EFFECTS OF COMMONLY USED ANALGESICS AND ANTIINFLAMMATORY DRUGS, IN ACUTE AND CHRONIC PAIN IN THE NAKED MOLE-RAT (HETEROCEPHALUS GLABER) USING THE FORMALIN TEST

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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TO:

My Mother, Father, Sisters and Brothers

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ABSTRACT

The aim of the present study was to investigate the effect of commonly used analgesic and antiinflammatory drugs in the naked mole-rat. Two centrally acting narcotic analgesics (pethidine hydrochloride and codeine phosphate), two non-steroidal antiinflammatory drugs (acetylsalicylic acid and naproxen) and two steroidal antiinflammatory drugs (hydrocortisone sodium succinate and dexamethasone phosphate) were used. The animals were kept under controlled laboratory conditions.

The formalin test was performed by injecting a dilute solution of formalin (20 µl of 10% formalin) subcutaneously in the right hind paw of both control and test animals. Two parameters, the number of licks and the time spent licking the paw (sec) were monitored in blocks of 5 min for either 1 h or 2 h. The vehicle or drug were injected 30 min prior to the formalin test.

The injection of dilute formalin produced two periods of pain behaviour characterized by licking and biting of the injected paw, the early (0-5 min) and the late (25-60 min) phase. Pethidine (20 or 30 mg/kg) and codeine (10, 25 or 50 mg/kg) significantly reduced licking activity in a dose-dependent manner, in both the early and late phase. In addition, pethidine and codeine administration also induced agonistic, hypersensitive, hyperactive behaviour and motor impairment that was naloxone (2 mg/kg) reversible. Acetylsalicylic acid (400 or 600 mg/kg), naproxen (200 mg/kg), hydrocortisone (75 or 150 mg/kg) and dexamethasone (30 mg/kg) significantly reduced licking and pain related activity in a dose-dependent manner but in the late phase only.

It is concluded that the naked mole-rat has anti-nociceptive

systems that can be activated by administration of the narcotic and non-narcotic drugs used. It appears that the opioid system, in the naked mole-rat is more involved in the regulation of agonistic and motor behaviour, than anti-nociception.

CHAPTER 1

1.0 LITERATURE REVIEW

1.1 Introduction

Animal experimental models are commonly used in pain research to obtain an understanding of pain mechanisms and for the screening of analgesic drugs (Zimmerman, 1983) that can be used in Veterinary and Human Medicine. The mouse, rabbit and rat have been used extensively in research for the evaluation of drug potency, their pharmacokinetic and pharmacodynamic properties and for screening of any side effects. During these experiments a novel laboratory rodent, the naked mole-rat, was used.

The naked mole-rat is a hairless rodent which lives in subterranean colonies, each having an average of 70-80 animals (sometimes the number can go up to 300). They are found in the arid regions of Kenya, Somalia and Ethiopia (Jarvis and Sale, 1971; Jarvis, 1978). They feed on roots and tubers that they obtain from the burrows (Jarvis and Sale, 1971; Jarvis, 1978).

The skin of the naked mole-rat is well vascularized but lacks sweat glands (Thigpen, 1940). These animals have high rates of thermal conductance (McNab, 1966). They have low basal metabolic rate, less than 40% of the expected value (McNab, 1966; 1968; Jarvis, 1978) and a body temperature of about 32^{0}C.

These naked mole-rats are highly social rodents with only one

breeding female, the queen (Jarvis, 1978). The queen breeds only once per year, except when the newborn litter dies, where she may then breed again (Jarvis, 1978; Faulkes *et al.*, 1987). The non-breeding females have reproductive suppression which seems to be due to a failure in ovulation (Faulkes *et al.*, 1987). Non-breeders have been found to have low urinary progesterone levels as compared to the queen (< 0.50 ng/mg creatinine cf. 0.73-148.40 ng/mg creatinine in the queen) (Faulkes *et al.*, 1987). Non-breeding females when removed from the suppressing influences of their colonies and housed with a male, become reproductively active (Jarvis, 1978). The non-workers stay deep in burrows with the queen. The worker mole-rats are responsible for digging burrows, locating food and transporting it to the nest areas (Jarvis, 1978).

The burrow systems of naked mole-rats consist of extensive foraging burrows running at root or tuber level and a deeper nest area. The burrows are extended in a random direction in order to increase the chances of getting food. Once the food is located, it is carried to the nest area and eaten there if it is small enough, whereas the larger tubers are gradually hollowed out (Jarvis and Sale, 1971; Jarvis, 1978). The humidity of the burrows is above 90% while the temperature is between 30-32^{0} C (McNab, 1966; 1968).

Naked mole-rats cause damage to roots and tubers, tea and coffee bushes, and impair soil, giving it a honeycomb appearance, with tunnels and mounds (Jarvis, 1969).

There is little information on the physiology of these rodents

(Brett, 1986), and only two studies on it's pharmacology have been performed (Kanui and Hole, 1990; Kanui and Hole, unpublished). The ability to detect aversive stimuli are basic features of animals, and mechanism of pain perception and pain regulation are basic functions of the nervous system (Kavaliers, 1988). It was of interest to obtain more information on the physiology of the naked mole-rat.

To study nociception, a modified formalin test was used (Kanui and Hole, unpublished), and to study analgesic mechanisms, 2 opioids (pethidine and codeine), 2 steroidal antiinflammatory drugs (dexamethasone and hydrocortisone) and 2 non-steroidal antiinflammatory drugs (acetylsalicylic acid and naproxen) were used. These drugs were chosen because their effects have been well demonstrated in the formalin test in other rodents and would therefore provide good comparative data.

The formalin test was used because it is a more superior test of nociception in several aspects: there is no restraint during the observation period and so the animals are not stressed, since stress can alter pain sensitivity. The pain stimulus bears a resemblance to most clinical pain and the two phases observed in the test may represent different types of pain, acute and chronic pain respectively (Dubuisson and Dennis, 1977; Alreja *et al.*, 1984; Hunskaar *et al.*, 1985a;1986; Hunskaar and Hole, 1987).

L2 Pain

1.2.1 **Definitions** (IASP, 1979)

Pain can be defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.

Analgesia refers to the absence of pain on noxious stimulation.

Hyperalgesia refers to increased sensitivity to noxious stimulation.

Hypoalgesia refers to diminished sensitivity to noxious stimulation.

A **nociceptor** is a receptor preferentially sensitive to a noxious or potentially noxious stimulus.

A **noxious** stimulus is a tissue damaging stimulus.

Pain threshold is the least stimulus intensity at which a subject perceives pain.

Lewis (1942) described two types of pain; superficial and deep. Superficial pain results from intense stimulation of the skin and can be well localized. Deep pain arises from skeletal muscles, tendons, periosteum, and joints and is poorly localized. Visceral pain shares many of the attributes of deep pain. Many authors further subdivide superficial pain into bright pricking pain and burning pain (Lewis, 1942). It has been proposed that first and second pain are produced by activation of A-δ and C fibers (Lewis, 1942; Price *et al.*, 1977).

Nociceptors, whether in the skin, muscle, or viscera, all seem to terminate as free nerve endings. There is no obvious structural distinction between the endings of various kinds of nociceptors. All of the nociceptors are supplied by small-sized afferent fibers, including both A-δ and C fibers (Zotterman, 1939).

1.2.2 Peripheral mechanisms of nociception

1.2.2.1 Cutaneous nociceptors

The bodies of primates and subprimates are endowed with receptors in the skin (Zotterman, 1939; Iggo, 1959; Burgess and Perl, 1967; Perl, 1968; Bessou and Perl, 1969; Adriaensen *et al.*, 1983) capable of detecting damaging or potentially damaging stimuli. The conduction velocities of these cutaneous nociceptive afferents are consistent with their being unmyelinated or fine myelinated fibers. Zotterman (1939) showed that burning and needle pricks elicited discharges in C and A-δ fibers. Conduction velocities of a range of 6 to 37 m/s and 5 to 28 m/s respectively have been reported (Iggo, 1959; Burgess and Perl, 1967).

The receptive fields of the cutaneous nociceptors have also been analysed in the above studies. The receptive fields are generally small and consist of responsive spots of under 1 mm in diameter (Burgess and Perl, 1967; Perl, 1968; Iggo, 1969)

Fibers activated by noxious mechanical stimuli can be divided into

specific mechanonociceptors and polymodal nociceptors. Specific mechanonociceptors are high threshold mechanoreceptors activated by mechanical stimulation and respond only to moderately intense or noxious mechanical stimuli (Burgess and Perl, 1967). They make up about 20% of the cutaneous A-δ fibers in the cat (Burgess and Perl, 1967) and are slowly adapting (Campbell *et al.*, 1979). The conduction velocity in the monkey is in the range of 5.2-53 m/s (Perl, 1968).

Polymodal C nociceptors respond to intense thermal, mechanical and chemical stimuli (Besson and Chaouch, 1987). Their conduction velocity is in the range of 0.5-1.4 m/s. These receptors increase their discharge with the intensity of stimulation and following a large initial discharge, the response adapts and settles to a lesser level that can outlast the stimulation (Adrian and Zotterman, 1926). They undergo fatigue following repetitive stimulation of the receptive field (Torebjork and Hallin, 1974; Kumazawa and Perl, 1977). Some A-δ polymodal nociceptors activated by thermal and mechanical stimulation have also been described in the cat (Beck *et al.*, 1974) and primates (Iggo and Ogawa, 1971). Their response to mechanical nociceptive stimulation is similar to that of the polymodal C nociceptors (Georgopoulos, 1976).

Some C fibers are activated by noxious thermal stimuli and also mechanical stimuli (Sumino and Dubner, 1981). Their threshold to heat stimulation is generally around 42^0C although some only respond to higher temperatures (Bessou and Perl, 1969). Their response to a suprathreshold stimulus occurs after a relatively short latency (Beitel

and Dubner, 1976; Georgopoulos, 1976). After the onset of the stimulus, a high level of activity is reached rapidly which then decreases but is maintained throughout the duration of the stimulation and can continue after cessation of the stimulus (Beitel and Dubner, 1976; Croze *et al.*, 1976). Some nociceptors continue to increase in activity above 53^0C yet others seem to reach a level of saturation at temperatures of above 50^0C (Beitel and Dubner, 1976; Georgopoulos, 1976). Increasing the skin temperature brings about a reduction in the latency and an increase in the response which is linear (Beck *et al.*, 1974) or exponential (Beitel and Dubner, 1976; Georgopoulos, 1976). Adriaensen *et al.*, (1983) reported that the discharge frequency to radiant heat, in humans, was higher in some A-δ polymodal fibers than in C fibers.

Receptors activated by both warming and cooling have been reported in the scrotal skin of the rat (Pierau *et al.*, 1974). A group of thermoreceptors that are excited at both non-noxious and noxious warm temperatures (above 43^0C) have been described by Sumino and Dubner, (1981) in the monkey.

There is little information on the responses of afferent fibers to noxious cold stimulation. These have response frequencies that increase with the degree of cooling (Georgopoulos, 1977). Dodt and Zotterman (1952b) described cold receptors on the tongue of the cat that responded to temperatures of above 45^0C. This paradoxical response has been confirmed (Dubner *et al.*, 1975; Long, 1977). Their responses commence after a latency, and then increase with the intensity of stimulation.

The conduction velocities of subprimate thermosensitive afferents are generally slow (Iggo, 1959) whereas those of the primates are faster (Iggo, 1969; Sumino and Dubner, 1981). The receptive fields consist of tiny spots of < 1 mm in diameter in both groups (Iggo, 1969; Duclaux and Kenshalo, 1980; Sumino and Dubner, 1981).

Polymodal nociceptors are sensitized by a previous nociceptive thermal stimulus. This is characterized by a reduction in threshold, an increase in response to a given set thermal stimulus, a reduction in the response latency and the appearance of spontaneous activity (Bessou and Perl, 1969; Beck *et al.*, 1974; Beitel and Dubner, 1976; Georgopoulos, 1976; Kumazawa and Perl, 1977; Lynn and Carpenter, 1982). This sensitization phenomenon is undoubtedly important in terms of the hyperalgesia of the skin in areas of damage due to repeated noxious stimulation (Hardy *et al.*, 1951).

The responses of the polymodal nociceptors to the cutaneous application of algogenic chemicals have been studied in animals (Bessou and Perl, 1969; Forster and Ramage, 1981; Lynn and Carpenter, 1982) and in humans (Van Hees and Gybels, 1972; Torebjork and Hallin, 1974). The response of A-δ polymodal nociceptors have also been described in humans (Adriaensen *et al.*, 1983). A good correlation exists between the activity produced by these stimuli and the pain sensation (pricking or burning) as reported by the human subjects tested (Adriaensen *et al.*, 1983).

In some situations, pain does not appear to result from the firing of

specific nociceptors, but is triggered instead, by activity of large diameter fibers that respond to gentle tactile stimulation. A good example here has been observed in painful neuropathies (Campbell *et al.*, 1988).

There is also a class of unmyelinated primary sensory neurones which do not respond to transient excessive mechanical or thermal stimuli, but have a chemical sensitivity that makes them responsive if tissues become inflammed. These have been referred to as silent afferents and have been identified in the monkey skin (Cohen *et al.*, 1990). These afferents significantly displayed receptive fields in the presence of experimental arthritis (Schaible and Schmidt, 1988), that could not be detected in the healthy joint.

1.2.2.2 Muscular and articular nociceptors

Muscle afferents of type III (fine myelinated fibers) and type IV (unmyelinated fibers) are activated by strong mechanical stimulation, intramuscular injection of hypertonic solutions and by thermal stimulation (Paintal, 1960; Bessou and Laporte, 1961; Iggo, 1961). Both the myelinated and unmyelinated fibers have similar characteristics. On the basis of their response thresholds to mechanical stimulation, two groups of afferent fibers can be distinguished. One group is only activated by intense stimuli (64% of group IV fibers and 56% of group III fibers), the other group is responsive to light mechanical stimuli such as innocuous indentations

of the tissue or active contractions of moderate force and innocuous stretch (Mense and Meyer, 1975).

Group III and group IV fibers also respond to algogenic substances such as bradykinin (Mense, 1977). However, some of these fibers are exclusively activated by chemical stimulation although a large number of group III and IV afferents resemble polymodal nociceptors (Kumazawa and Mizumura, 1977).

A-δ afferent fibers of the cat posterior articular nerve responding to noxious articular stimulation have been reported (Burgess and Clark, 1969). A detailed analysis of the joint afferents has indicated that some of the group III and IV sensory units in joint nerves may be involved in signalling innocuous joint movements and position, whereas other fine fibers are probably specific nociceptors since they are only activated by extreme rotation which could be considered as noxious (Schaible and Schmidt, 1983a,b). These fine afferent articular units are also activated by bradykinin and acute joint inflammation (Coggeshall *et al.*, 1983).

1.2.2.3 Visceral nociceptors

It is generally held that under normal conditions, the activation of visceral receptors does not give rise to any painful sensation (Cervero, 1988). However, a painful sensation on occasion referred to a cutaneous zone can appear after various visceral disorders (ischaemia, irritation of mucosa or serosa, torsion or traction of the mesenteries,

contraction or excessive distension of the viscera) (Cervero, 1988). It is not yet clear whether visceral pain results from the activation of specific nociceptors or from the excessive activity of receptors involved in reflex regulations of visceral functions in normal conditions (Cervero, 1988).

Some fine diameter afferent fibers in the heart are activated by myocardial ischaemia (Brown, 1967; Uchida and Murao, 1974) and by application of KCl or lactic acid to the myocardium (Uchida and Murao, 1975). A large number of A-δ and C fibers emanating from the heart, lungs and large vessels are excited by the local application of bradykinin and also by light mechanical stimulation. Only a small number of cardiac afferents activated by bradykinin are also excited by strong pressure and pinch and so could be considered as nociceptors (Barker *et al.*, 1980).

In the respiratory system, receptors localized in the superficial tracheobronchial tree which are activated by irritants and the receptors situated in the intra-alveolar space and excited by pulmonary congestion could be considered as nociceptors (Paintal, 1973).

Some receptors capable of inducing vegetative reactions have been characterized in the bile duct (Cervero, 1982). Similarly, the rectal mucosa possesses receptors that respond to strong mechanical stimulation and on occasion also to thermal and or chemical stimuli applied to their discrete receptive fields. They resemble cutaneous C polymodal nociceptors and are similar to those of the spermatic

nerve of the dog testicle (Kumazawa and Mizumura, 1980). These A-δ and C fibers in the superior spermatic nerve are activated as well by intense mechanical stimuli. They are also highly excited by noxious heat and algogenic compounds (Kumazawa and Mizumura, 1980). The pain that results from excessive distension or contraction of hollow viscera may be due to activity of the "in series" tension receptors (Leek, 1972).

1.2.2.4 Mechanisms of nociceptor activation

1.2.2.4.1 Direct or indirect action of nociceptive stimulation

The short latency and relatively low threshold to mechanical and thermal stimuli of some cutaneous nociceptors argues for a direct activation of nociceptors by the stimulus (Besson and Chaouch, 1987). Using a very brief thermal stimuli produced by a laser beam, it was demonstrated that the latency of C polymodal nociceptors in humans was longer by only 21 ms than that evoked by transcutaneous electrical stimulation, which probably bypasses the terminal transducing processes. This very minor difference in latency (taking into account the time taken for heat to be transferred from the skin surface towards the terminals) does not seem to favour a role for a chemical mediator in the action of natural stimuli (Paintal, 1976). However, a variety of studies have shown excitatory action of some chemical substances on polymodal nociceptors, suggesting an intermediate action in certain conditions (Handwerker, 1976; King *et al.*, 1976).

The effect of heat on some cutaneous nociceptors can under certain conditions be potentiated by bradykinin or prostaglandins (Handwerker, 1976). Furthermore, analgesic effects of ASA, which is mediated through inhibition of prostaglandin synthesis (Vane, 1971) may further support an indirect action through chemical mediators.

1.2.2.4.2 Involvement of nociceptors in neurogenic inflammation

When nociceptive stimulation is applied to cutaneous areas, a characteristic set of responses (neurogenic inflammation) consisting initially of a local reddening at the site of injury and that spreads outwards from the initial stimulus site is produced (Lewis, 1942). Antidromic stimulation sufficient to activate nociceptive afferents produces a reduction in the threshold of nociceptors (Fitzgerald, 1979) and a peripheral vasodilatation (Garcia and Hamamura, 1974) resulting from the release of various substances.

Pain associated with inflammation is derived from the stimulation of chemoreceptors by inflammatory mediators (Ferreira, 1982). These chemoreceptors are unmyelinated free nerve endings localized in the connective tissue spaces close to the capillaries and venules. During inflammation, sensory nerves may be injured, but hyperalgesia derives possibly from the concomitant action of inflammatory mediators, sometimes enhanced by the presence of physical stimulation like pressure (Ferreira, 1982).

Prostaglandins (PG) are probably the most important inflammatory

mediators (Ferreira, 1982). Ferreira, (1982) showed that a slow rate of prostaglandin release during a long period of time, as in inflammation, is capable of causing hyperalgesia. PGE_1 potentiated pain caused by bradykinin or histamine (Ferreira *et al.*, 1973). Infusion of PGE_1 together with histamine, produced itching (Ferreira *et al.*, 1973). The effects of PGE_1 and PGE_2 are cummulative and sustained. Thus, continuous generation of minute amounts of PGs at site of injury will sensitize the nerves so that mechanical stimulation or mediators such as bradykinin and histamine can cause pruritus or pain (Ferreira, 1982).

Central release of PGs is also thought to increase peripheral hyperalgesia. This has been seen in the cerebral cortex and in frog spinal cord (Ramwell and Shaw, 1966; Ramwell *et al.*, 1966). Using a modification of the Randall-Selitto test which measures the sensitivity to pressure in a carageenin injected paw, Ferreira *et al.* (1978) demonstrated both central and peripheral components of inflammatory hyperalgesia in rats. The hyperalgesia was counteracted by several prostaglandin synthesis inhibitors including aspirin, indomethacin and paracetamol, regardless of route of administration. Hyperalgesia to thermal stimulation in rats tested with the hot-plate has been reported after administration of $PGF_{2\alpha}$ into the subarachnoid space (Ferreira, 1983). Hyperalgesia has also been reported after intrathecal administration of $PGF_{2\alpha}$ in rats (Ferreira, 1983).

