is that the appetitive decrements seen to follow *k* opioid receptor stimulation in the mesolimbic pathway might correspond to the "relative" refractory phase. An association between the copulation latency and the post-ejaculatory refractory period is not a new idea. Beach (1956) suggested that a single mechanism might regulate both factors, and a statistical relation between the two has since been demonstrated (Sachs, 1978; Dewsbury, 1979). Additionally, the severe consummatory *cum* appetitive deficits, seen in the present study to follow the joint stimulation of mesolimbic and mPOA *k* opioid receptors resemble, and hence might correspond to the "absolute" refractory phase.

A second possible function for the observed *k* opioid receptor inhibition of male sexual behaviour is that *k* receptors might regulate the temporal pattern of sexual behaviour. Interestingly, this function might be compatible with, and even related to the mediation of refractory periods suggested above. As discussed earlier, a certain minimum amount of time from the first intromission to ejaculation would appear to be necessary to optimize the probability of impregnating the female (Adler, 1969). As it has been demonstrated that males have the capacity to ejaculate in a briefer time span than they normally exhibit, it would seem that mechanisms are in place to ensure that rapid ejaculations are avoided. Although exogenous *k* agonist administration did not extend the post-ejaculatory refractory period, it did lengthen both the ejaculation latency and the *III* among those males that began to copulate. Possibly, the delayed ejaculation is attributable to the potentiated brief refractoriness that is seen to follow intromissions. Augmented intromission-induced refractory periods might result from brain levels of exogenous *k* agonists that exceed the levels of endogenous *k* agonists speculated to be released by intromissions.

Finally, endogenous inhibitory mechanisms might prevent the expression of sexual behaviour in the absence of appropriate sexual stimuli. If we can presume that males are

not continually aroused sexually, then it may be plausible to suggest that in the absence of sexually arousing stimuli, the same mechanism suggested to mediate refractory periods might also serve to inhibit a chronic search for sexual union. As Beach suggested, it may only be human beings' ability to cognitively maintain symbolic representations of sexual activity that leads us to subjectively experience periods of sexual yearning.

Though compatible with the present data, the proposed functional significance of refractory period mechanisms, and their suggested evocation by *k* receptor stimulation, are clearly speculative. The present experiments did not constitute explicit tests of *k* receptor mediated refractory periods, but more explicit tests might be possible. As noted above, U-50,488H did not increase the post-ejaculatory refractory period. It was suggested that this reflected a reduced release of the endogenous *k* agonist, DYN, attributable to the presence of the exogenous *k* agonist, U-50,488H, during the pre-ejaculatory phase; *k* receptor stimulation prior to ejaculation might decrease the DA release that would normally elicit DYN release. If the presence of U-50,488H does inhibit the release of DYN, then this might be a suitable explanation for why the refractory period is not extended. Moreover, it suggests an experimental test: if U-50,488H were administered immediately following ejaculation, as opposed to before ejaculation as done in the present study, then U-50,488H might well summate with released endogenous DYN to extend the post-ejaculatory period. Should a prolonged refractory period be found to result from the post-ejaculatory administration of U-50,488H, it would more clearly indicate the involvement of both *k* receptors and their endogenous ligands in the regulation of refractoriness. The evidence presently available from the current study is circumstantial: U-50,488H inhibits the onset of copulation; it increases the refractory period between intromissions, the III; and it induces body flattening, a common post-ejaculation behaviour.

A second common post-ejaculatory behaviour, that was not measured in the current study, is the post-ejaculatory vocalization. Lesion studies indicate that there are species differences in the mediation of these "songs." In hamsters, mPOA lesions decrease post-

ejaculatory "song" production (Floody, 1989), whereas in mice the same lesions leave these vocalizations undisturbed (Bean, Numez & Conner, 1981). Currently, it is not known how mPOA lesions affect rat vocalizations. It seems an intriguing possibility that the administration of *k* agonists might induce the production of these "songs" independent of an ejaculation. Interestingly, the post-ejaculatory vocalizations have been suggested to reverse the increases in mPOA temperature that accompany copulatory behaviour (Blumberg et al., 1987; Blumberg & Moltz, 1987, 1988).

In summary, the stimulation of intra-cranial *k* opioid receptors would appear to inhibit multiple aspects of male sexual behaviour. Although the neuroanatomical limits of these effects were not explicitly demarcated, it would seem that the stimulation of *k* opioid receptors in or near either the VTA or NAS decreases a male rat's appetitive motivation to interact with a sexually receptive female. Conversely, *k* receptors in or near the mPOA would appear to act in cooperation with coinciding inhibitory actions in the mesolimbic system to inhibit both appetitive and consummatory dimensions of male sexual behaviour. Compatibly, the administration of a *k* opioid receptor antagonist to any of the three tested brain regions increased female-directed behaviour. Although DA release was not measured, it seems probable that these copulatory deficits were attributable to the known *k* agonist-induced inhibitions of DAergic neurotransmission. Similarly, decreased DA release might account for the U-50,488H-induced decreased bodily grooming. *K* opioid receptor stimulation in or near the NAS also evoked body flattening that might account for the U-50,488H-induced decreased body temperature and these effects might reflect interactions between *k* opioid and serotonergic systems. Finally, the administration of *k* opioid receptor agonists to all three tested brain sites effectively decreased locomotor activity, compatible with both decreased DA neurotransmission and aversive central motivational states.