In another study, PGE, administered systemically produced analgesia in rats. This study implied that some of the PGs may inhibit

the central transmission of nociceptive information (Sanyal *et al.*, 1979). PGs are widely distributed in the central nervous system and it is likely that they interact with various systems related to modulation of nociception and the different effects reported may reflect their different site of action (Berge, 1986).

PGs are thought to affect pain transmission by inhibiting the release of neurotransmitters from terminals of descending monoaminergic pathways. Inhibitory effects of PGs on the release of norepinephrine have been demonstrated in *in vitro* preparations of brain tissue (Chiu and Richardson, 1985).

Substance P (SP) also plays an important role in peripheral hyperalgesia. Peripheral terminals of fine-diameter afferents contain substance P (Hokfelt *et al.*, 1975). During activation of fine nociceptive afferents, SP is released locally and at a distance via axonal reflexes (White and Helme, 1985). This peptide induces both vasodilatation and produces both direct and indirect effect on nociceptors. Substance P can cause release of histamine from adjacent mast cells which produces vasodilatation and activates or sensitizes the surrounding nerve endings (Foreman *et al.*, 1983). The involvement of SP is mainly supported by the effect of it's antagonists which have been shown to reduce both neurogenic vasodilatation and plasma extravasation (Lembeck *et al.*, 1982). This is in agreement with observations made after systemic or local administration of capsaicin, which produces a depletion of SP (Fitzgerald, 1983). Such studies have underlined the importance of chemical factors in the modulation of sensitivity of nociceptors at the peripheral terminal

level.

Morphine and enkephalin have analgesic effect when tested directly on hyperalgesic paws (Ferreira, 1983) and in the writhing test in mice using acetic acid (Bentley *et al.*, 1981). These results support the idea of the presence of peripheral opiate receptors. Intradermal injection of low doses of morphine is able to reduce nociceptive responses evoked from the injected area (Ferreira, 1983). These results suggest the presence of peripheral opiate receptors. A possible involvement of adrenergic receptors at the peripheral terminals has been indicated (Coderre *et al.*, 1984). Therefore the activation of nociceptors could involve both direct or indirect mechanisms.

1.2.3 Central mechanisms of nociception

1.2.3.1 Neuroanatomy and physiology of the dorsal horn of the spinal cord

The spinal cord of the cat has been subdivided into 10 laminae (Rexed, 1952, 1954). These subdivisions reflected neuronal groupings as seen in cytoarchitectonic studies (using Nissl's stains) based on shapes, sizes, density and distribution of neuronal cell bodies. Similar lamination has been noted in the rat (Steiner and Turner, 1972), and in the monkey (Scheibel and Scheibel, 1968; Light and Perl, 1979a,b; Ralston and Ralston, 1979). Laminae I-VI make up the dorsal horn.

Lamina I consists of small, medium and large-sized cells that are scattered and their cell bodies have a primarily horizontal arrangement (Rexed, 1952, 1954; Scheibel and Scheibel, 1968; Light and Perl, 1979a). Waldeyer (1888) referred to the large lamina I neurones as marginal cells since they occupy the dorsal margin of lamina I (LI). This layer of the spinal cord is usually referred to as the marginal zone of Waldeyer.

The marginal cell bodies are flattened between the overlying white matter and the underlying LII (Waldeyer, 1888; Rexed, 1952, 1954; Scheibel and Scheibel, 1968). Gobel (1978) classified LI cells into 2 groups, the pyramidal and multipolar cells that can be further subdivided into 2 each. The dendrites of the marginal cells travel between the plane of the white matter and the outer cells of LII (Cajal, 1909; Scheibel and Scheibel, 1968; Kumazawa and Perl, 1978; Light *et al.*, 1979). These dendrites are usually confined in LI (Gobel, 1978; Light *et al.*, 1979) but occasionally, dip down into LII. Marginal cells of LI have axons that project for long distances but also have local connexions with other LI neurones via short axons or collaterals via Lissauer's tract (Szentagothai, 1964; Scheibel and Scheibel, 1968). The axonal projections of the cells seem to be primarily to the thalamus or propriospinal (Szentagothai, 1964).

Christensen and Perl (1970) reported that the marginal cells in LI respond to peripheral stimulation in one of 3 ways: the first group of cells responded only to mechanical nociceptive stimulation by slowly conducting (small) myelinated axons. The second group was

responsive to both mechanical and thermal nociceptive fibers, the former transmitted by small myelinated fibers, the latter by unmyelinated axons. Finally, the third group of cells responded to innocuous temperature changes as well as mechanical and thermal nociceptive stimuli. Later studies also have confirmed the presence of nociceptive neurones in LI (Menetrey *et al.*, 1977; Kumazawa and Perl., 1978; Cervero *et al.*, 1979a). This lamina has also been demonstrated to contain neurones that are excited by both innocuous and noxious mechanical stimulation in the monkey (Handwerker *et al.*, 1975), in the cat (Cervero *et al.*, 1979a) and in the rat (Menetrey *et al.*, 1977).

Lamina II (Rexed, 1952, 1954) is otherwise known as the substantia gelatinosa. This layer consists of small and closely packed cells with radial orientation with respect to the surface of the cord (Rexed, 1952, 1954; Szentagothai, 1964). 2 cell types were described by Cajal (1909), the central cells and the border cells, also called the islet and stalked cells respectively (Gobel, 1975, 1978). On the basis of cellular density, the substantia gelatinosa has been subdivided into LII outer and LII inner (Gobel, 1978; Light and Perl, 1979b; Ralston and Ralston, 1979).The dendrites of LII cells remain largely within LII and are extensively branched (Szentagothai, 1964; Scheibel and Scheibel, 1968).

The central cells of the substantia gelatinosa can be divided into 2 groups on the basis of their axonal projections; funicular cells and short-axoned cells (Cajal, 1909; Szentagothai, 1964; Scheibel and Scheibel, 1968). The axons of both these cells are thought to end

within the substantia gelatinosa (Szentagothai, 1964; Sugiura, 1975) and on this basis, the substantia gelatinosa is regarded as a closed system. Central cells appear to have axons that remain within LII (Gobel, 1975; 1978; Bennet *et al.*, 1980).

The border cells of LII are large and are situated in the dorsal part of the substantia gelatinosa. Their dendrites pass longitudinally over the dorsal horn as well as radially into the substance of the substantia gelatinosa (Cajal, 1909; Rexed, 1952, 1954; Sugiura, 1975). There is evidence that border cells of LII send their axons into LI (Gobel, 1975; 1978; Bennet *et al.*, 1980).

Electrophysiological recording of dorsal horn LII neurones is difficult because of their small size (Besson and Chaouch, 1987; Brown, 1982). Part of the difficulty is due to the relatively small samples of recordings from neurones that have been definitely established as LII neurones and to the use of different preparations (anaesthetized, spinal, decerebrate), and different types of microelectrodes. Different groups of workers classify the response of the units according to different criteria (e.g. based on inhibitory response or excitatory response) (Besson and Chaouch, 1987).

Neurones in the substantia gelatinosa that respond exclusively to noxious stimuli have been reported (Light *et al.*, 1979; Wall *et al.*, 1979; Bennet *et al.*, 1980). Neurones responding exclusively to innocuous mechanical stimulation of the skin have also been reported by the same groups. Furthermore, neurones that respond to both noxious and innocuous stimulus have also been reported (Price *et al.*,

1979; Wall *et al.*, 1979; Bennet *et al.*, 1980).

Melzack and Wall (1965) and (Wall, 1978) assigned a modulatory role to the neurones of the substantia gelatinosa in their gate control theory of pain. They proposed in their theory, that the substantia gelatinosa neurones modulate afferent signals before they influence the tract cells. These tract cells (T cells) which may be the origin of the spinothalamic tract (Nathan, 1976) were thought by Melzack and Wall (1965) and Wall (1978) to be the neural mechanism which comprises the action system for response and perception. The small myelinated and the unmyelinated fibers were assigned the important role for keeping the gate open whereas the large fibers tended to close the gate at some stage. The tonic activity in small fibers would keep the gate partly open while an input over large fibers would close the gate, limiting the discharge of the T cells. A prolonged stimulus would result in adaptation of the large afferents and the small fibers would get the upper hand, opening the gate further. The gate could be returned to a closed position by adding a large fiber input. Descending pathways could alter the gate control system. The activity in such pathways need to be appropriate to the situation and so a central control trigger was proposed. The dorsal column pathway and the spinocervicothalamic pathway were considered as likely candidates to provide the discriminative information needed for a central decision to alter the sensitivity of the gate mechanism.

The remaining laminae of the dorsal horn (LIII-LVI) can be considered together since their neurones have dendrites that cut across these laminae (Brown, 1982). Not much has been studied

about these laminae. Lamina III contains a large number of small neurones about which little is known at present; some of them have similar properties to LII neurones according to Wall *et al.*, (1979). Neurones from LIII-VI do not have dendritic trees limited to their own lamina and are capable of sampling inputs from wide areas (Brown, 1982). With Laminae III-VI, most neurones even those whose main axons project out of the gray matter, have axonal projections that are directed to deeper laminae (Brown, 1982). Several major ascending systems arise from the cells whose somata lie within them (Brown, 1982).

1.2.3.2 Primary afferent input to the dorsal horn of the spinal cord

It is in the dorsal horn of the spinal cord where inputs from the skin and deep somatic and visceral structures are received. It is also here that descending influences from supraspinal structures can exert themselves on dorsal horn neurones and the primary afferents, and therefore, perhaps modify information from the periphery tremendously.

The branching patterns of axon collaterals arising from the axons after they enter the spinal cord is quite specific and varies according to the type of the afferent unit (Brown, 1982). Large myelinated axons innervating sensitive mechanoreceptors distribute their axons to some or all of LIII, IV, V and dorsal parts of LVI, with occasional branches in the inner LII (Light and Perl, 1979a; Brown *et al.*, 1981). The small myelinated and unmyelinated fibers form what is called

Lissauer's tract (Earle, 1952; Pearson, 1952). The lateral part of this tract is propriospinal and some of it's fibers project from the substantia gelatinosa (Szentagothai, 1964; Light and Perl, 1979a). This lateral portion shows a preponderance of small diameter fibers. The A-δ group of axons, some of which innervate cutaneous and deep nocireceptors have been shown by intra-axonal horseradish peroxidase injection (Light and Perl, 1979b; Mense *et al.*, 1981) to provide boutons to LI, the marginal cell layer. In addition, they may send terminals to LV.

With the use of Golgi stain, Rethelyi, (1977) suggested that fine nonmyelinated (C fiber) axons were distributed to LII. Degeneration and autoradiographic studies (LaMotte, 1977; Ralston and Ralston, 1979) also showed C fiber terminations in LII. Light *et al.*, (1979), basing their studies on response properties and dendritic distribution of neurones within LI and LII, suggested that C fibers innervating sensitive high threshold mechanoreceptors project to inner LII whereas those innervating cutaneous nociceptors project to outer LII and LI. The large and small fibers mix to a certain degree and there is no absolute segregation (Light and Perl, 1979a).

Substance P, a putative transmitter in small axons, has been demonstrated to be mainly localized in LI and LII (Hokfelt *et al.*, 1975; Takahashi and Otsuka, 1975).

The pooling of data from experiments in which single cutaneous axons are injected with horseradish peroxidase show that the map of receptive fields recorded from dorsal horn neurones and the map of

the body surface laid down by the primary afferent fibers are similar (Brown *et al.*, 1980; Brown, 1982). This map of the receptive field of the body surface represented in the spinal cord is referred to as the somatotopic representation laid down by the primary afferent fibers. This map consists of concentric shells with the innermost representing the most distal and medial parts of the body, whereas the proximal parts are represented more laterally (Brown *et al.*, 1980; Brown, 1982). The map has a steep gradient mediolaterally and a gentle gradient rostrocaudally. These gradients reflect the fact that primary afferent fibers form long sagittally running columns of terminals within the dorsal horn (Brown, 1982).

1.2.3.3 Viscero-somatic convergence in the spinal cord

It has been observed that pain from the viscera is sometimes referred to the skin and hence the term viscero-somatic convergence. Viscero-somatic convergence occurs in LV and LVIII of the spinal cord (Selzer and Spencer, 1969; Milne *et al.*, 1981).

In the study carried out by Milne *et al.*, (1981) in the monkey, it was demonstrated that viscero-somatic convergence of both visceral (testicular) and cutaneous nociceptors occured on spinothalamic neurones, thus some of the neurones showing convergence project to the thalamus.

The viscero-somatic convergence onto the same dorsal horn neurone has been used to explain referred pain and the inhibitory

interactions have been used to explain the alleviation of pain of visceral structures on stimulation of the skin or more superficial structures (Ruch, 1946).

1.2.3.4. Propriospinal inputs into the spinal cord

The marginal cells of LI seem to have a primary role in intersegmental connexions (Burton and Loewy, 1976). Less is known about propriospinal inputs into the dorsal horn than either primary afferent input and those from the brain because these are short ranging connexions and therefore more difficult to study. About a third of the axons from Lissauer's tract arise from the dorsal horn neurones most of which are marginal cells of LI or neurones in LII (Chung *et al.*, 1979; Chung and Coggeshall, 1979). These axons are propriospinal and run for short distances in Lissauer's tract connecting LI and LII.

Axons of ascending pathways may give off collaterals (in the white matter) that enter the dorsal horn. This has been reported for axons of both spinocervical tract neurones and also neurones belonging to the post-synaptic dorsal column system (Brown *et al.*, 1977; Brown and Fyffe, 1981).

1.2.3.5 Neurotransmitter release by the nociceptive afferents at the spinal level

1.2.3.5.1 Substance P (SP)

Since it's discovery by Von Euler and Gaddum (1931), many studies have pointed to the role of SP, an 11-amino acid polypeptide, to be the neuromediator for thin nociceptive afferent fibers. SP is found in great concentrations at the level of the dorsal roots and dorsal horn (Otsuka and Konishi, 1976; Takahashi and Otsuka, 1975).

Anatomical studies using SP antibodies and electron microscopy have demonstrated the presence of SP in afferent terminals in LI and outer parts of LII of the dorsal horn of the spinal cord (Hokfelt *et al.*, 1975; Jessell *et al.*, 1979; Difiglia *et al.*, 1982; DeLanerolle and LaMotte, 1983). Dorsal root rhizotomy resulted in reduction of SP levels in the dorsal horn showing that most of this substance originates in the periphery (Hokfelt *et al.*, 1980). SP has also been shown to be present in intrinsic spinal neurones (Hokfelt *et al.*, 1980; Gibson *et al.*, 1981), and in fibers descending in the brain stem (Chan-Palay *et al.*, 1978; Pelletier *et al.*, 1981).

Release of immunoreactive SP has been evoked from various spinal cord preparations *in vitro* (Otsuka and Konishi, 1976; Jessell and Iversen, 1977), and into the subarachnoid space of the spinal cord of anaesthetized cat after stimulation of the sciatic nerve, but only at high stimulus intensity neccessary to stimulate A-δ and C fibers (Yaksh *et al.*, 1980). A similar release of SP has been induced by

noxious natural stimulation (Kuraishi *et al.* 1985). In one study, diminished level of SP were observed in spinal cord substantia gelatinosa of patients with reduced pain sensitivity (Pearson *et al.*, 1982). Henry (1976) showed that iontophoretic administration of SP excited dorsal horn neurones that were also activated by noxious heat stimulation of the cutaneous receptive field.

Systemic administration of SP during neonatal period caused a degeneration of primary sensory neurones including a diminution in the number of C afferent fibers (Jancso *et al.*, 1977; Scadding, 1980). Similarly, biochemical studies have demonstrated a reduction in SP at the level of the primary sensory neurones after administration of capsaicin (Fitzgerald, 1983). Results from electrophysiological studies in adult rats, after neonatal capsaicin pretreatment, have shown a mean reduction in the number of neurones responding to C fiber inputs in the dorsal horn (Cervero *et al.*, 1984). Local application of capsaicin onto a peripheral nerve blocks axonal transport and depletes the neurone of SP (Gibson *et al.*, 1982). This treatment, several days later, reduces postsynaptic excitation and decreases the number of noxious heat-responsive dorsal horn neurones (Fitzgerald and Woolf, 1982). A reduction of the responses to spinothalamic tract neurones to both noxious mechanical and thermal stimulation has been described in monkeys after acute topical application of capsaicin onto a peripheral nerve (Chung *et al.*, 1985). In summary, SP present in primary afferent fibers appears to be involved in excitatory transmission processes related to the passage of nociceptive information in the spinal cord. The nature of this involvement is

however, unclear and evidence has been confusing, since no effect, analgesia, hyperalgesia and behavioral effects have been reported in many studies (Besson and Chaouch, 1987). A more recent speculation, therefore is that SP may modulate the excitability of dorsal horn neurones, possibly in combination with a rapidly acting neurotransmitter (Henry, 1982). Moreover, the fact that SP coexists with other substances in the same nociceptive fiber complicates the issue even further. In one study, calcitonin-gene-related-peptide (CGRP), coexisting with SP in the same sensory neurones potentiated hyperalgesia induced by intrathecal SP (Wieselfield-Hallin *et al.*, 1984).

Other substances such as excitatory amino acids (Glutamate, aspartate) and adenosine 5'-triphosphate could also act as neurotransmitters in primary afferent fibers (Salt and Hill, 1983).

1.2.3.6 Ascending systems that transmit information from nociceptors

Various ascending tracts are involved in relaying information from cutaneous and deep structures to supraspinal structures. These include the spinothalamic tract (Mehler, 1957; Mehler *et al.*, 1960; Boivie, 1971b), post-synaptic dorsal column pathway (Angaut-Petit, 1975a,b; Rustioni, 1977; Brown and Fyffe, 1981), the spinocervical tract (Brown, 1982) and the spinoreticular tract (Rossi and Brodal, 1957; Mehler *et al.*, 1960).

The existence of the spinothalamic projections has been demonstrated in many species including the cat (Mehler, 1966; Boivie, 1971b), dog (Hagg and Ha, 1970), rat (Lund and Webster, 1967b; Mehler, 1969), monkey (Mehler *et al.*, 1960; Kerr,1975b) and man (Bowsher, 1957; Mehler, 1969).

There are regional and species differences in the the cells of origin of the spinothalamic tract (Willis and Coggeshall, 1978). Electrophysiological mapping experiments have shown that the cells of origin in cervical enlargement of the cat are concentrated in LI, V and VI (Dilly *et al.*, 1968) while those in the cat's lumbar enlargement are in LI and LV-VIII (Trevino *et al.*, 1972) but mostly in LVII and VIII. Later studies by Trevino and Carstens (1975), using retrograde horseradish peroxidase injected into the diencephalon as a marker, confirmed these sites of origin in the cat. In the lumbar cord of the monkey (Trevino *et al.*, 1973; Albe-Fessard *et al.*, 1974; Trevino and Carstens, 1975) and the rat (Giesler *et al.*, 1976), the sites of origin of spinothalamic cells are in LI and IV to VIII, but mostly in V. Some spinothalamic tract cells project to the ipsilateral diencephalon, but most project contralaterally (Trevino *et al.*, 1972; 1973). This has been confirmed using horseradish peroxidase (Trevino and Carstens, 1975). The decussation is probably in the same segment as the cell body.

By means of electron microscopy and degeneration studies of the spinothalamic tract, it has been revealed that the ascending axons appear to be myelinated. Only very few unmyelinated fibers were

identified (Lippman and Kerr, 1972).

The tract has a roughly somatotopic organization with the caudal body represented dorsolaterally and the rostral body ventromedially (Applebaum *et al.*, 1975). The termination sites of the tract in the thalamus include the ventrobasal complex, the posterior nuclear group, the intralaminar nuclei, the nucleus paracentralis, ventrocaudal nucleus and nucleus centralis lateralis (Clark,1936; Anderson and Berry, 1959; Mehler *et al.*, 1960; Jones and Burton, 1974).

Some spinothalamic cells respond only to noxious stimuli, and many of these are located in LI (Price and Mayer, 1974; 1975; Willis *et al.*, 1975), although high threshold cells are also found deeper in the dorsal horn. Spinothalamic tract cells show vigorous response to the injection of algesic chemicals into the arterial circulation (Levante *et al.*, 1975; Foreman *et al.*, 1977). Other spinothalamic tract cells can be activated by low threshold stimulation and often also by noxious stimulation (Price and Mayer, 1974; 1975; Willis *et al.*, 1974; 1975; Applebaum *et al.*, 1975).

Thus, although there is evidence that points to the role of the spinothalamic tract neurones in pain signalling, it is not the sole tract involved in transmission of pain. In one experiment, Cadwalader and Sweet (1912) reported that dogs whose ventrolateral tracts had been sectioned, responded slowly to pain and extreme heat.

The post-synaptic dorsal column neurones are located in LIII, IV, and the medial parts of V (Angaut-Petit, 1975a; Rustioni, 1977;

Brown and Fyffe, 1981). Apart from receiving information about light touch from sensitive cutaneous mechanoreceptors, this pathway also relays information emanating from nociceptors (Angaut-Petit, 1975b; Brown and Fyffe, 1981). Axons exclusively driven by nociceptors have been reported (Angaut-Petit, 1975b). Thus, this system is capable of transmitting information from nociceptors. This pathway terminates in the dorsal column nuclei (Angaut-Petit, 1975a).

The spinocervical tract is also involved in transmission of pain (Cervero *et al.*, 1977; Brown, 1982). The cells of origin are located in LIII, IV, and V of the dorsal horn (Brown, 1982). The spinocervical tract terminates in the lateral cervical nucleus in the upper cervical cord.

The cells of origin of the spinoreticular tract activated antidromically following stimulation in the reticular formation were found to be concentrated in LVII and VIII in the cat spinal cord (Fields *et al.*, 1975; 1977a), although there are also some cells in the dorsal horn. In degeneration studies, this pathway has been shown to terminate in various brain stem areas including the nucleus reticularis lateralis, nucleus reticularis gigantocellularis, and nucleus reticularis ventralis (Rossi and Brodal, 1957; Anderson and Berry, 1959). The electrophysiological properties have been well investigated (Fields *et al.*, 1977a,b; Menetrey *et al.*, 1980). The inputs to neurones of this tract include nociceptor afferents although the exact role of the tract in nociception is uncertain.

1.2.3.7 Descending systems acting on dorsal horn neurones

The dorsal horn also receives inputs from neurones located at various sites in the brain. These descending systems include the corticospinal tract (Nyberg-Hansen and Brodal, 1963; Coulter and Jones, 1977), the raphe-spinal system (Basbaum *et al.*, 1978) and the reticulo-spinal system (Basbaum *et al.*, 1978).

The corticospinal tract cells terminate in LIII-LVI or even VII in the cat (Nyberg-Hansen and Brodal, 1963), and are absent from LI and II. Using autoradiography in the monkey, the origin of the corticospinal tract was demonstrated to be from cytoarchitectonic regions 4, 3a, 3b, 1, 2, and 5 (Coulter and Jones, 1977).

Wall (1967) investigated the influence of the corticospinal pathway upon dorsal horn interneurones and reported no effect on LIV neurones, prominent inhibition in LV and excitation in LVI. Fetz (1968) reported that inhibition is more prominent dorsally and excitation ventrally. The inhibitions reported by Wall (1967) and Fetz (1968) are in agreement with earlier reports of primary afferent depolarization (Carpenter *et al.*, 1963b; Andersen *et al.*, 1964e), indicating the operation of presynaptic inhibition of cutaneous and group Ib and II muscle afferents and inhibition and excitation of various dorsal horn neurones including those giving rise to ascending pathways (Wall, 1967; Fetz, 1968; Coulter *et al.*, 1974).

The raphe spinal system arises from midline raphe nuclei of the brain stem and consists of bilateral pathways descending in the

dorsolateral funiculi (Basbaum and Fields, 1977). Terminations are in LI, LII, LV and medial parts of LVI and VII (Basbaum and Fields, 1977; Basbaum *et al.*, 1978). The parts of the dorsal horn receiving inputs from the raphe nuclei are those parts considered to be concerned with nociception and to give rise to the spinothalamic and spinoreticular tracts.

Fluorescence histochemistry has revealed the existence of numerous noradrenaline and serotonin containing neurones in the raphe spinal fibers (Dahlstrom and Fuxe, 1965). The monoaminergic terminals in the spinal cord disappear 6-8 days after transection of the cord. Iontophoresis of serotonin onto dorsal horn neurones leads to depression of their activity (Engberg and Ryall, 1966; Randic and Yu, 1976). These investigators demonstrated the inhibition of both spontaneous and noxious evoked activity of dorsal horn neurones on administration of serotonin.

Electrical stimulation of the nucleus raphe magnus produces inhibition of the dorsal horn neurones in LI, V and VI that receive a noxious mechanical input (Fields *et al.*, 1977b; Guilbaud *et al.*, 1977b). Willis *et al.*, (1977) also reported that electrical stimulation of the nucleus raphe magnus in the monkey inhibited cells of origin of the spinothalamic tract. In another study by Proudfit and Anderson (1974), it was demonstrated that electrical stimulation of the nucleus raphe magnus leads to primary afferent depolarization indicating presynaptic inhibition of the dorsal horn neurones.

The nucleus reticularis gigantocellularis and nucleus reticularis

magnocellularis also contribute to the descending system to the spinal cord (Basbaum *et al.*, 1978). These workers showed by means of autoradiography that the pathway arising in the nucleus reticularis magnocellularis descends in the ipsilateral dorsolateral part of the spinal cord and terminates in LI, II, V, VI and also VII in the ventral horn. The descending system from nucleus reticularis gigantocellularis was shown by the use of radioactive leucine to terminate ipsilaterally in LVII and LVIII and contralaterally in LVII in the ventral horn (Basbaum *et al.*, 1978). These regions are related to the motor system. Electrical stimulation within the reticular nucleus produces primary afferent depolarization and both dorsal and ventral root potentials (Proudfit and Anderson, 1974), exerting presynaptic control on the spinal cord neurones. Thus even pathways that are generally regarded as part of the motor system produce actions at the spinal cord level that must have consequences for sensations.

Finally, many other descending pathways are capable of influencing either directly or indirectly the activity of the dorsal horn (Willis and Coggeshall, 1978). For example, stimulation of the vestibular nerve has been shown to excite interneurons in both the dorsal and ventral horn (Erulkar *et al.*, 1966). This action could be mediated by way of either vestibulospinal or reticulospinal tracts.

1.2.3.8 Neuropharmacology of the descending systems

Studies using fluorescence histochemistry (Dahlstrom and Fuxe, 1965) have shown serotonin (5-HT) containing cell bodies and

neuronal projections to be associated with brain stem raphe nuclei. Following brain stem stimulation, release of 5-HT in the spinal cord has been demonstrated *in vitro* (Anden *et al.*, 1964) and *in vivo* (Yaksh and Tyce, 1980).

In the dorsal horn there are many 5-HT terminals particularly associated with LI, II and V (Ruda and Gobel, 1980). Recent neuropharmacological experiments support the role of 5-HT as a neurotransmitter in the dorsal horn influencing antinociceptive mediation. Serotonin receptor blockade by administration of mianserin or metergoline shortened the response latencies in rats in the hot-plate and tail-flick tests (Berge *et al.*, 1983). A tonic inhibitory influence mediated by descending 5-HT pathways has been suggested (Berge, 1982; Berge *et al.*, 1983). Chemical lesioning of the spinal 5-HT neurones by intrathecal 5,6,-dihydroxytryptamine also increased sensitivity to noxious stimulation (Fasmer *et al.*, 1983). Depletion of 5-HT by *p*-Chlorophenylalanine reduced stimulation produced analgesia and administration of the precursor of 5-HT, 5-hydroxytryptophan restored the effect (Akil and Mayer, 1972; Akil and Liebeskind, 1975). Thus, descending 5-HT neurones may be involved in the tonic regulation of nociception (Berge and Ogren, 1984).

The site of action is unclear; 5-HT containing terminals appear to synapse largely with dorsal horn neurones forming few, if any, direct contacts with sensory terminals (Ruda and Gobel, 1980). Thus the evidence is strong that descending serotonergic neurones modulate pain sensitivity, despite the fact that some authors have found

inconsistent results. This may be due to different methodologies used (Fasmer *et al.*, 1983; 1984).

Evidence is increasing that descending noradrenergic pathways also participate in spinal modulation of nociceptive information. Stimulation of brain stem nuclei from which noradrenergic neurones originate have analgesic effect (Segal and Sandberg, 1977). Noradrenaline (NA) applied iontophoretically inhibits the activity of nociceptive neurones in the dorsal horn (Belcher *et al.*, 1978; Headley *et al.*, 1978; North and Yoshimura, 1984). Behavioral studies using intrathecal adminstration of NA also show a spinal depression of nociceptive messages (Kuraishi *et al.*, 1979; Kuraishi *et al.*, 1985).

The analgesic effects induced by intrathecal administration of NA are mediated by activation of α-adrenoceptors (Howe *et al.*, 1983). Belcher *et al.*, (1978), found that the inhibitory effects of NA in the dorsal horn are more marked and more selective than those of 5-HT. The inhibitory effects have been demonstrated on neurones located both superficially and in deeper laminae of the dorsal horn (Belcher *et al.*, 1978; North and Yoshimura, 1984).

These descending noradrenergic fibers arise mostly from the locus coeruleus, the subcoeruleus, and from area 5 of the cerebral cortex (Westlund and Coulter, 1980; Bryum *et al.*, 1984). These descending projections terminate in the marginal layer, LII, IV, VI, and the ventral horn (Westlund *et al.*, 1983). Their effect on spinal nociceptive transmission could involve both pre- and postsynaptic

mechanisms (Belcher *et al.*, 1978; Wilcockson *et al.*, 1984), although the details are still not clear.

In a recent study, lesions of descending catecholaminergic pathways were found to alter responses to noxious stimuli in the hot-plate and formalin tests (Fasmer *et al.*, 1986). These pathways tonically inhibit nociceptive sensitivity recorded with the hot-plate test, but tonically enhanced the behavioral responses to pain induced by formalin (Fasmer *et al.*, 1986). This showed that mechanisms involved in the spinal modulation of nociception (by catecholaminergic systems) may be different for different types of pain. There is also evidence for tonic regulation of nociceptive sensitivity by spinal catecholaminergic pathways (Howe *et al.*, 1983).

There is strong evidence supporting the role of endogenous opioids in pain modulation. From the original *in vitro* observations a model was proposed whereby enkephalinergic interneurones found locally in regions of primary afferent synapses provided axo-axonic terminals on SP-containing primary afferents and hence provided an inhibitory system for selective blockade of nociceptive information by opioids (Jessell and Iversen, 1977). Since axo-axonic connections as required have not been found (LaMotte and DeLanerolle, 1981) a modification proposed by Henry (1982) suggests the importance of circulating opioids crossing into the spinal cord and selectively depressing SP release.

Enkephalin and β-endorphin are also present in supraspinal sites allied to pain pathways. Enkephalin has been demonstrated in the

periaqueductal gray matter (PAG) and nucleus raphe magnus (NRM) (Hokfelt *et al.*, 1977). In comparison, β-endorphin is linked with a major neuronal system in the brain originating in the arcuate nucleus of the hypothalamus with axonal projections to many areas including the PAG and nucleus locus coeruleus (Bloom *et al.*, 1979). Intracerebroventricular injection of β-endorphin leads to profound antinociception (Loh *et al.*, 1976) as does the enkephalin analogues (Beddell *et al.*, 1977). Regions around the PAG are among the most sensitive of all sites to elicit antinociceptive effects suggesting the participation of opioid peptides to the descending spinal inhibitory systems (Smith, 1984).

Recently, increasing interest has grown on the role of opioid peptides as neuromodulators, modulating the changes produced by other putative neurotransmitters, eg., 5-HT and NA. Thus, many neurochemical studies have implicated descending 5-HT and NA fibers in the analgesia induced by morphine, and most studies have shown generally a reduction in analgesia induced by morphine after lesioning of 5-HT and NA descending pathways (Deakin and Dostrovsky, 1978; Yaksh, 1979; Proudfit and Hammond, 1981; Berge *et al.*, 1983; Kuraishi *et al.*, 1983).

Thus, the implication of monoamines and endogenous opioids in the mechanism of control exerted by the brain stem is supported by many studies. However, many other substances are found at brain stem level. For example, analgesic effects have been described after the injection of neurotensin in the PAG (Behbehani and Pert, 1984) or acetylcholine in the parabrachial region (Katayama *et al.*, 1984).

Acetylcholine iontophoresis has been shown to excite NRM (Wilcockson *et al.*, 1983). Furthermore the phenomenon of coexistence of neurotransmitters in the same neurones complicates their functions even further.

1.2.3.9 The role of the thalamus and cerebral cortex in nociception

Most earlier studies on the role of the thalamus and cerebral cortex in the modulation of pain were done on war patients with brain lesions (Sweet, 1971).

In man, when vascular lesions destroy the nucleus ventralis posterolateralis of the thalamus, severe sensory loss is found in the contralateral limbs and trunk (Sweet, 1971). Gardner and Cuneo (1945) were able only to follow a few degenerating spinothalamic fibers beyond the midbrain and into the thalamic nucleus ventralis posterolateralis (VPL) after thoracic cordotomy in man.

Using stains for axonal degeneration, Mehler (1957) found that true spinothalamic fibers to the nucleus VPL constitute 30% of the ascending fibers in the chimpanzee. He also saw terminations in other thalamic nuclei; parafascicularis, paracentralis, nucleus centralis lateralis. Using degeneration studies, Bowsher (1957), found degeneration in the ipsilateral nucleus VPL after spinothalamic tractotomy, as well as a little degeneration on the contralateral VPL nucleus. Bowsher (1957) also found terminations in the large nucleus centrum medianum of man, and a few in the rostral part of the

thalamic reticular nucleus.

Gaze and Gordon (1955) were among the first to study electrophysiological properties of the thalamic nociceptive neurones. These authors recorded electrical activity of single units in the thalamus while stimulating the saphenous fibers. They found that about 9 out of the 63 units found, responded only to very strong stimuli like squeezing, pinching, tapping or pricking. These stimuli activated saphenous δ fibers. Monkeys with chronic implanted electrodes (Sweet, 1971) also exhibited behaviour, suggesting pain, when the thalamic nucleus ventralis posterior was stimulated. Electrical lesions in the regions of the centrum medianum caused hypoalgesia to analgesia in man, over varying extents of the contralateral half of the head, limbs or body. There was either a reduction or elimination of complaints about pain on the contralateral side (Sweet, 1971). Units in the ventrocaudal region of the thalamus in the rat responded exclusively to noxious stimulation of the tail (Hellon and Mitchell, 1975). Foreman *et al.*, (1976) antidromically activated spinothalamic cells by stimulation of the thalamus. They showed that spinothalamic tract nociceptors both in LI and V could be excited from either the posterior thalamic nuclei or ventrobasal complex.

Electrophysiological recordings from the medial thalamus of arthritic rats indicated existence of neurones responding to nociceptive stimuli in mediodorsal, anteromedial, ventromedial and ventrolateral nuclei of the medial thalamus and the nearby submedian nucleus (Dostrovsky and Guilbaud, 1990). This suggests that all these

regions may be involved in mediating various aspects of nociception (Dostrovsky and Guilbaud, 1990). The intralaminar nuclei, consisting of centromedial, centrolateral, paracentral, centre median and the parafascicular region, have been implicated in nociception (Perl and Whitlock, 1961; Price and Dubner, 1977; Peschanski *et al.*, 1981; Duncan *et al.*, 1988; Dostrovsky and Guilbaud, 1990). The pulvinar of the thalamus, the ventrocaudal nucleus and the dorsomedian nucleus have also been implicated in pain perception (Richardson, 1974; Fukushima *et al.*, 1976). Some of these nuclei, e.g., pulvinar and dorsomedian nucleus, have been used as targets for stereotaxic surgery for the relief of chronic pain (Richardson, 1974). The ventrobasal complex and intralaminar nuclei have also been shown to consist of neurones that respond exclusively to noxious stimuli in the cat and monkey (Mountcastle and Henneman, 1949; 1952). Thus, the thalamus plays a major role in pain mechanisms.

It is however, in the cortex where pain is perceived. Foerster (1927), one of the early workers who explored the responses in man on electrical stimulation of the cortex, observed that stimulation of the postcentral gyrus (consisting of sensory areas) or the superior parietal lobule elicited contralateral paraesthesias, occasionally so strong so as to be painful. Cardiac pain and severe abdominal pain have also been reported by Foerster (1936) when areas in the postcentral gyrus for the upper trunk and the lower trunk were stimulated.

Stimulation of areas in the postcentral gyrus, precentral gyrus (the motor zone) and a few points anterior or posterior to these two gyri

elicited behaviour suggestive of pain or at least activity in pain pathways (Penfield and Boldrey, 1937).

Erickson *et al.*, (1952) recorded similar results in patients afflicted with either a painful phantom limb (an illusion of persistent presence of a limb after it's amputation, and in this case, a painful illusion) or with the syndrome of thalamic pain. These patients' spontaneous pain in each phantom limb was stopped dramatically on injection of the appropriate areas of the postcentral gyrus with procaine. Lewin and Phillips (1952) reproduced preoperative pain upon stimulation of the postcentral gyrus and obtained relief by removal of this area of the cortex.

War patients with cortical wounds and showing disturbances in pain and thermal senses (Sweet, 1971), had lesions in areas 3a and 3b of the cortex. Eleven patients with isolated cortical lesions were also reported to have impairment of pain perception (Marshall, 1951). Biemond (1956) studied patients with cerebrovascular lesions, neoplasms confined to the cerebral cortex and some with cerebral infarctions. He found all lesions located in the second somatosensory cortex and postulated that this area participates in pain perception as the patients had various degrees of hypoanalgesia to spontaneous pain. Davis and Stokes (1966) reported two case reports of relief of pain for 18 months after excision of the postcentral gyrus.

In experiments with lower primates, application of strychnine locally to a small area of the cortex set up irritation in the corresponding skin area represented. The scratching was more

vigorous contralaterally than ipsilateral to the side of the application of the drug (Dusser and Sager, 1937). This study also points to the ipsilateral cerebral representation of the body.

Degeneration studies using the marchi stain, have shown that thalamic nuclei (posterolateralis and ventralis posteromedialis (VPM) project to the postcentral gyrus of the same cerebral hemisphere (Walker, 1942). The nucleus VPM sends fibers to the lowest or facial sector of the postcentral gyrus, and the most lateral parts of the nucleus ventralis posterior project to the superior part of the gyrus. Stimulation of the thalamocortical projections deep to the second somatosensory cortex reproduced chronic pain in patients. Lesions of the thalamocortical radiations have been used to relieve pain in patients (Tasker *et al.*, 1982). This shows that the thalamus receives nociceptive information and sends it to the cortex.

In the cerebral cortex, nociceptive stimuli appears to project to the somatosensory areas 1 and 2 in cats and dogs (Amassian, 1951) and monkey (Ruch *et al.*, 1952) and in man, mainly area 2 (Penfield and Rasmussen, 1950).

1.3 Tests used in nociception for the evaluation of analgesics and study of nociceptive mechanisms

Nociceptive tests using animal models are used to study pain mechanisms and for testing the efficacy of drugs developed for the management of clinical pain in both humans and animals (Pong *et al.*,

1985). Several tests of nociception have been developed over the years and will be reviewed.

1.3.1 Chemically induced writhing

This test was first introduced by Siegmund *et al.*, (1957). The procedure involves an intraperitoneal injection of phenylquinone which induces "writhing" where the animal has contractions of the abdomen, twisting and turning of the trunk, and extension of the hind limbs. Acetic acid (Koster *et al.*, 1959), bradykinin (Emele and Shanaman, 1963) and acetylcholine (Collier *et al.*, 1968) have also been used to induce writhing. This test is commonly employed as a screening method because of it's simplicity and sensitivity (Taber, 1974). This test, however, lacks specificity as many drugs without analgesic effects in man can effectively inhibit the writhing response in laboratory animals (Chernov *et al.*, 1967). The mechanism of the syndrome is not known, but many mediators have been proposed, including prostaglandins (Deraedt *et al.*, 1980).