Conclusion

In conclusion, it appears that central *k* opioid receptors and their endogenous ligands regulate the expression of sexual behaviour in the male rat. When mesolimbic *k* opioid receptors are stimulated, they would appear to inhibit appetitive components, and when stimulated in conjunction with mPOA *k* receptors, to also inhibit consummatory dimensions of male sexual behaviour. Possibly, these distinctive regulatory mechanisms underlie the "relative" and "absolute" refractory periods that are seen to follow both intromissions and ejaculations.

These experiments do not, however, unequivocally indicate which neural systems are engaged beyond the *k* opioid receptor. Phenomenologically, certain characteristics of the U-50,488H-induced behavioural syndrome resemble specific alterations that are induced by neuroleptics - decreased female-directed behaviour, increased copulation and ejaculation latencies, decreased locomotor activity, decreased body grooming - whereas others resemble those elicited by 5-HT_{1a} agonists and 5-HT₂ antagonists - increased copulation latencies, decreased mounts and intromissions prior to ejaculation, increased body flattening, decreased body temperature. These behavioural parallels suggest that *k* opioid receptor stimulation might diminish both DA and 5-HT post-synaptic activity, a mélange of their individual behavioural effects composed by U-50,488H in the present study.

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APPENDIX

Experiment 6A: VTA Drug Infusions

Intra-VTA NBNI plus Systemic Saline vs Systemic Saline

Mount Latency: High vs Low Subgroups:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	2	790706.2	395353.1	5.473	.0122
Error	21	1516940.4	72235.2		

Intromission Latency: High vs Low Subgroups:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	2	23051.0	11525.5	6.303	.0079
Error	19	34743.2	1828.6		

Mean No. Ejaculations: High vs Low Subgroups:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	2	13.9	7.0	4.981	.0170
Error	21	29.3	1.4		

Intra-VTA NBNI plus Systemic U50 vs Systemic U50

Mount Latencies: High vs Low Subgroups:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	2	2072637.3	1036318.7	4.328	.0363
Error	13	3112902.6	239454.0		

Intromission Latencies: High vs Low Subgroups:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	2	2618941.4	1309470.7	4.821	.0271
Error	13	3530968.2	271612.9		

Intra-VTA Saline vs Intra-VTA NBNI vs Systemic U50 vs Intra-VTA NBNI plus Systemic U50

Mean No. Ejaculations:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	21.6	7.2	4.708	.0071
Error	36	55.1	1.5		

Inter-Intromission Interval:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	58064.1	19354.7	13.243	.0000
Error	34	49691.6	1461.5		

Female-Directed Behaviour:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	1434.9	478.3	18.273	.0000
Error	46	1204.1	26.2		

Bodily Grooming:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	746.4	248.8	16.098	.0000
Error	46	710.9	15.4		

Genital Grooming:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	935.1	311.7	10.083	.0000
Error	46	1422.0	30.9		

Laying Down:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	6197.5	2065.8	26.321	.0000
Error	46	3610.4	78.5		

Body Flattening:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	364.3	121.4	7.473	.0004
Error	46	747.4	16.2		

Experiment 6A: NAS Drug Infusions

Intra-NAS NBNI plus Systemic Saline vs Systemic Saline

Mount Latency: High vs Low Subgroups:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	2	1573455.6	786727.8	8.597	.0019
Error	21	1921859.2	91517.1		

Intromission Latency: High vs Low Subgroups:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	2	937411.4	468705.7	4.985	.0182
Error	19	1786605.5	94031.9		

Mean No. Ejaculations: High vs Low Subgroups:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	2	22.6	11.3	11.146	.0005
Error	21	21.3	1.0		

Intra-NAS Saline vs Intra-NAS NBNI vs Systemic U50 vs Intra-NAS NBNI plus Systemic U50

Mount Latency:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	2205936.1	735312.0	4.626	.0083
Error	33	5244963.7	158938.3		

Intromission Latency:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	2169827.3	723275.8	4.700	.0081
Error	31	4770054.3	153872.7		

Inter-Intromission Interval:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	44961.1	14987.0	10.757	.0001
Error	31	43189.5	1393.2		

Intromissions Preceding Ejaculation:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	454.6	151.5	2.471	.0833
Error	27	1655.9	61.3		

Female-Directed Behaviour:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	927.7	309.2	9.940	.0000
Error	46	1431.2	31.1		

Body Flattening:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	369.2	123.1	10.010	.0000
Error	46	565.5	12.3		

Experiment 6A: mPOA Drug Infusions

Intra-mPOA Saline vs Intra-mPOA NBNI vs Systemic U50 vs Intra-mPOA NBNI plus Systemic U50