1.3.2 Yeast or carageenin induced hyperalgesia

In this test, inflammation and hyperalgesia are induced in the rat hind paw by injection of yeast (Randall and Selitto, 1957) or carageenin (Vinegar *et al.*, 1976). Nociception is then quantified by applying pressure on the inflammed paw by means of a metal cylinder and the pressure (mmHg) at which the animal begins to vocalize or

struggle is recorded. The contralateral non-injected paw is used as a control. Several modifications of the test have been described. Drugs can be administered before, at the time of, or after the injection of the inducing agent (Hunskaar, 1987a). This test has been used to distinguish between drugs acting in the CNS and locally at the site of inflammation (Randall and Selitto, 1957; Vinegar *et al.*, 1976). This test is simple to perform and is sensitive to non-narcotic analgesics (Randall and Selitto, 1957; Vinegar *et al.*, 1976).

1.3.3 Adjuvant induced arthritis

This is a purely chronic model of pain where the stimulus is tonic (Pircio *et al.*, 1975). Polyarthritis is induced in the rat by an intradermal injection of *Mycobacterium butyricum* with Freund's adjuvant into the tails of rats (Pircio *et al.*, 1975). The polyarthritis produced is similar to various human conditions and results from the tests are predictive of the effect of such agents in man (Pircio *et al.*, 1975). The disadvantage is that these animals suffer from an immunological disease (induced by *Mycobacterium butyricum*) which does not necessarily reflect all chronic conditions (Hunskaar, 1987a). Drug effects are usually measured as a reduction in the amount of foot swelling and this may not be indicative of nociception (Hunskaar, 1987a). Vocalization on manipulation of the tibio-tarsal joint has been used to indicate nociceptive threshold (Pircio *et al.*, 1975). This technique does not induce pain in normal (control) rats (Hunskaar, 1987a). Several modifications of this test have been developed. In one

modification, simultaneous measurement of oedema (paw volume) and recording of vocalization have been claimed to separate antiinflammatory from antinociceptive activity of NSAIDs and other drugs (Capetola *et al.*, 1980).

1.3.4 The tail-flick test

This test was first introduced by D'Amour and Smith (1941) and it uses radiant heat focused on the tip of the tail and measures the latency before the rat "flicks" it's tail out of the beam as a sign of nociception. The tail-flick reflex is a spinally integrated reflex (Irwin *et al.*, 1951) not disrupted by spinalization. This test is commonly used in pain research not only for screening drugs but also for study of spinal mechanisms of nociception (Berge *et al.*, 1980; Berge, 1982). These authors noted that the test allowed repeated testing with no conditioning effect, little individual variation, and that it's potency ranking of opiate analgesics correlated well with accepted clinical ratings.

1.3.5 The hot-plate test

The hot-plate test described by Woolfe and MacDonald (1944) is one of the most commonly used tests of nociception in rodents. Originally, the test measured nociceptive responses (kicking and dancing, licking the fore paw, the hind paw or both) of mice placed on the hot-plate at temperatures varying from 55 to 70^{0}C. This test

was later modified (Eddy *et al.*, 1950: Eddy and Leimbach,1953) where a constant hot-plate temperature of about 55^0C was used. The nociceptive responses measured were; shaking of the foot, holding the foot tightly against the body, and licking the fore paw, hind paw or both. Recently, a modified hot-plate test for use in mice and rats has been developed (Hunskaar *et al.*, 1986). The temperature is slowly increased from non-noxious levels upto the end point which is the temperature when the first hind paw lick occurs. If no hind paw lick is observed, the test is terminated at 52^0C (cut off value). This modified increasing temperature hot-plate test is more sensitive and gives more consistent, valid and reliable results (Hunskaar *et al.*, 1986). Many different behavioral criteria have been used as the end point in the hot plate-test, but licking of a fore paw or hind paw is commonly used (Ankier, 1974; Hunskaar *et al.*, 1986).

1.3.6 The formalin test

The formalin test was first described for rats and cats by Dubuisson and Dennis (1977). Subcutaneous administration of 0.05 ml and 0.1 ml of 5% formalin respectively induced pain in the fore paw of these animals. A biphasic pain response was produced. Pain intensity was rated according to a visual analog scale and was given a numerical value from "0" to "3". "0" indicated that the injected paw bore the animal's weight and that there was no discernable difference in how the two fore paws were used during sitting or locomotion. "1" indicated that the fore paw rests lightly on the floor and during

locomotion. The animal had a definite limp. "2" indicated that the injected paw was elevated off the ground, and "3" indicated that the animal licked, bite or shook the affected paw. The formalin test has been modified during subsequent studies (Takahashi *et al.*, 1984; Hunskaar *et al.*, 1985a; Shibata *et al.*, 1989). Only one behavioral response (licking the hind paw) has since then been monitored because it is easy to observe and to quantify and is very consistent (Hunskaar *et al.*, 1985a). Also, scoring the hind paw lick gave more consistent and reliable results because of less interference with rearing and grooming behaviour (Berge *et al.*, 1983).

The formalin test has several advantages over the other tests (Dubuisson and Dennis, 1977). There is no restraint during the observation period. The animals are not stressed as stress can alter pain sensitivity of the animal. The pain stimulus bears a resemblance to most clinical pain. The stimulus elicits a continuous response that enables a temporal nociceptive profile to be measured.

Formalin was found to be a useful tool for obtaining neurogenic inflammation (Brown *et al.*, 1968). Subcutaneous injection of formalin in the animals produces a biphasic response with an early and late phase of high licking activity. The two phases observed in the test represent different types of pain. It is thought that the early phase is evoked by the direct stimulation of nociceptors by formalin and central release of substance P, whereas the late phase is caused by inflammation (Dubuisson and Dennis, 1977; Alreja *et al.*, 1984; Hunskaar *et al.*, 1985a, 1986; Hunskaar and Hole 1987; Shibata *et al.*, 1989). The late phase is mediated by mediators of inflammation e.g.,

bradykinin, prostaglandins, histamine and serotonin (Shibata *et al* ., 1989).

The details of the mechanism of action of formalin are still obscure. In a recent study, it was observed that subcutaneous injection of formalin in the hind paw induced a transient activation of enkephalinergic neurones segmentally in the spinal cord of the rat (Bourgoin *et al.*, 1990). The release of met-enkephalin-like material (MELM) in the cerebrospinal fluid perfusates from the lumbar level took place 5-10 minutes after the formalin injection and was of short duration (5-10 minutes). The transient decrease in nociception in the formalin test, 5-15 minutes after formalin injection in mice (Hunskaar *et al.*, 1985a), is concomitant with the enhancement of spinal MELM release (Bourgoin *et al.*, 1990) and may explain the reduction in nociception during that period of time. Electrophysiological studies have demonstrated a biphasic increase in the excitability of dorsal horn cells following formalin injection into their receptive fields (Dickenson and Sullivan, 1987). It has been demonstrated that the central changes induced in the early phase of the formalin test may contribute to the development of the late phase, suggesting that mechanisms other than inflammation may also be involved (Dickenson and Sullivan, 1987).

Formalin injection into the hind paw produces an increase in the amount of immunoreactive SP in the dorsal horn after 1 hour (Kantner *et al.*, 1985; McCarson and Goldstein, 1989). The increases in dorsal horn SP-like-immunoreactivity may be due to decreased SP release from primary afferent neurones (Henry, 1976; Jancso and Kiraly, 1980). Formalin injection may cause a decrease of SP release.

Lesioning the descending serotonergic pathways using the neurotoxin 5,6-dihydroxytryptamine indicates that the early and the late response in the formalin test may be modulated differently in the central nervous system (Fasmer *et al.*, 1984).

Whatever the mechanism of action, the formalin test is very useful in studies of pain mechanisms, and for evaluation of analgesic drugs, for use in the treatment of either acute or chronic pain (Shibata *et al.*, 1989). Intraperitoneal injections of morphine (0.8 mg/Kg) induces analgesia, in both the early and late phase, in about 10-15 minutes with no return of pain in cats (Dubuisson and Dennis, 1977). Pethidine (8 mg/kg), given intraperitoneally, produced analgesia in the early and late phase but showed greater individual variability than was observed with morphine. In rats, when 0.05 ml of 5% formalin was used, morphine (2 mg/kg) produced only slight analgesia in both phases. Morphine (6 mg/kg) produced clear analgesia and also an increase in the animals' general activities. Pethidine (25 mg/kg) produced analgesia in the early and late phase, although of a shorter duration than with morphine. The rats fell into a stupor 10-20 minutes after drug administration (Dubuisson and Dennis, 1977).

In studies using the modified formalin test in mice, 20 µl of 1 or 5% formalin was used (Hunskaar *et al.*, 1985a; Hunskaar *et al.*, 1986; Hunskaar, 1987b). Nociceptive behaviour in the early phase (0-5 minutes after formalin injection) and the late phase (20-30 minutes after formalin injection) was scored as the amount of time spent licking the injected hind paw. Morphine (2.5-10 mg/kg) inhibited the formalin induced biphasic pain response dose-dependently.

Aspirin, 200-400 mg/kg, inhibited the early response whereas 300-400 mg/kg inhibited the late response (Hunskaar *et al.*, 1985a; Hunskaar *et al.*, 1986; Hunskaar, 1987b). In a later study, aspirin (200-400 mg/kg) inhibited the biphasic response in a dose dependent manner (Hunskaar and Hole, 1987). Naproxen (50, 100 mg/Kg) induced a dose-dependent antinociception in the late phase only. Hydrocortisone (75, 150 mg/kg) and dexamethasone (5, 10 mg/kg) suppressed licking activity in the late phase only (Hunskaar and Hole, 1987).

In a recent study using 20 µl of 0.5% formalin in mice (Shibata *et al.*, 1989), morphine (1, 3, 6 mg/kg), dexamethasone (0.25, 0.5, 1 mg/kg) and hydrocortisone (3, 6.5, 12.5 mg/kg) administration gave similar results to those obtained in earlier experiments. In this study, however, aspirin (100, 200, 300 mg/kg) inhibited only the late response dose-dependently (Shibata *et al.*, 1989).

Recently, the effect of different formalin concentrations on the nociceptive response in the formalin test was studied in mice (Rosland *et al.*, 1990). Using formalin concentrations of 0.02-0.2% only the early phase was observed while a concentration of 1% or more induced both the early and the late phase. When low formalin concentrations (0.2%) were used, repeated testing using the same paw could be performed at intervals of 1 week without any significant change in the response and tissue damage. It was concluded that the formalin concentration should be as low as possible to minimize suffering of the animal. Formalin concentrations of 0.05-0.2% are recommended for studying the early phase, whereas 1% or higher are

recommended for inducing the late phase (Rosland *et al.*, 1990).

Ambient temperature has been shown to influence the licking response in the late phase of the formalin test (Rosland, 1991). An increase in ambient temperature caused an increase in the intensity and duration of licking in the late phase. It is recommended that the ambient temperature in the testing chamber should be carefully controlled to obtain reliable results especially in the late phase of the formalin test (Rosland, 1991).

1.4 Modes of action of narcotic analgesics and antiinflammatory drugs

1.4.1 Narcotic analgesics

Based on different pharmacological characteristics observed in *in vitro* and *in vivo* studies, at least five major opioid receptor subtypes have been postulated; μ, κ, σ (Martin *et al.*, 1976), δ (Lord *et al.*, 1977) and ε (Wuster *et al.*, 1980;1981). Furthermore, subclasses of some of these receptor types have also been identified (Pasternak and Wood, 1986; Zukin *et al.*, 1988). The μ_1 and the μ_2 subclasses of receptors have been postulated, based on the ability of certain irreversible opioid ligands (naloxazone and naloxonazine) to alter high affinity opioid binding (Ling *et al.*, 1986; Pasternak and Wood, 1986). The κ-opioid binding site has been subdivided into three components, κ_1, κ_2 (Zukin *et al.*, 1988) and κ_3 (Clark *et al.*, 1989). These receptors are thought to mediate the actions of opiates as will

be reviewed later.

Autoradiographic binding studies have demonstrated opioid binding sites in many brain areas (Yaksh 1984a) and throughout the spinal gray with the highest density in the substantia gelatinosa (Fields *et al.*, 1980; Czlonkowski *et al.*, 1983; Yaksh 1984b). A significant reduction in opioid binding is observed following rhizotomy or ganglionectomy (LaMotte *et al.*, 1976), and after chemical destruction of small primary afferents with the neurotoxin capsaicin (Gamse *et al.*, 1979). These results suggest the existence of opioid binding sites pre- and postsynaptic to small afferent terminals.

Electrophysiological studies using microiontophoretic application of opioids in the dorsal horn or systemic administration in spinally transected animals have shown a depression in activity evoked by stimulation of high-threshold, slowly conducting afferents (Duggan and North, 1984; Martin, 1984; Yaksh and Noueihed, 1985).

By use of several modalities of nociceptive stimulation (thermal, chemical, mechanical), studies of the effects of spinally administered agents in animals, have provided firm evidence that the spinal opioids induce a significant reduction in pain behaviour (Yaksh and Rudy, 1976, 1977).

The mechanism at the biochemical level through which morphine and related compounds mediate analgesia is not very clear. Morphine and related compounds inhibit the release of substance P from terminals of afferent neuronal pathways (Jessell and Iversen, 1977). This is thought to occur through inhibition of prostaglandin-induced

production of cyclic adenosine monophosphate (c'AMP) (Stone and Perkins, 1979). Opiate receptor stimulation has been linked with a reduction in adenylate cyclase activity and a concomitant reduction in cyclic AMP concentration (Collier and Roy, 1974). This was demonstrated in the rat brain homogenate. Opiates specifically inhibit the stimulation by PGE of c'AMP formation.

Evidence from electrophysiological studies strongly support the idea that the action of opioids is mediated via alterations of ionic fluxes. Chapman and Way (1980) postulated that narcotic drugs exert their effects by producing a decrease in Ca^{2+} flux or binding at the synapse. This results in reduced neurotransmitter release and also a selective decrease in calcium levels. A homeostatic mechanism then comes into effect which tends to reverse the effects of the drug, resulting in an increased calcium content in synaptic vesicles. Therefore, more opiate is needed to produce a response and hence this adaptation results in development of tolerance. Opioids inhibit the calcium-dependent release process and decrease the duration of the calcium ion action potential recorded from the cell body. Changes in voltage-dependent Ca^{2+} influx may be either direct or secondary to K^+ conductance changes (North and Williams, 1983).

A presynaptic site of action of opiates has been suggested from studies using cultured spinal neurones (MacDonald and Nelson, 1978). It was postulated that enkephalins inhibit the release of substance P from these primary afferent neurones (Jessell and Iversen, 1977). These authors also demonstrated that K^+-evoked release of SP from slices of rat trigeminal nucleus was inhibited by

opiates and opioid peptides in a naloxone-sensitive manner. Yaksh *et al* ., (1980) also reported a decrease in SP release evoked by fine fiber stimulation after administration of morphine.

In electrophysiological studies a local hyperpolarization after opioid administration in dorsal root ganglion cells has been recorded supporting the presence of presynaptic opioid receptors (Duggan and North, 1984). These observations suggest a clear presynaptic effect of these agents on primary afferent terminal excitability.

The presynaptic action of these opioids could be due to a depolarization or hyperpolarization of the presynaptic membrane in the afferent neuron. The depolarization could result in decreased calcium ion flux thus resulting in reduced neurotransmitter release (Chapman and Way, 1980). The hyperpolarization could be due to an opening of K^+ channels associated with a reduction in calcium entry and a release of less neurotransmitter (Werz and McDonald, 1983).

Post-synaptic action of opioids have also been demonstrated. Post-synaptic hyperpolarization has been described in the locus coeruleus (Pepper and Henderson, 1980) and in the dorsal horn (Barker *et al.*, 1978). Enkephalin caused a hyperpolarization of dorsal horn neurones via an increase in potassium conductance (Yoshimura and North, 1983).

Recently, it has been hypothesized that the spinal analgesic action of morphine is due in part to the release from primary afferent neurone terminals and activation of A_1 and A_2 adenosine receptors (Sweeney *et al.*, 1987a; Sosnowski *et al.*, 1989). Occupation of

adenosine receptors have been shown to produce mild analgesia (Sweeney *et al.*, 1987b; Sosnowski *et al.*, 1989).

Studies based on the selective μ, δ and κ agonists (Yaksh, 1987), the distinguishable affinity of naloxone for the sites acted upon by the spinal agents (Yaksh, 1987), and the ability to differentially antagonize the effects with agents selective for the receptors (Cotton *et al.*, 1984; Portoghese and Takemori, 1985) showed that the μ, δ, and κ opiate receptors modulate nociceptive processing.

Current evidence has suggested that the opioid receptor is coupled to it's membrane function by a second messenger protein of the guanine nucleotide type (G-protein) (North *et al.*, 1987). This protein has three subunits (α, β, γ). The α-subunit contains the guanine nucleotide binding site and has receptor specificity (Simonds, 1988; Weiss *et al.*, 1988). The G-protein α-subunit in the active state binds 5'-triphosphate, dissociates from the β and γ subunits and directly modulates a number of cellular functions (Allende, 1988) such as adenylate cyclase, phospholipase C and ion channels (Simonds, 1988).

At the membrane level, μ– and δ-opioid agonists have been shown to inhibit neuronal activity by hyperpolarizing the membrane through an increase in potassium conductance. This hyperpolarization leads indirectly to an inhibition of calcium entry during an action potential (North *et al.*, 1987). These effects are mediated through the G-protein which couples the opioid receptor directly to the potassium channel. The κ-agonists inhibit directly the entry of calcium through

voltage dependent calcium channels, involving another G-protein (McFadzean, 1988; Rosenthal *et al.*, 1988).

In addition to their action on terminal receptors, high concentration of opiates produce a weak local anaesthetic effect on isolated nerves (Shefner *et al.*, 1981), and inhibit neuronal firing (Karras and North, 1979).

1.4.2 Steroidal antiinflammatory drugs

Corticosteroids suppress the classic signs of inflammatory reactions (heat, pain and swelling) (Booth, 1988). They inhibit the enzyme phospholipase A_2 activity (Nijkamp *et al.*, 1976; Blackwell *et al.*, 1978), which is necessary for the release of arachidonic acid. Thus, cortiscoteroids ultimately inhibit the formation of some important mediators of inflammation, i.e. prostaglandins, thromboxanes, and the leukotrienes (see Fig. 1) (Vane and Botting, 1987).

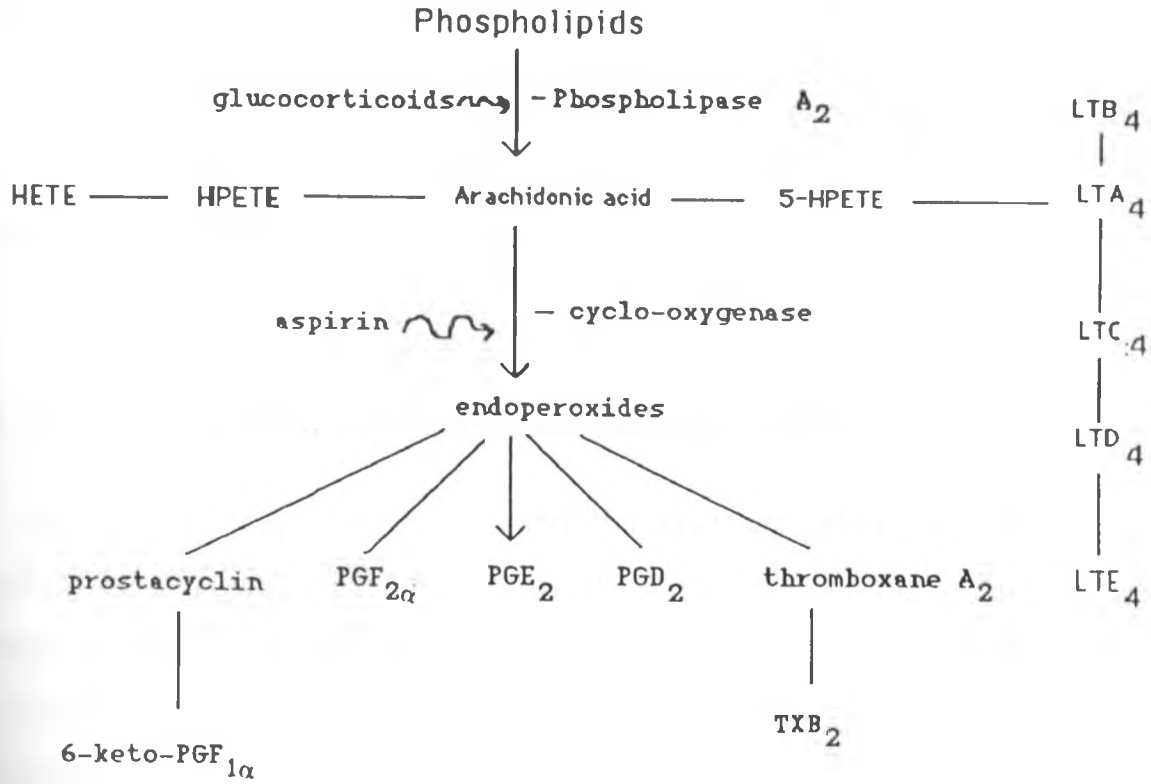

Fig. 1. Catabolic pathways of arachidonic acid (Vane and Botting, 1987).

Antiinflammatory steroids, inhibit phospholipase A_2 indirectly by the release of an inhibitory protein. Glucocorticoids interact with specific membrane receptors (Di Rosa and Persico, 1979; Flower and Blackwell, 1979; Russo-Marie *et al.*, 1979) and after transcriptional events (Danon and Assouline, 1978) lead to the formation of the inhibitory protein (Blackwell *et al.*, 1980). This protein has been variously termed macrocortin (Blackwell *et al.*, 1980), lipomodulin (Hirata *et al.*, 1980) or renocortin (Cloix *et al.*, 1983). The name

lipocortin has been agreed upon (Flower, 1985).

It is now thought that the action of lipocortin is indirect by binding onto calcium and phospholipid rather than the direct inhibition of phospholipase A_2 (Flower, 1985).

1.4.3 Non-steroidal antiinflammatory drugs (NSAID)

Lim and colleagues (1964) used the crossed-perfused dog spleen preparation to demonstrate that NSAIDs produce analgesia peripherally (Lim *et al.*, 1964). However, it was in 1971 that Vane demonstrated that aspirin inhibits the synthesis of prostaglandins by inhibiting the enzyme cyclooxygenase which catalyzes the conversion of arachidonic acid to endoperoxides (Vane, 1971) (Fig. 1). Studies using local injection of small amount of NSAIDs into inflammatory lesions confirmed that there was a peripheral site of action (Ferreira *et al.*, 1978; Flower *et al.*, 1980). Inhibition of PG synthesis by NSAIDs has been demonstrated both *in vitro* (Vane, 1971) and *in vivo* (Willis *et al.*, 1972). However, the correlation between analgesia and the inhibition of PGs is not clear. Prostaglandins are able to sensitize nociceptors to mechanical or chemical stimulation in concentrations found in inflammatory exudates (Flower *et al.*, 1980).

Since primary prostaglandins are probably the most important hyperalgesic mediators present at the site of the inflammatory reactions (Ferreira, 1983), the NSAIDs should be more appropriately referred to as antalgics because they prevent the induction of

hyperalgesia (Ferreira, 1983). Some authors claim that only inflammatory pain can be reduced by these drugs or they are only effective in conditions where prostaglandins are synthesized locally (Ferreira, 1972; Ferreira *et al.*, 1973; Moncada *et al.*, 1975).

Acetylsalicylic acid (ASA) and paracetamol may have analgesic properties independent of inhibition of PG synthesis (Hunskaar *et al.*, 1986). In arthritic rats, ASA has been shown to depress the responsiveness of joint capsule sensory receptors that have an enhanced sensitivity, in comparison to normal rats (Guilbaud and Iggo, 1984).

There is growing interest on the central effects of NSAIDs. A number of studies have provided evidence in support of the central effects of the prototype, ASA. Yaksh and Hammond (1982), demonstrated attenuation of the nociceptive response in the writhing test in rats after intrathecal ASA. Central effects of ASA have been demonstrated in the formalin test (Hunskaar, 1987b) and using capsaicin (Hunskaar, 1985b). In another study, the central effect of ASA, paracetamol, phenacetin and indomethacin were clearly shown on hyperalgesia induced by carageenin (Ferreira *et al.*, 1978). Sodium salicylate increased the nociceptive threshold in rats on stimulation of the lateral hypothalamus (Dubas and Parker, 1971). Activity in single neurones in the rat thalamus elicited by electrical stimulation of afferent C fibers in the sural nerve is depressed by paracetamol, and ASA (Carlsson and Jurna, 1987).

Indomethacin and diclofenac administered intracerebroven-

tricularly inhibited nociceptive responses in arthritic rats (Okuyama and Aihara, 1984). In a more recent study indomethacin and diclofenac, dose-dependently depressed activity evoked by electrical stimulation of afferent C fibers in the ipsilateral or contralateral sural nerve, in single neurones of the rat thalamus (Jurna and Brune, 1990).

The mechanism of action of these NSAIDs at the central level is not clear. Ferreira *et al.*, (1978), suggested that the effect involved inhibition of central release of prostaglandins which lower the threshold of the central pain circuits. Since the cyclooxygenase enzyme from different tissues show differential sensitivity to ASA-like drugs (Flower and Vane 1972), Ferreira *et al.*, (1978) suggested that the antialgesic effect of these drugs was due to their selective action on nervous tissue cyclooxygenase. Subsequent reports, also indicate that prostaglandins may contribute to nociception by an action in the CNS. For example, noxious stimuli elicit the release of prostaglandins from the frog spinal cord (Ramwell *et al.*, 1966) and from the cat cerebral cortex (Ramwell and Shaw, 1966). Low doses of prostaglandins applied via a spinal intrathecal cannula lower nociceptive thresholds (Ferreira *et al.*, 1978; Yaksh, 1982; Ferreira, 1983). Indomethacin (Raffel *et al.*, 1976), aspirin and naproxen (Chiu and Richardson, 1985) decrease prostaglandin output from brain tissue.

The exact site of action at which prostaglandins exert their central effects on nociception is unknown. They are assumed to act centrally on neurones that transmit the nociceptive message (Yaksh, 1982).

They could act at several spinal sites of nociceptive control, including neural circuitry mediating opioid-induced analgesia. Prostaglandins have been reported to block bulbospinal projection neurones that operate in pain control circuits (Yetunde and Levine, 1988). Prostaglandins may facilitate nociceptive signal transmission in the CNS, and non-opioid analgesics or NSAIDs abolish this facilitatory action (Ferreira *et al.*, 1982; Yaksh, 1982). This proposition was made since salicylic acid and some of it's derivatives are known to markedly change the electrophysiological properties of neuronal membranes (Barker and Levitan, 1971; Levitan and Barker, 1972) and block impulse conduction in nerve fibers (Riccippo Neto and Narahashi, 1976).

Shyu *et al.*, (1984) demonstrated that increased activity of central 5-HT pathways were associated with dental analgesia and enhanced aspirin-induced analgesia, whereas decreased activity of these pathways correlated with dental hyperalgesia and diminished aspirin-induced analgesia. The preoptic anterior hypothalamic area seems to be the most sensitive site of the brain for this central aspirin mediated effect. Serotoninergic and catecholaminergic mechanisms could be involved (Shyu and Lin, 1985). Intravenous administration of acetylsalicylate of lysine, a soluble salt of aspirin, reduced the firing discharge of thalamic neurones evoked by noxious stimuli (Groppetti *et al.*, 1988). Microinjections of ASA into the preoptic region of the hypothalamus depressed nociceptive responses in monkeys in a way that depended on intact functioning of monoaminergic pathways (Tagliamonte *et al.*, 1971).

1.5 The effect of opiates on behaviour

Morphine along with other opiate drugs produces excitatory effects that include hyperkinesis (Vasko and Domino, 1978). The excitatory effects of morphine are more prominent when low doses are used (Clark, 1979). With higher doses, a characteristic biphasic response is observed; the drug produces depressant and then excitatory effects (Mucha *et al.*, 1981; Numan and Lal, 1981). Martin *et al.*, (1963) showed that a 60 mg/kg dose of morphine produced depressant effects including hypokinesis, whereas a 5 mg/kg dose produced excitatory effects including hyperkinesis. Bartoletti *et al.*, (1983) also reported that morphine's kinetic effect differs markedly with dose, excitatory kinetic action being prominent at low doses, whereas sedation increases at higher doses. The fact that larger doses of a drug can produce less effect than some optimal dose was interpreted in terms of differences in the population and distribution of receptors occupied by the drug at different doses (Messing *et al.*, 1979).

It has been reported that organisms appear to develop tolerance to the depressant actions of morphine (Hinson and Siegel, 1983) whereas the excitatory actions rarely diminish and often increase in intensity after repeated administration (Eposito *et al.*, 1979; Bartoletti *et al.*, 1983), which could be due to sensitization. Some authors have suggested that the excitatory effects such as hyperkinesis and hyperthermia could reflect direct actions of opiates (Mucha *et al.*, 1981) whereas others have suggested that the

excitatory effects are secondary conditioned responses for the initial unconditioned depressant effects of opiates (Hinson and Siegel, 1983).

The behavioral effect of morphine may depend on the emotional state of the individual (D'Amato and Castellano, 1989). Morphine has been shown to have anti-emotional properties. Opiates influence behaviour through a decrease of emotional levels (File and Rodgers, 1979; Castellano *et al.*, 1984), particularly in stressful conditions (File and Rodgers, 1979). For example, a decrease in emotionality can account for morphine-induced memory impairment in rodents (Gaungher and Kapp, 1978; Castellano *et al.*, 1984).

Apart from hyperkinesis (Vasko and Domino, 1978), the other excitatory effects of morphine and other opiate drugs include hyperthermia (Cox *et al.*, 1979) and hypermetabolism (Martin *et al.*, 1963; Lin, 1982).

There also appears to be a species difference in the prominent response observed on injection of opiate drugs (Simon and Hiller, 1978). Central nervous system (CNS) depression is seen in the dog, monkey and man while stimulatory behaviour is elicited in the cat, horse, goat, sheep, pig and cow following systemic administration of morphine (Simon and Hiller, 1978). The distribution pattern of the opiate binding sites differed in the amygdala and the frontal cortex. These regions are at least two times higher in receptor level for the species that show CNS depression than for the species that show CNS excitation to opiates (Simon, 1977). Administration of 5, 10 and

20 mg/kg of morphine hydrochloride in cats, i.p., produced hyperexcitement and aggressive behaviour (Booth, 1988). Convulsive seizures have been induced in the dog and rabbit with large doses of morphine (Booth, 1988). The ability of opiates to induce generalized convulsive seizures has been considered to be an undesirable effect (Martin, 1984).

The mechanism of induction of excitatory/depressant effects on injection of opiates is not as yet clear. Geller *et al.*, (1983) hypothesized that the stimulatory/inhibitory effects of morphine reflect distinct agonistic actions of opiates on opiate receptors. Jorenby *et al.*, (1988) proposed that the stimulatory behaviour could be due to activation of an excitatory opioid receptor subtype.

Opiate receptors are widely distributed in the brain (Kuhar *et al.*, 1973: Kuhar and Atweh, 1977) but are very highly concentrated in the limbic system (Simon and Hiller, 1978), which is strongly implicated in the control of emotional behaviour.

Frenk *et al.*, (1978) demonstrated specific opiate receptor types involved in the different effects observed on administration of opiate drugs. They showed that the same dose of enkephalin (120µg) caused analgesia without seizures when injected near the periaqueductal grey (PAG), and induced seizures without analgesia when administered near the dorsomedial nucleus of the thalamus. Seizures were accompanied by myoclonic twitches, catalepsy, muscular rigidity and "wet-dog" shakes which were naloxone reversible (Frenk *et al.*, 1978). These effects suggest that the enkephalin-induced

analgesia and seizures are mediated by opiate receptors located in different regions of the brain that are pharmacologically different. They concluded that enkephalin-induced seizures could have been mediated by δ-receptors in the dorsomedial thalamus, and analgesia, by μ-receptors in the PAG. The σ-opiate receptors mediate mania (Snyder, 1984) and are concentrated in the hippocampus. The κ-opiate receptors are localized in the deep layers of the cerebral cortex and their stimulation causes muscular contractions (Chavkin *et al.*, 1982).

It is also possible that stimulatory effects of morphine and related compounds are due to indirect action of these drugs on other brain systems. Biphasic effects of 10 mg/kg morphine on brain acetylcholine utilization in rats were observed in the hippocampus, thalamus and hypothalamus (Vasko and Domino,1978). Basbaum *et al.*, (1973) abolished the locomotor depressant effects of morphine by depleting brain serotonin levels using *p*-Chlorophenylalanine and the stimulant actions by depleting catecholamines with α-methyl-tyrosine. Morphine's hyperkinetic effect has also been reported to reflect agonistic action of opiates on mesolimbic dopaminergic neurones (Stewart and Vezina, 1987), and on cerebral noradrenergic functions (Booth, 1988). Thus, the actions of opiates are complex and may be mediated by multiple neurotransmitters and different pathways.

1.6 Objectives

The objectives of this study were:-

1. To investigate whether formalin induces both acute and chronic pain in the naked mole-rat, as has been observed in other rodents, the rat and mouse.

2. To investigate the analgesic effects of commonly used analgesics and antiinflammatory drugs in the formalin test.

3. To provide information on the nervous system and particularly the nociceptive system of the naked mole-rat.

It is hoped that the information provided may be useful to zoo workers taking care of such animals.

CHAPTER 2

2.0 MATERIALS AND METHODS

2.1 Experimental animals

Naked mole-rats were obtained from Kathekani in the Machakos district of Kenya (240 km South East of Nairobi), an arid region characterized by an ambient temperature of 27-34^{0}C and an annual rainfall of less than 700 mm. They were caught by opening foraging burrows which were recognized by the presence of fresh soil covering the burrow inlets. The entrances of the burrows were cleared of soil and pieces of food (sweet potatoes) were placed there to attract them. Immediately a naked mole-rat appeared at the entrance while coming to investigate the damaged burrow or to fetch the food, the tunnel was quickly blocked using a hoe and the mole caught. The animals were then placed in large tins with food and soil as bedding and transported by railway to the laboratory in Nairobi.

They were kept in opaque metal cages of size 1m x 0.5m x 0.5m, with a fitting lid which had a few holes made into it to allow circulation of air. The aim of using the opaque cages was to subject them to total darkness (24 hours per day), to simulate their natural environment (the dark burrows). Since these naked mole-rats are colony rodents, they were kept in colonies of 20-50 animals per cage. After 2 weeks, when it was observed that they had started breaking through the joints of their metal cages with their sharp teeth and escaping through the holes they created, they were transferred to

smooth round opaque plastic cages (50cm diameter x 20cm perpendicular height).

Tissue paper was used as bedding instead of soil. This helped keep the animals warm and it also absorbed their urine. The bedding was changed every day. The ambient temperature was kept at 29-30^{o}C by using two 250 W infrared lamps that were centrally placed above the cages. The height of the infrared lamps above the cages was adjusted to maintain the temperature at the required level.

The animals were fed with sweet potatoes and carrots *ad-libitum* and were allowed to adapt to the laboratory environment for at least a month before the start of the experiments. During the acclimatization period, the animals were handled twice daily. Animals weighing 35-40g were used in the experiments. All experiments were done in light (8.00-13.00 hrs and 14.00-16.00 hrs). No attempt was made to determine the sex of the animals since this can only be done after laparotomy. A total of 265 animals were used.

2.2 Experimental procedure

2.2.1 Drugs and dosages

The analgesic and anti-inflammatory drugs used during the experiments were as follows:-

Acetylsalicylic acid (Svaneapoteket, Bergen, Norway; 200, 400, 600 mg/kg) was dissolved in 0.1M Tris buffer, pH = 7.4. Codeine phosphate (Norsk medisinaldepot, Bergen; 10, 25, 50 mg/kg), pethidine hydrochloride (Roche, England; 10, 20, 30 mg/kg), dexamethasone phosphate (Merck, Sharp and Dohme, U.S.A.; 10, 20,

30 mg/kg), hydrocortisone sodium succinate (Lyka labs, Bombay, India; 40, 75, 150 mg/kg), naproxen (Astra, Sweden; 50,100, 200 mg/kg) and naloxone hydrochloride (Endo laboratories, U.S.A.; 2mg/kg) were all dissolved in 0.9% NaCl.

2.2.2 Experimental design

A complete randomized design was used in the experiments (Steel and Torrie, 1981). The animals were chosen at random and were used only once. The drugs or vehicle were injected blindly (the experimenter was not aware of the drugs or vehicle used until after data analysis).

2.2.3 Drug administration

The naked mole-rats were handled as follows for drug administration:-

The animal was carefully picked out of the cage by holding the loose skin on the dorsal side of the neck using the right hand, and placing it on the palm of the left hand. The tail was held with the little finger of the same (left) hand. The ventral surface of the animal was then exposed. Using a micro-litre syringe (100µl) or a plastic syringe (1ml), the drug or vehicle was injected intraperitoneally (i.p.) (1cm to the left of the midline and on the lower abdomen). A 26-gauge needle was used. Aspiration was performed to ensure that the drug or vehicle was injected i.p. but not into the intestines.

All injections were performed i.p. 30 minutes before injection of the formalin solution. The injection of naloxone was repeated at 30 minute intervals for 4 hours, in the experiments done to study agonistic behaviour after pethidine hydrochloride or codeine phosphate administration. In the control experiments, an equal volume of vehicle was similarly injected.

2.2.4 Formalin test

The modified formalin test (Kanui and Hole, unpublished) was used for nociceptive testing. The animals were adapted to the observation chamber (Perspex box, 30cm x 30cm x 30cm) 20 minutes prior to the formalin injection. Using a micro-litre syringe, 20 µl of 10% formalin in 0.9% NaCl was injected subcutaneously into the dorsal right hind paw of each animal. A 26-gauge needle was used. The animal was returned to the observation chamber immediately after the formalin injection and the observation period started. Two parameters were recorded simultaneously as follows:-

a) The total number of licks were counted using a manual counter and recorded over a one hour observation period, in blocks of 5 minutes.

b) The amount of time (seconds) the animal spent licking the injected hind paw was recorded using a stop-watch over a one hour observation period, also in blocks of 5 minutes.

Pain behaviour was also studied over a 2 hour observation period.

2.2.5 Agonistic behaviour

Based on preliminary experiments, agonistic behaviour was studied immediately after injection of pethidine hydrochloride (10, 20, 30 mg/kg), codeine phosphate (10, 25, 50, mg/kg) and/or after naloxone hydrochloride (2mg/kg). Naloxone hydrochloride was injected i.p. alone or in combination with the opiate drugs. Naloxone injections were repeated every 30 minutes for 4 hours. The animals were transferred to their home cages, 10 in each cage (colony cages), or to single cages (1 in each cage), after administration of the drugs. The animals were observed continuously for 60 minutes. The occurrence or not of aggressive (in single cages excitement) and hyperactive behaviour was scored. The number of animals participating in a fight were counted and recorded. 18 hours later, the number of dead mole-rats, the wounds and blood spots on the animals were counted and recorded. The effect of pethidine hydrochloride and codeine phosphate on motor impairment was also studied. The onset of the impairment after the i.p. injection of the drug or vehicle, the duration, and the frequency of reccurrence were recorded using a timer.

2.2.6 Statistical analysis

Data was analysed using analysis of variance (ANOVA). Student's t-test subsequent to ANOVA was performed where comparisons were restricted to two means. The level of significance was set at 5% ($P<0.05$). Results are presented as mean $\pm$ standard error of mean (s.e.m.).

CHAPTER 3

3.0 RESULTS

3.1 The formalin test

Injection of 20 µl of 10% formalin subcutaneously into the dorsal right hind paw produced distinct behavioural responses, licking and biting of the injected paw. Two distinct periods of high pain behaviour were identified; the early phase lasting for the first 5 minutes and a second, the late phase, starting 20-30 min after injection of formalin (Fig. 2). The late phase could also be demonstrated 120 minutes after the injection of formalin (Fig. 3). The vehicle (0.9% NaCl) induced only minimal pain response (Fig. 2).