Mount Latency:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	2419365.4	806455.1	8.746	.0002
Error	35	3227176.8	92205.0		

Intromission Latency:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	2328936.5	776312.2	7.615	.0005
Error	33	3364167.8	101944.5		

Ejaculation Latency:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	578123.3	192707.8	3.593	.0249
Error	30	1608976.6	53632.6		

Inter-Intromission Interval:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	61273.8	20424.6	15.549	.0000
Error	33	43346.6			

Intromissions Preceding Ejaculation:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	260.8	86.9	2.766	.0590
Error	30	942.9			

Female-Directed Behaviour:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	1259.7	419.9	16.805	.0000
Error	41	1024.4	25.0		

Genital Grooming:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	768.2	256.1	7.579	.0004
Error	41	1385.3	33.8		

Laying Down:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	4943.4	1647.8	22.179	.0000
Error	41	3046.0	74.3		

Experiment 6B: VTA Drug Infusions

Intra-VTA Saline vs Intra-VTA NBNI vs Systemic U50 vs Intra-VTA NBNI plus Systemic U50

Mount Latency:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	2868836.2	956278.7	12.487	.0000
Error	39	2986760.3	76583.6		

Intromission Latency:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	2990723.5	996907.8	12.410	.0000
Error	39	3132806.3	80328.4		

Ejaculation Latency:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	961807.7	320602.6	9.178	.0001
Error	36	1257516.2	34931.0		

Mean No. of Ejaculations:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	29.8	9.9	11.662	.0000
Error	39	33.2	0.8		

Inter-Intromission Interval:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	73442.9	24481.0	20.071	.0000
Error	39	47569.6	1219.7		

Intromissions Preceding Ejaculation:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	281.7	93.9	4.066	.0138
Error	36	831.5	23.1		

Female-Directed Behaviour:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	570.7	190.2	9.886	.0000
Error	45	865.9	19.2		

Bodily Grooming:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	1438.0	479.3	20.658	.0000
Error	45	1044.1	23.2		

Genital Grooming:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	784.4	261.5	10.446	.0000
Error	45	1126.4	25.0		

Laying Down:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	5175.4	1725.1	29.550	.0000
Error	45	2627.1	58.4		

Body Flattening:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	338.0	112.7	9.049	.0001
Error	45	560.3	12.4		

Experiment 6B: NAS Drug Infusions

Intra-NAS Saline vs Intra-NAS NBNI vs Systemic U50 vs Intra-NAS NBNI plus Systemic U50

Mount Latency:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	2418432.5	806144.2	10.288	.0001
Error	31	2429192.4	78361.0		

Intromission Latency:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	2513392.2	837797.4	10.448	.0001
Error	31	2485730.6	80184.8		

Ejaculation Latency:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	817304.5	272434.8	4.496	.0110
Error	31	1636115.4	60596.9		

Mean No. Ejaculations:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	19.3	6.4	5.409	.0041
Error	31	36.8	1.2		

Inter-Intromission Interval:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	54525.3	18175.1	13.137	.0000
Error	31	41504.9	1383.5		

Intromissions Preceding Ejaculation:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	261.8	87.3	3.609	.0260
Error	27	653.0	24.2		

Female-Directed Behaviour:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	348.7	116.2	4.162	.0121
Error	38	1061.2	27.9		

Genital Grooming:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	835.9	278.6	9.2	.0001
Error	38	1148.8	30.2		

Body Flattening:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	270.3	90.1	5.953	.0020
Error	38	575.2	15.1		

Experiment 6B: mPOA Drug Infusions

Intra-mPOA Saline vs Intra-mPOA NBNI vs Systemic U50 vs Intra-mPOA NBNI plus Systemic U50

Mount Latency:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	2459295.8	819765.3	9.044	.0002
Error	34	3081823.6	90641.9		

Intromission Latency:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	2576544.4	858848.1	8.523	.0002
Error	34	3426155.1	100769.3		

Ejaculation Latency:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	813804.1	271268.0	6.381	.0017
Error	34	1317800.4	42509.7		

Mean No. Ejaculations:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	22.1	7.4	7.547	.0005
Error	34	33.2	1.0		

Inter-Intromission Interval:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	64844.9	21615.0	18.066	.0000
Error	34	40678.2	1196.4		

Intromissions Preceding Ejaculation:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	331.1	110.4	2.797	.0565
Error	31	1223.3	39.5		

Female-Directed Behaviour:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	379.1	126.4	4.691	.0066
Error	41	1104.4	26.9		

Bodily Grooming:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	790.9	263.6	16.807	.0000
Error	41	643.1	15.7		

Genital Grooming:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	845.6	281.9	5.306	.0035
Error	41	2178.2	53.1		

Laying Down:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	5208.2	1736.1	29.694	.0000
Error	41	2397.1	58.5		

Body Flattening:

Source of Variation	df	Sum of Squares	Mean Square	F	P
Group	3	349.6	116.5	9.710	.0001
Error	41	492.0	12.0		