3.1.1 Pethidine hydrochloride

Intraperitoneal pethidine (20 or 30mg/kg) significantly reduced licking activity during the early phase of the formalin test (20 and 30 mg/kg: $F_{(1,18)} = 7.34$ and 6.85 respectively, $P < 0.05$) (Table 1 and Fig. 4). The mean number of licks were notably lower after pethidine 20 mg/kg (25.9 ± 10.35) and 30 mg/kg (31.5 ± 7.42) than in the vehicle-treated controls (78 ± 16.39). In the late phase, there was a highly significant reduction in the number of licks in pethidine-treated animals (20 or 30 mg/kg ; $F_{(1,18)} = 22.12$ and 23.51 respectively, $P < 0.001$)(Fig.4)) when

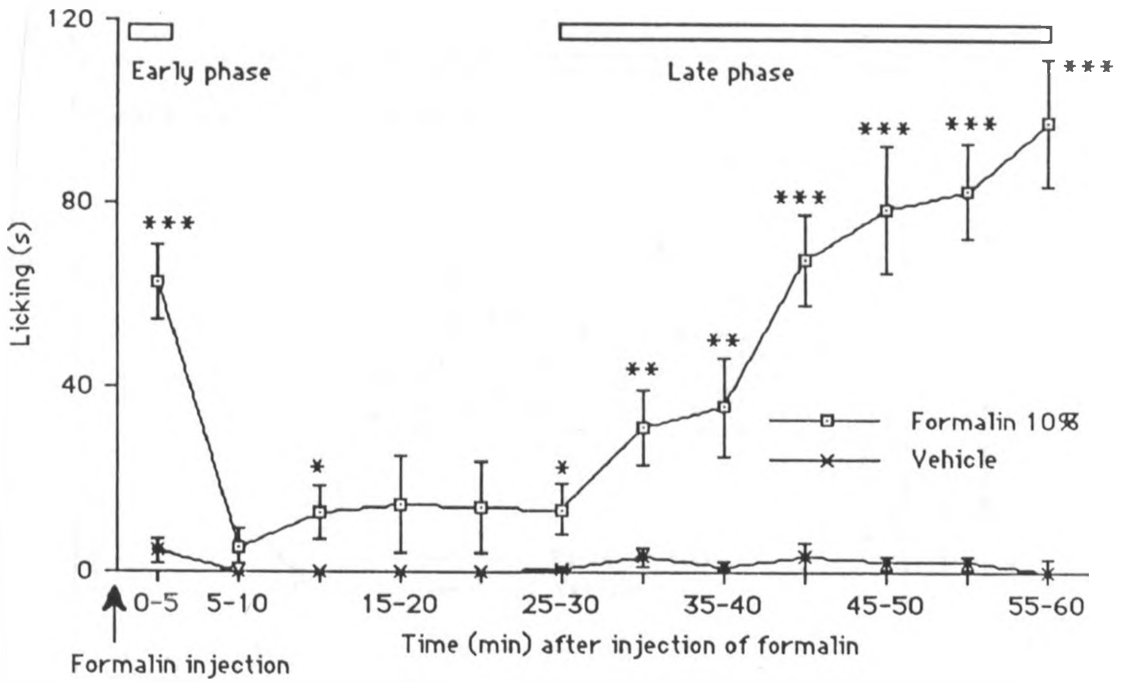

Fig. 2.: Time-course of paw-licking after a subcutaneous injection of 20 µl of 10% formalin into the dorsal right hind paw (mean ± s.e.m.; n = 10; *P< 0.05,**P <0.01,***P < 0.001, Student's t-test subsequent to ANOVA). Each point represents the amount of time the animals spent licking the injected hind paw during a 5 minute observation period.

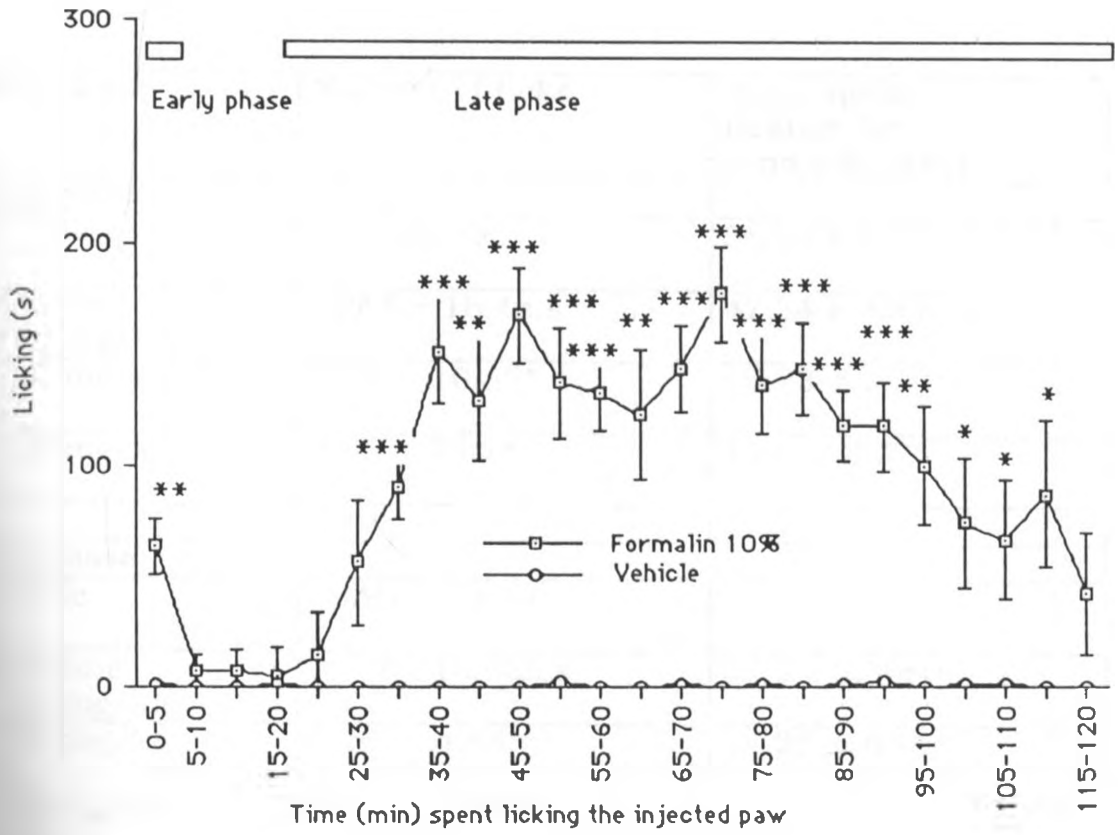

Fig. 3.: Time-course of paw-licking after a subcutaneous injection of 20 µl of 10% formalin or vehicle into the dorsal right hind paw (mean ± s.e.m.; n = 5; *P< 0.05,**P <0.01,***P < 0.001, Student's t-test subsequent to ANOVA). Each point represents the amount of time the animals spent licking the injected hind paw during a 5 minute observation period.

Table 1: Number of licks and time spent licking the injected hind paw (mean ± s.e.m.) after administration of vehicle or pethidine (10, 20, 30 mg/kg) in the early and late phase of the formalin test. In this and subsequent tables, 10 naked mole-rats were used in each group.

Drug/ Dose	**Number of licks**	**Time spent licking the hind paw (sec)**
Early phase		
Vehicle	78 ± 16.39	36.94 ± 6.51
Pethidine 10 mg/kg	109.6 ± 16.4n.s.	47.34 ± 9.39n.s.
20 mg/kg	25.9 ± 10.35*	15.23 ± 5.71*
30 mg/kg	31.5 ± 7.42*	15.6 ± 3.77*
Late phase		
Vehicle	79.94 ± 14.64	35.26 ± 6.63
Pethidine 10 mg/kg	70.16 ±16.62n.s.	32.2 ± 9.38n.s.
20 mg/kg	7.73 ± 4.64***	14.25 ± 6.85*
30 mg/kg	5.89 ± 4.36***	2.57 ±1.9***

* - Significant difference at $P < 0.05$.
*** - Significant difference at $P < 0.001$.
n.s. - Not statistically significant.
Student's t-test subsequent to ANOVA.

compared to the controls. Pethidine 20 or 30 mg/kg-treated animals licked less (7.73 ± 4.64 and 5.89 ± 4.36 respectively) than the controls (79.94 ± 14.64).

Pethidine (20 or 30 mg/kg) significantly reduced the time spent licking the injected hind paw in the early phase of the formalin test (20 or 30 mg/kg: $F_{(1,18)} = 6.29$ and 8.06 respectively, $P < 0.05$) (Table 1 and Fig. 5). The 20 and 30 mg/kg-treated groups spent 15.23 ± 5.71 and 15.6 ± 3.77 secs licking the injected hind paw respectively. The controls spent over twice the amount of time (36.94 ± 6.51 sec) licking the injected paw. In the late phase, pethidine (20 mg/kg) significantly reduced the time spent licking the injected hind paw ($F_{(1,18)} = 2.53$, $P < 0.05$) (Fig. 5). The control animals spent 35.26 ± 6.63 sec licking while the pethidine (20 mg/kg)-treated animals spent 14.25 ± 6.85 sec licking the injected paw. Administration of pethidine (30 mg/kg) caused a more marked reduction in the time spent licking the injected hind paw during the late phase. The animals spent 2.57 ± 1.9 sec licking the paw. The reduction was statistically significant ($F_{(1,18)} = 22.48$, $P < 0.001$) (Fig. 5).

Administration of pethidine (10 mg/kg) failed to cause any significant reduction in licking activity in the early phase ($F_{(1,18)} = 1.79$, $P = 0.2$) and the late phase ($F_{(1,18)} = 0.2$, $P = 0.66$) of the formalin test (Fig. 4). Similarly, there was no significant reduction in time spent licking the injected paw in the early phase ($F_{(1,18)} = 0.83$, $P = 0.37$) and

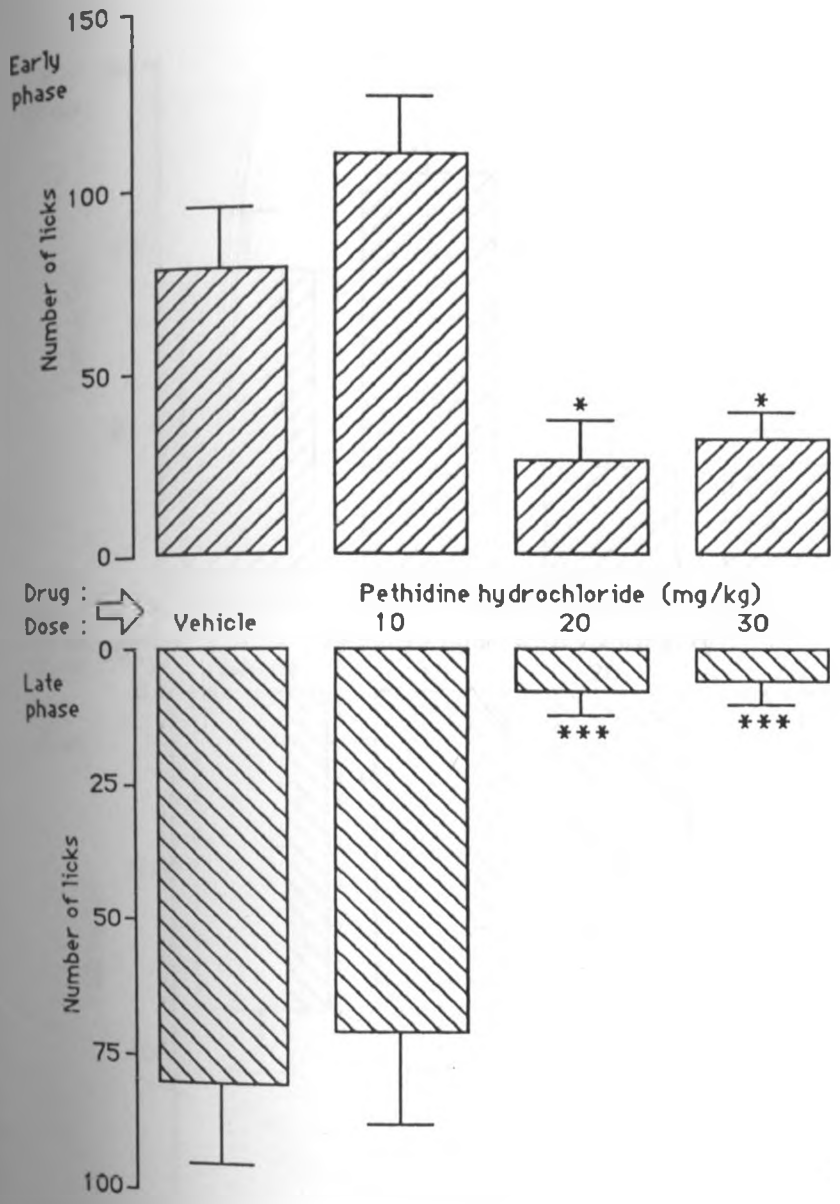

Fig. 4.: Antinociceptive effect of intraperitoneally administered pethidine (10, 20, 30 mg/kg) or vehicle on licking activity, after a subcutaneous injection of formalin in the hind paw, in the early and late phase of the formalin test (mean ± s.e.m.; n = 10; $F_{(1,18)}$ =1.79, 7.34, 6.85 and 0.2, 22.12, 23.51 for the early and late phase respectively; *P< 0.05, ***P < 0.001, Student's t-test subsequent to ANOVA).

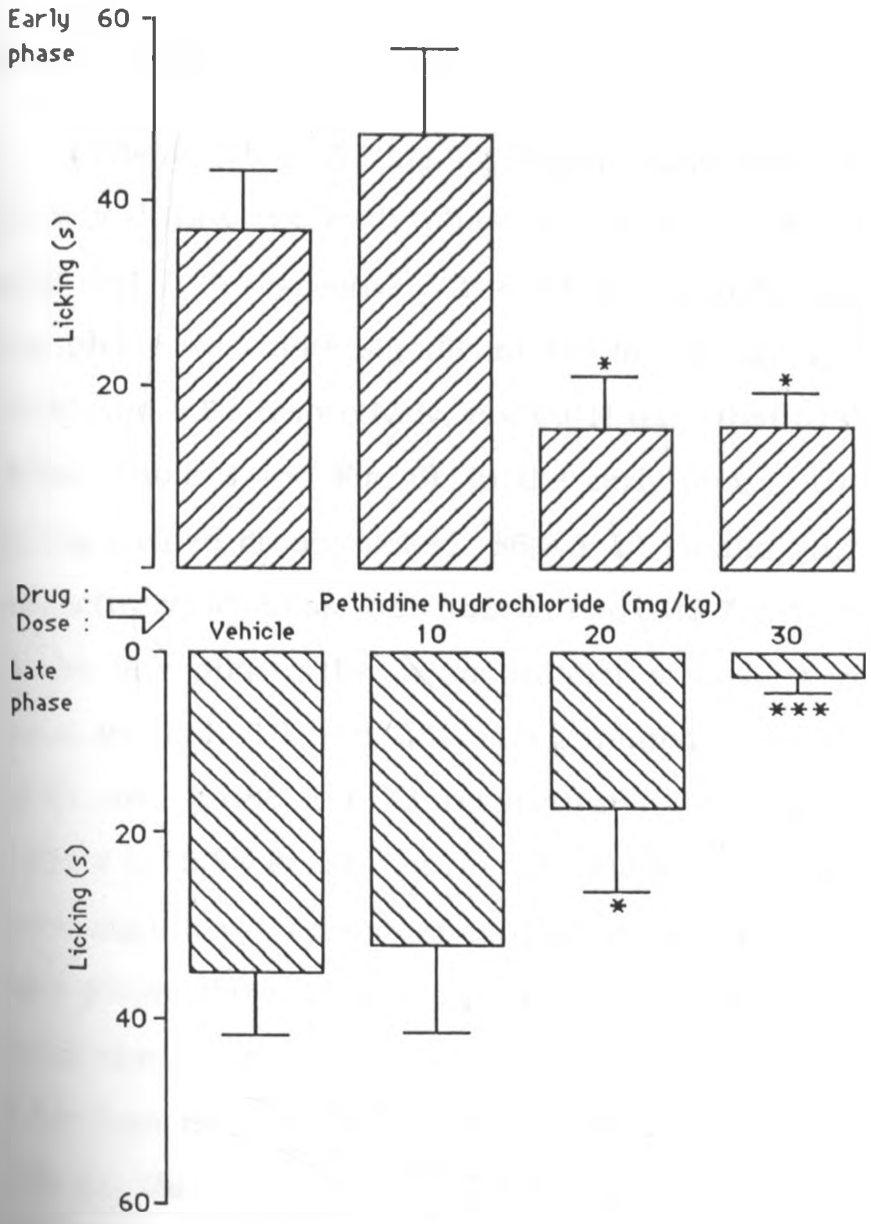

Fig. 5.: Effect of intraperitoneally administered pethidine (10, 20, 30 mg/kg) or vehicle on time spent licking the injected hind paw in the early and late phase of the formalin test (mean ± s.e.m.; n = 10; $F_{(1,18)}$ = 0.83, 6.29, 8.06 and 0.07, 2.53, 22.48 for the early and late phase respectively; *P< 0.05, ***P < 0.001, Student's t-test subsequent to ANOVA).

the late phase ($F_{(1,18)}$ = 0.07, P = 0.79) of the formalin test (Fig 5). This data is summarized in Table 1.

3.1.2 Codeine phosphate

Codeine (10 or 25 mg/kg) significantly reduced licking activity during the early phase (10 or 25 mg/kg: $F_{(1,18)}$ = 4.58 and 7.99 respectively, P < 0.05). The reduction in the late phase was more significant (10 or 25 mg/kg: $F_{(1,18)}$ = 10.35 and 9.51 respectively, P < 0.01) than that in the early phase (Table 2 and Fig. 6). In the early phase, 10 and 25 mg/kg-treated groups licked 86.3 ± 13.15 and 76.7 ± 9.47 respectively, whereas the controls had 133.7 ± 17.81 licks. In the late phase, the mean number of licks were much lower in both 10 and 25 mg/kg-treated groups (57.3 ± 10.21 and 64.86 ± 6.73 respectively) than the controls (118.03 ± 15.88 licks). Codeine (50 mg/kg) caused an even more significant reduction in the licking activity in the early phase (50 mg/kg: $F_{(1,18)}$ = 36.13, P < 0.001)(Fig. 6) where the mean number of licks were much lower (20 ± 6.38) than in controls (133.7 ± 17.81). In the late phase, codeine (50 mg/kg) completely abolished the licking activity (50 mg/kg: $F_{(1,18)}$ = 55.28, P < 0.001)(Fig.6).

The total time spent licking the injected right hind paw was less significant after codeine 10 mg/kg in both the early (41.3 ± 5.58 sec) and the late phase (32.41 ± 5.67 sec) (10 mg/kg: $F_{(1,18)}$ = 4.58, P < 0.05 and 10.35, P < 0.01

Table 2: Number of licks and time spent licking the injected hind paw (mean ± s.e.m.) after administration of vehicle or codeine (10, 25, 50 mg/kg) in the early and late phase of the formalin test.

Drug/Dose	**Number of licks**	**Time spent licking the hind paw (sec)**
Early phase		
Vehicle	133.7 ± 17.81	62. 64 ± 8
Codeine 10 mg/kg	86.3 ± 13.15*	41.3 ± 5.58*
25 mg/kg	76.7 ± 9.47*	39.66 ± 5.81*
50 mg/kg	20 ± 6.38***	10.77 ± 3.53***
Late phase		
Vehicle	118.03 ± 15.88	61.28 ± 7.76
Codeine 10 mg/kg	57.3 ± 10.21**	32.41 ± 5.67**
25 mg/kg	64.86 ± 6.73**	33.61 ± 3.69**
50 mg/kg	0 ± 0***	0 ± 0***

* - Significant difference at $P < 0.05$.
** - Significant difference at $P < 0.01$.
*** - Significant difference at $P < 0.001$.
n.s. - Not statistically significant.
Student's t-test subsequent to ANOVA.

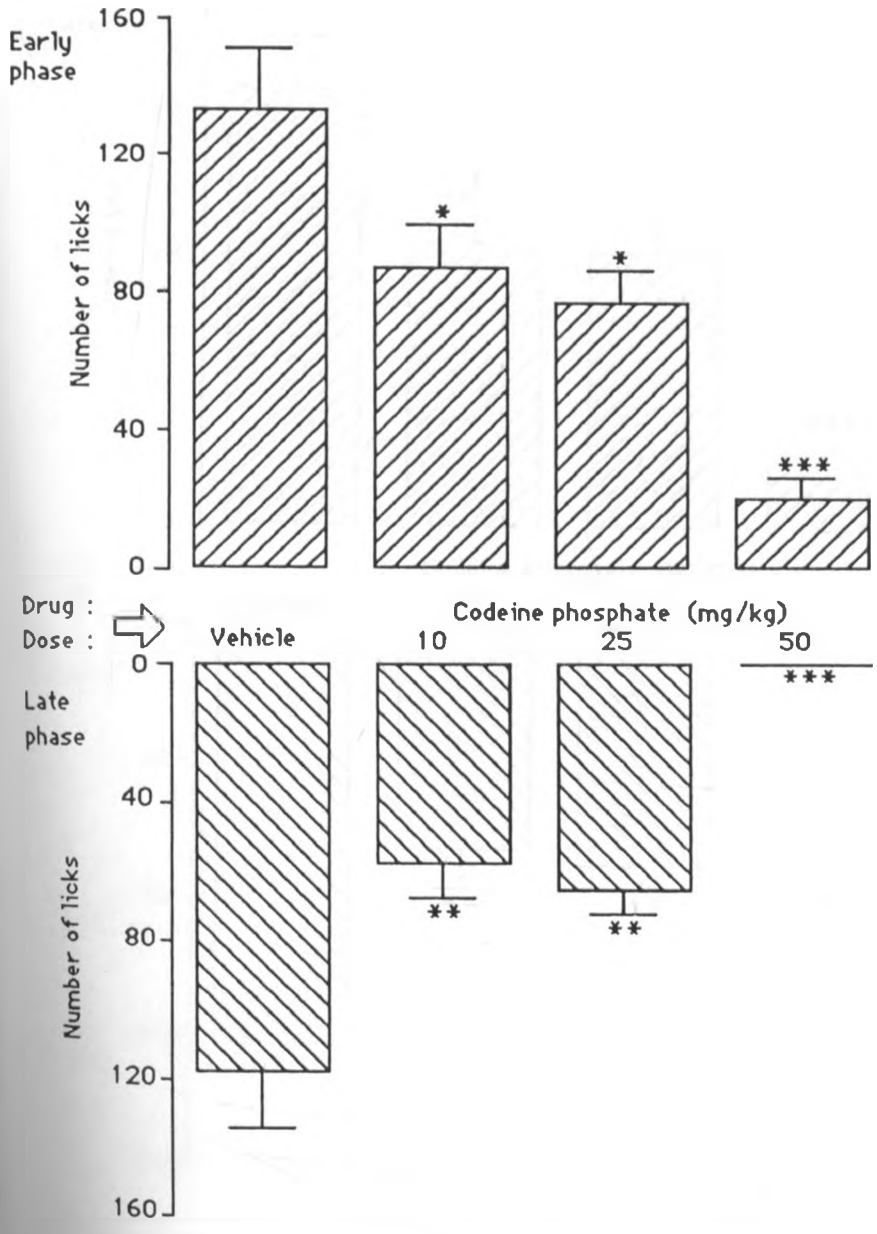

Fig. 6.: Effect of intraperitoneally administered codeine (10, 25, 50 mg/kg) or vehicle on licking activity in the early and late phase of the formalin test (mean ± s.e.m.; n = 10; $F_{(1,18)}$ = 4.58, 7.99, 36.13 and 10.35, 9.51, 55.28 for the early and late phase respectively; *P< 0.05,**P <0.01,***P < 0.001, Student's t-test subsequent to ANOVA).

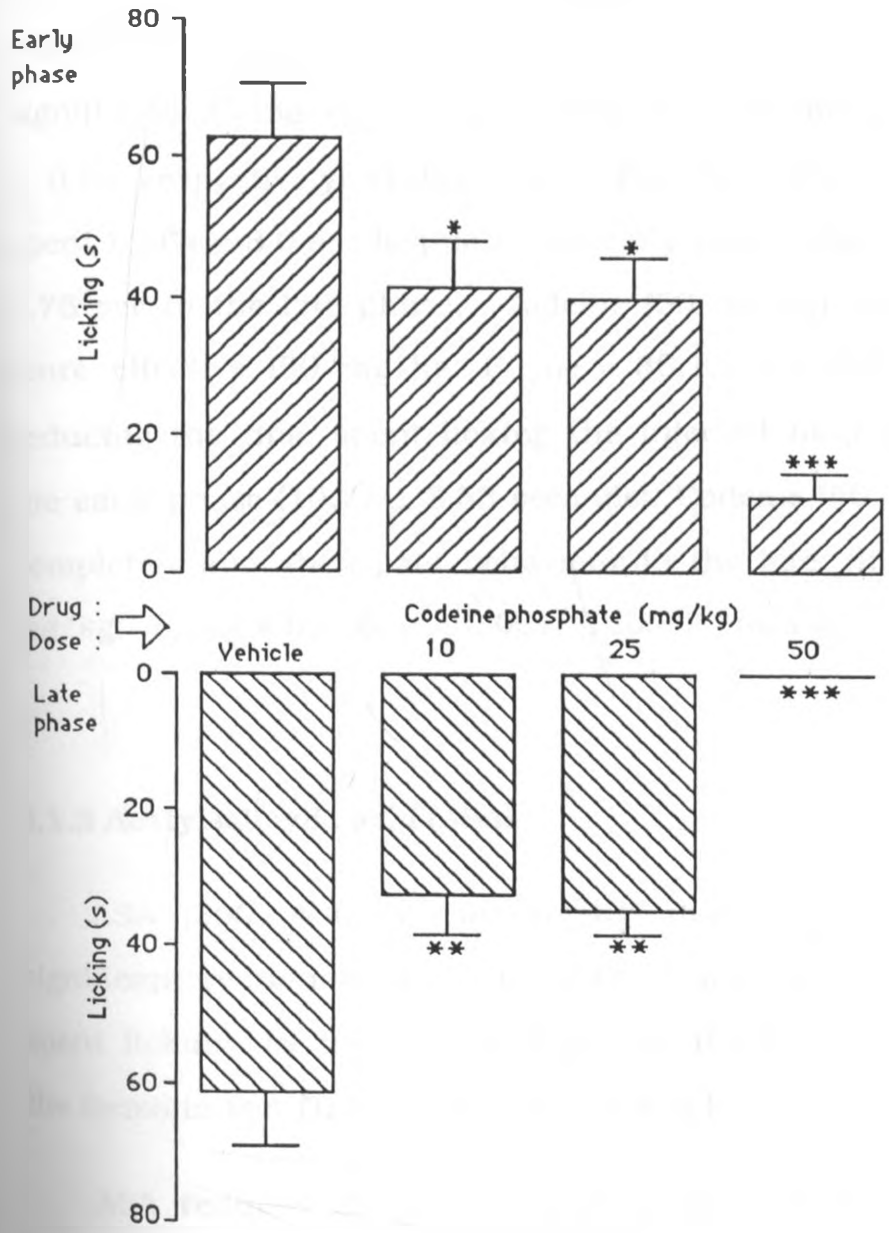

Fig. 7.: Effect of intraperitoneally administered codeine (10, 25, 50 mg/kg) or vehicle on time spent licking the injected hind paw in the early and late phase of the formalin test (mean ± s.e.m.; n = 10; $F_{(1,18)}$ = 4.58, 7.99, 36.13 and 10.35, 9.51, 55.28 for the early and late phase respectively; *P< 0.05,**P <0.01,***P < 0.001, Student's t-test subsequent to ANOVA).

respectively). Codeine (25 mg/kg) caused a reduction of the time spent licking the hind paw in both phases. The time spent licking in both phases was 39.66 ± 5.81 and 33.61 ± 3.69 sec respectively. The reduction was even more significant (25 mg/kg: $F_{(1,18)}$ = 7.99, $P < 0.05$ and 9.51, $P < 0.01$ respectively) (Table 2 and Fig. 7). The controls spent 62.64 ± 8.0 sec licking in the early phase and 61.28 ± 7.76 sec in the late phase. Codeine (50 mg/kg) was even more effective (50 mg/kg: $F_{(1,18)}$ = 36.13, $P < 0.001$) in reducing the time spent licking the injected hind paw in the early phase (10.77 ± 3.53 seconds). Codeine (50 mg/kg) completely abolished pain behaviour in the late phase (50 mg/kg: $F_{(1,18)}$ = 55.28, $P < 0.001$) (Table 2 and Fig. 7).

3.1.3 Acetylsalicylic acid (ASA)

ASA (200, 400 or 600 mg/kg) did not cause any significant reduction in the number of licks and the time spent licking the injected hind paw in the early phase of the formalin test (Table 3 and Figs. 8 and 9).

ASA reduced the licking activity and the amount of time spent licking the injected hind paw in the late phase only. The mean number of licks in the 400 mg/kg injected group (78.59 ± 8.5) were significantly lower ($F_{(1,18)}$ = 4.90, $P < 0.05$) than those of the controls (118.5 ± 15.9) (Table 3 and Fig. 8). Similarly, the amount of time spent licking the injected hind paw was significantly less in the 400 mg/kg-

Table 3: Number of licks and time spent licking the injected hind paw (mean ± s.e.m.) after administration of vehicle or ASA (200, 400, 600 mg/kg) in the early and late phase of the formalin test.

Drug/dose	Number of licks	Time spent licking the injected hind paw (sec)
Early phase		
Vehicle	150 ± 17.52	65.13 ± 8.52
ASA 200 mg/kg	122.7 ±13.78n.s.	60.37 ± 6.81n.s.
400 mg/kg	114.3 ±14.39n.s.	58.744 ± 8.17n.s.
600 mg/kg	110.8 ±15.13n.s.	56.92 ± 8.45n.s.
Late phase		
Vehicle	118.5 ±15.9	82.47 ± 14
ASA 200 mg/kg	80.74 ±13.32n.s.	53.254 ± 8.19n.s.
400 mg/kg	78.59 ± 8.5*	51.82 ± 3.37*
600 mg/kg	34.99 ± 5.7***	23.67 ± 4.49***

* - Significant difference at $P < 0.05$.
*** - Significant difference at $P < 0.001$.
n.s. - Not statistically significant.
Student's t-test subsequent to ANOVA.

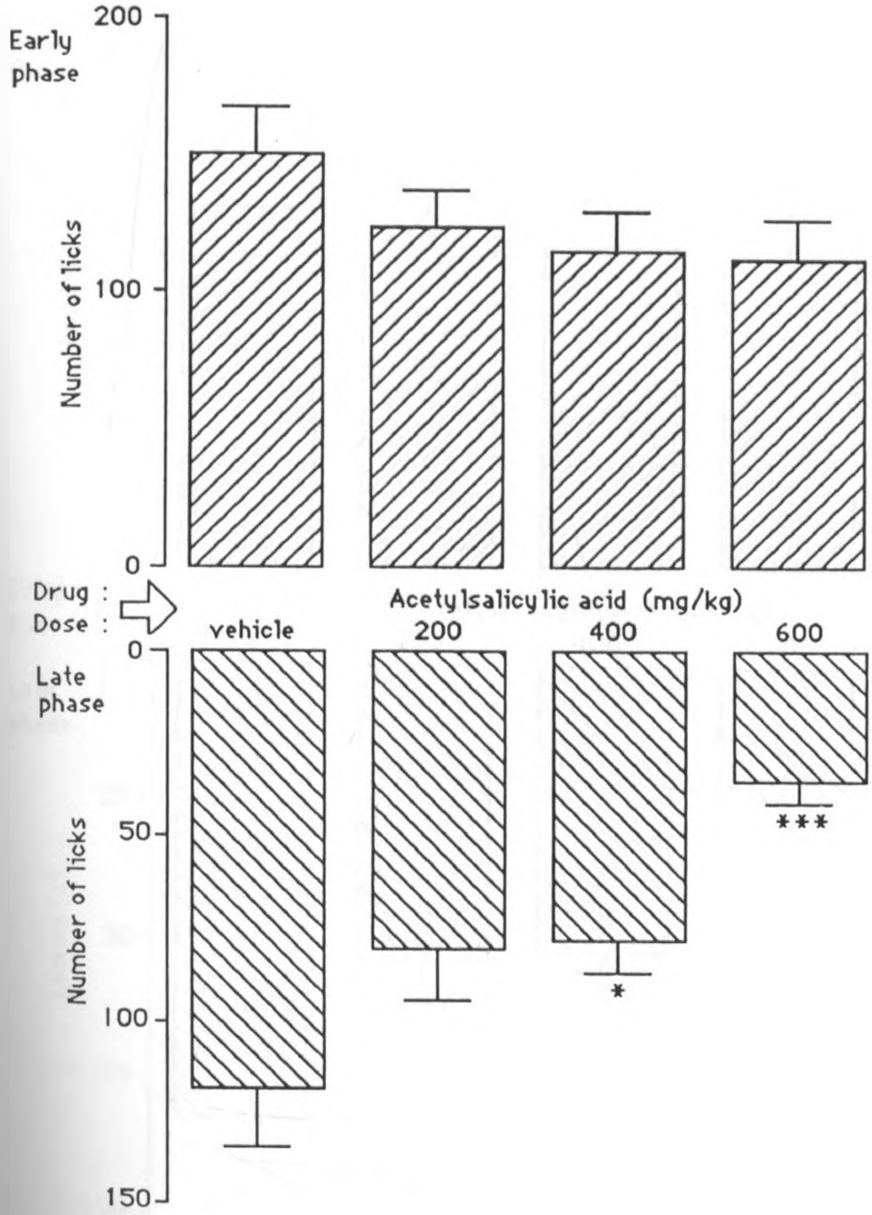

Fig. 8.: Effect of intraperitoneally administered ASA (200, 400, 600 mg/kg) or vehicle on licking activity in the early and late phase of the formalin test (mean ± s.e.m.; n = 10; $F_{(1,18)}$ =0.23, 2.49, 2.88 and 3.316, 4.91, 24.46 for the early and late phase respectively; *P< 0.05,***P < 0.001, Student's t-test subsequent to ANOVA).

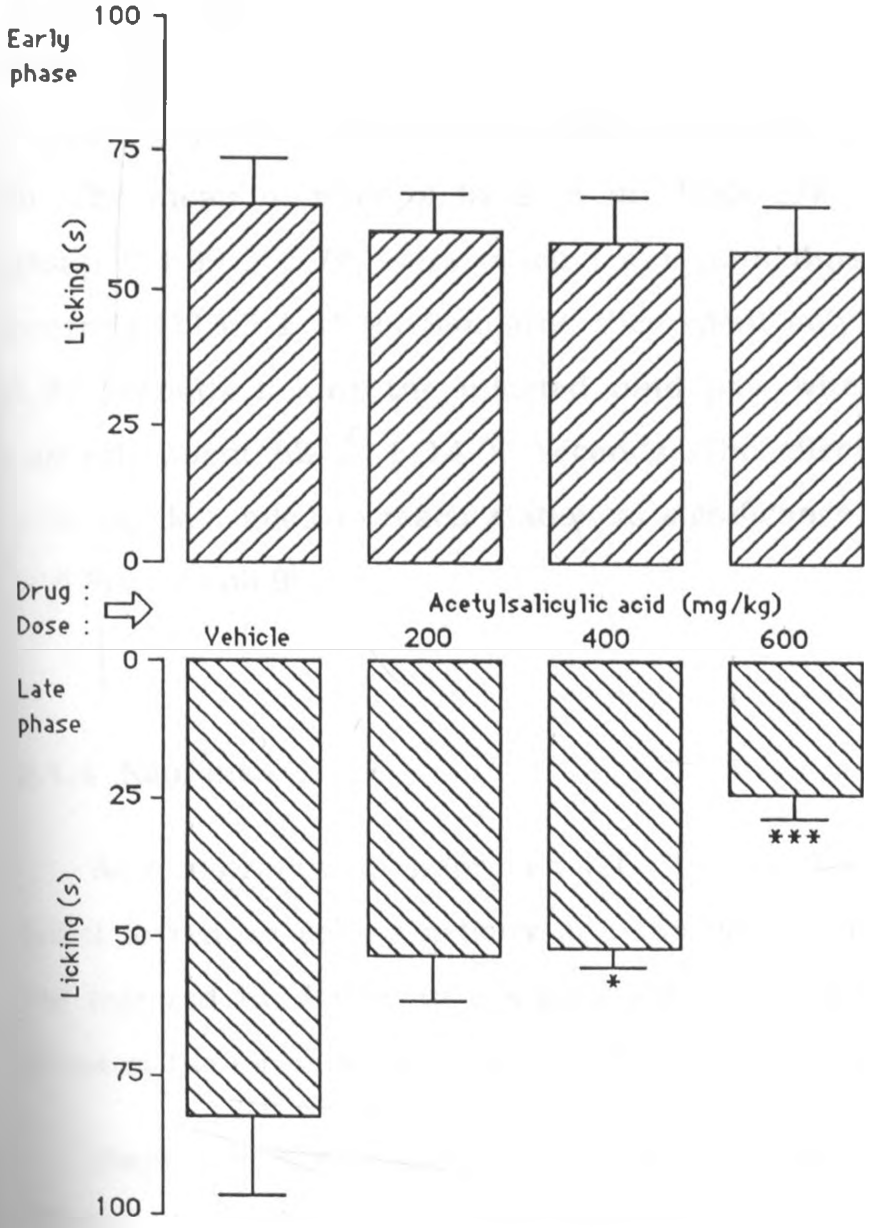

Fig. 9.: Effect of intraperitoneally administered ASA (200, 400, 600 mg/kg) or vehicle on time spent licking the injected hind paw in the early and late phase of the formalin test (mean ± s.e.m.; n = 10; $F_{(1,18)}$ = 0.19, 0.29, 0.47 and 3.25, 4.53, 15.99 for the early and late phase respectively; *P< 0.05,***P < 0.001, Student's t-test subsequent to ANOVA).

treated group (51.82 ± 3.37 seconds) than that by the controls (82.47 ± 14.00 seconds)(400 mg/kg: $F_{(1,18)} = 4.53$, $P < 0.05$)(Fig. 9). ASA (600 mg/kg) caused a more significant reduction in the licking activity and the time spent in the pain behaviour ($F_{(1,18)} = 24.46$, $P < 0.001$ and $F_{(1,18)} = 15.99$, $P < 0.001$ respectively)(Table 3, Figs. 8 and 9). The mean number of licks in the 600mg/kg treated-group (34.99 ± 5.70) were much fewer than those of the controls (118.5 ± 15.9). Similarly, they spent only 51.82 ± 3.37 seconds licking the injected hind paw whereas the controls spent 82.47 ± 14.00 seconds. The effect of ASA (200 mg/kg) failed to reach statistical significance (Table 3 and Figs. 8 and 9).

3.1.4 Naproxen

Administration of naproxen (50, 100 or 200 mg/kg) failed to reduce licking activity and the time spent licking the injected hind paw to a significant level, in the early phase of the formalin test (Table 4, Figs. 10 and 11).

Naproxen 200 mg/kg reduced the licking activity (43.23 ± 11.63) and the time spent licking the injected hind paw (28.23 ± 8.09 seconds) to a significant level (200 mg/kg: $F_{(1,18)} = 11.58$, $P < 0.05$ and $F_{(1,18)} = 4.88$, $P < 0.01$ respectively). Naproxen (50 or 100 mg/kg) caused an insignificant reduction in the number of licks and the time

Table 4: Number of licks and time spent licking the injected hind paw (mean ± s.e.m.) in vehicle- and naproxen-treated animals in the early and late phase of the formalin test.

Drug/dose	Number of licks	Time spent licking the hind paw (sec)
Early phase		
Vehicle	120.5 ± 16.63	59.33 ± 7.35
Naproxen 50 mg/kg	138 ± 18.55n.s	84.49 ± 11.95n.s.
100 mg/kg	136.7 ±18.83n.s	84.96 ± 12.35n.s.
200 mg/kg	109.8 ±17.58n.s.	72.98 ±14.37n.s.
Late phase		
vehicle	92.84 ± 8.79	48.48 ± 4.32
Naproxen 50 mg/kg	79.54 ± 9.94n.s.	48.42 ± 6.62n.s.
100 mg/kg	67.37 ± 11.63n.s.	46.84 ± 9.84n.s.
200 mg/kg	43.23 ±11.63 *	28.23 ± 8.09**

* - Significant difference ($P < 0.05$).
** - Significant difference ($P < 0.01$).
n.s. - Not statistically significant.
Student's t-test subsequent to ANOVA.

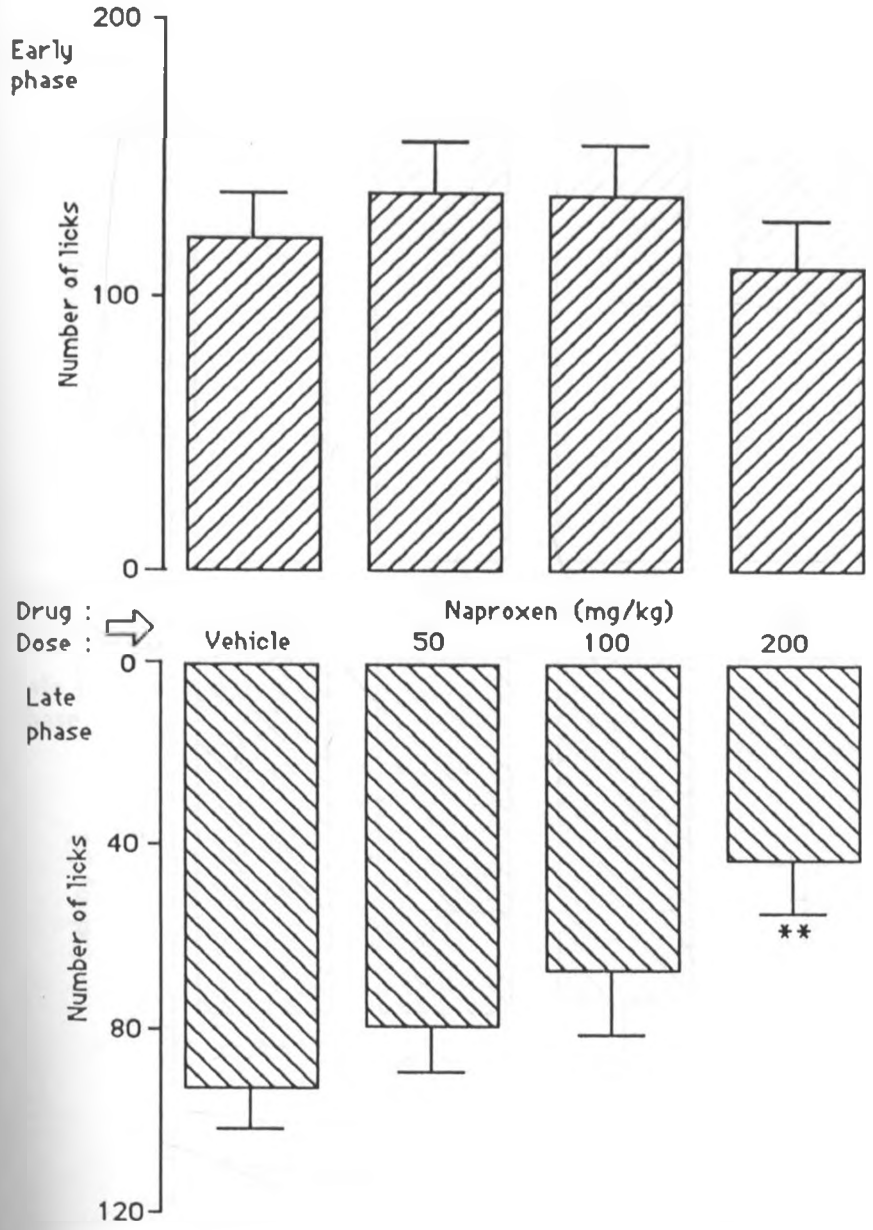

Fig. 10.: Effect of intraperitoneally administered naproxen (50, 100, 200 mg/kg) or vehicle on licking activity in the early and late phase of the formalin test (mean ± s.e.m.; n = 10; $F_{(1,18)}$ = 0.49, 0.42, 0.2 and 1.16, 2.36, 11.58 for the early and late phase respectively; **P< 0.01, Student's t-test subsequent to ANOVA).

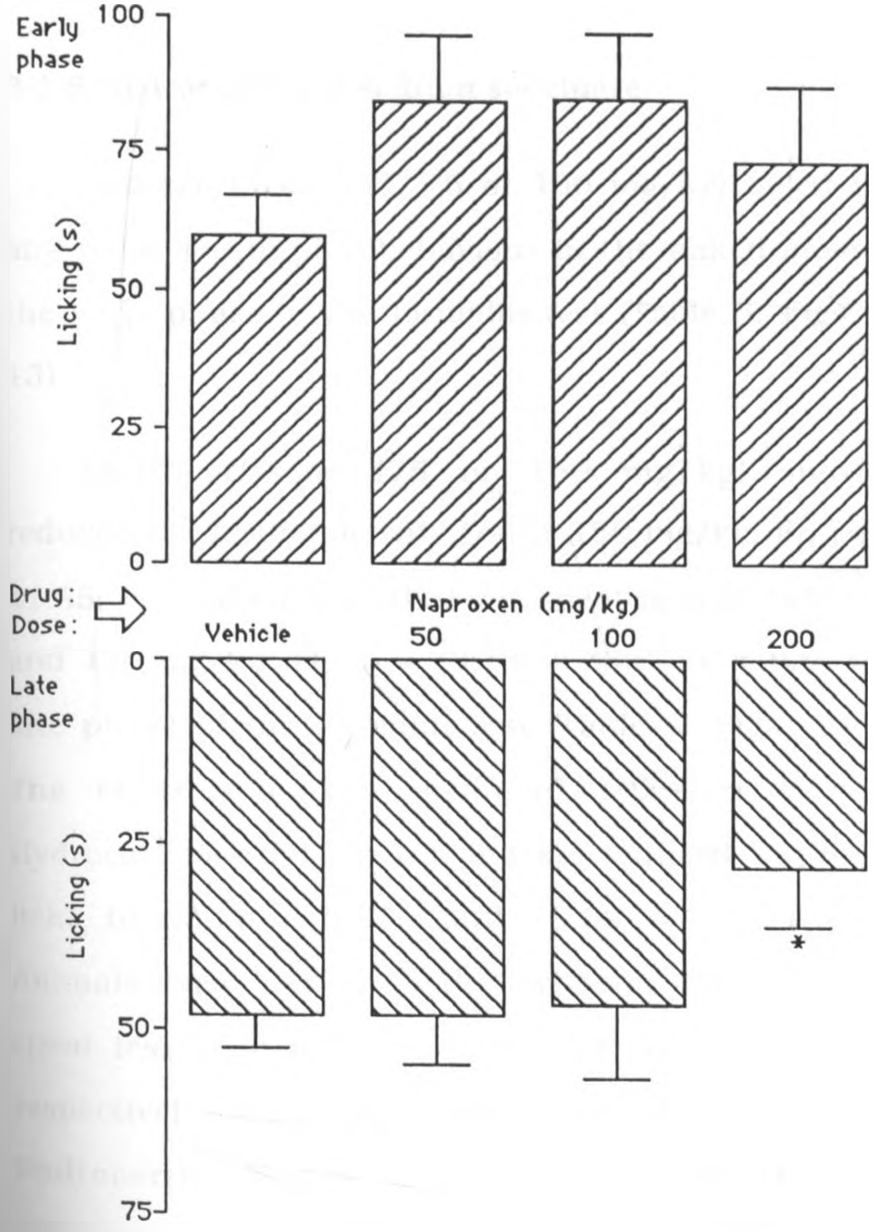

Fig. 11.: Effect of intraperitoneally administered naproxen (50, 100, 200 mg/kg) or vehicle on time spent licking the injected hind paw in the early and late phase of the formalin test (mean ± s.e.m.; n = 10; $F_{(1,18)}$ = 3.22, 3.18, 0.72 and 5.2, 0.02, 4.88 for the early and late phase respectively; *P< 0.05, Student's t-test subsequent to ANOVA).

spent licking the injected hind paw in the late phase of the formalin test (Table 4 and Figs. 10 and11).

3.1.5 Hydrocortisone sodium succinate

Hydrocortisone (40, 75 or 150 mg/kg) failed to cause any reduction of pain behaviour in the naked mole-rats, in the early phase of the formalin test (Table 5, Figs. 12 and 13).

Hydrocortisone (75 or 150 mg/kg) significantly reduced the licking activity (75 or 150 mg/kg: $F_{(1,18)} = 32.7$, 41.55, $P < 0.001$) and the time spent in pain behaviour (75 and 150 mg/kg: $F_{(1,18)} = 28.13$ or 33.84, $P < 0.001$), in the late phase of the formalin test (Table 5, Figs. 12 and13). The controls had a mean of 102.4 ± 10.88 licks. Hydrocortisone (75 or 150 mg/kg) reduced the number of licks to 33.03 ± 5.37 and 18.59 ± 7.13 respectively. Animals treated with hydrocortisone 75 or 150 mg/kg spent less time licking (19.2 ± 3.27 or 11.45 ± 4.60 sec respectively) than the controls (59.63 ± 6.89 seconds.). Hydrocortisone (40 mg/kg) caused an insignificant reduction in licking in the late phase of the formalin test (Table 5, Figs. 12 and13).

Table 5: Number of licks and time spent licking the injected hind paw (mean ± s.e.m.) after injection of hydrocortisone (40, 75, 150 mg/kg) in the early and late phase of the formalin test.

Drug/dose	Number of licks	Time spent licking the hind paw (sec)
Early phase		
Vehicle	137.1 ± 18.97	74.51 ±11.047
Hydrocortisone 40 mg/kg	154.3 ±14.86n.s.	83.18 ± 9.6n.s.
75 mg/kg	122.2 ±15.68n.s.	66.66 ± 9.07n.s.
150 mg/kg	131.6 ±16.06n.s.	73.6 ± 8.97n.s.
Late phase		
Vehicle	102.4 ± 10.88	59.63 ± 6.89
Hydrocortisone 40mg/kg	73.11 ±13.1n.s.	43.22 ± 7.79n.s.
75mg/kg	33.73 ± 5.37***	19.2 ± 3.27***
150 mg/kg	18.59 ± 7.12***	11.45 ± 4.6***

*** - Significant difference at $P < 0.001$.
n.s. - Not statistically significant.
Student's t-test subsequent to ANOVA.

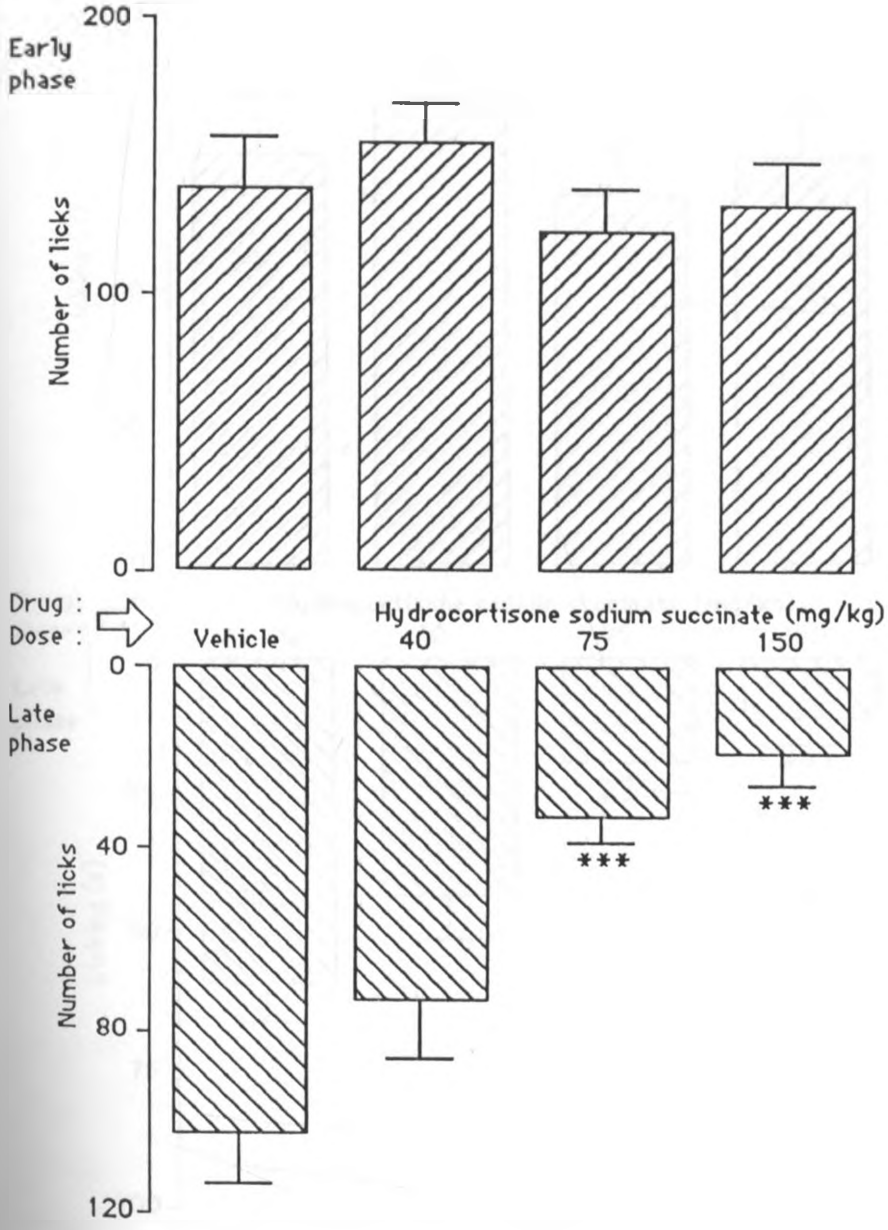

Fig. 12.: Effect of intraperitoneally administered hydrocortisone (40, 75, 150 mg/kg) or vehicle on licking activity in the early and late phase of the formalin test (mean ± s.e.m.; n = 10; $F_{(1,18)}$ = 0.51, 0.37, 0.05 and 2.96, 32.7, 41.55 for the early and late phase respectively; ***P< 0.001, Student's t-test subsequent to ANOVA).

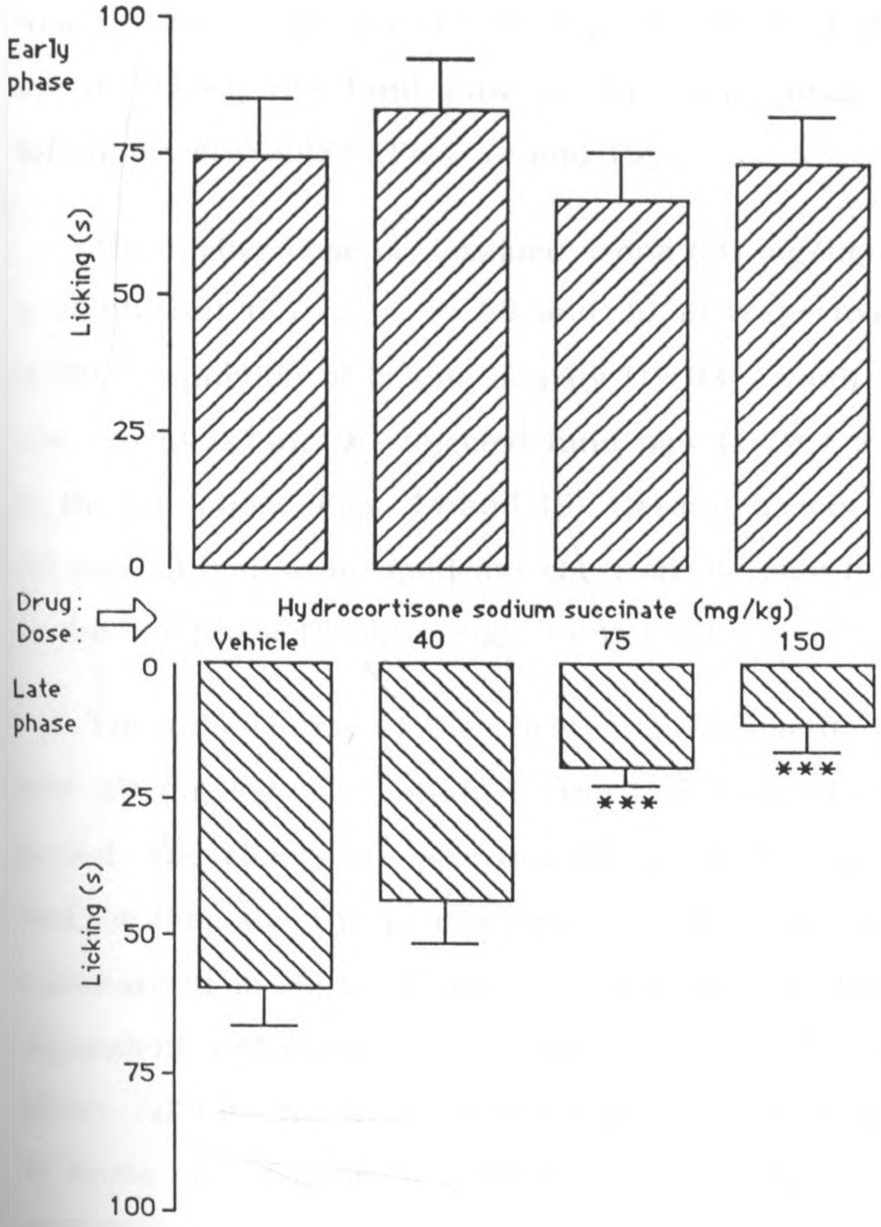

Fig. 13.: Effect of intraperitoneally administered hydrocortisone (40, 75, 150 mg/kg) or vehicle on time spent licking the injected hind paw in the early and late phase of the formalin test (mean ± s.e.m.; n = 10; $F_{(1,18)}$ = 0.35, 0.30, 0.00 and 2.49, 28.13, 33.84 for the early and late phase respectively; ***P< 0.001, Student's t-test subsequent to ANOVA).

3.1.6 Dexamethasone phosphate

Dexamethasone (10, 20 or 30 mg/kg) had an insignificant effect on the licking activity and the time spent licking the hind paw in the early phase of the formalin test (Table 6, Figs. 14 and 15).

The higher dose of dexamethasone (30 mg/kg) caused a significant ($F_{(1,18)}$ = 81.98 and 36.39 respectively, $P < 0.001$) reduction of licking activity (15.93 ± 4.66) and the time spent licking the injected hind paw (7.19 ± 2.13 sec) in the late phase (Figs. 14 and 15). Dexamethasone (10 and 20 mg/kg) had an insignificant effect on the pain behaviour in the late phase (Table 6, Figs. 14 and 15).

The time-course of the effects of dexamethasone (20 and 30 mg/kg) were studied over a 2 hour observation period. The effects of these two doses on licking activity and the time spent in pain behaviour in this experiment are summarized in Figs. 16 and 17. There was a clear dose-dependent reduction in the licking activity in the late phase. Administration of dexamethasone (20 mg/kg) tended to cause an elevation of licking activity and time spent in pain behaviour. Dexamethasone (30 mg/kg) caused a reduction in licking activity and time spent licking the injected hind paw during the first 90 mins after which there was an increase up to the end of the 120 min observation period. There was no overall significant difference between the dexamethasone-treated groups (20

Table 6: Number of licks and time spent licking the injected hind paw (mean ± s.e.m.) after administration of dexamethasone (10, 20, 30 mg/kg) in the early and late phase of the formalin test.

Drug/dose	**Number of licks**	**Time spent licking the injected hind paw (sec)**
Early phase		
Vehicle	112 ± 9.97	55.24 ± 8.47
Dexamethasone 10 mg/kg	105.9 ± 10.04n.s.	64.13 ± 9.714n.s.
20 mg/kg	135.9 ± 13.93n.s.	64.73 ± 8.26n.s.
30 mg/kg	103.9 ± 16.64n.s.	55.95 ± 9.56n.s.
Late phase		
Vehicle	101.04 ± 8.16	68.48 ± 9.94
Dexamethasone 10 mg/kg	132.77 ±15.9n.s.	59.25 ± 5.02n.s.
20 mg/kg	94 ± 20.78n.s.	52.87 ±12.60n.s.
30 mg/kg	15.93 ± 4.66***	7.19 ± 2.13***

*** - Significant difference at $P < 0.001$.
n.s. - Not statistically significant.
Student's t-test subsequent to ANOVA.

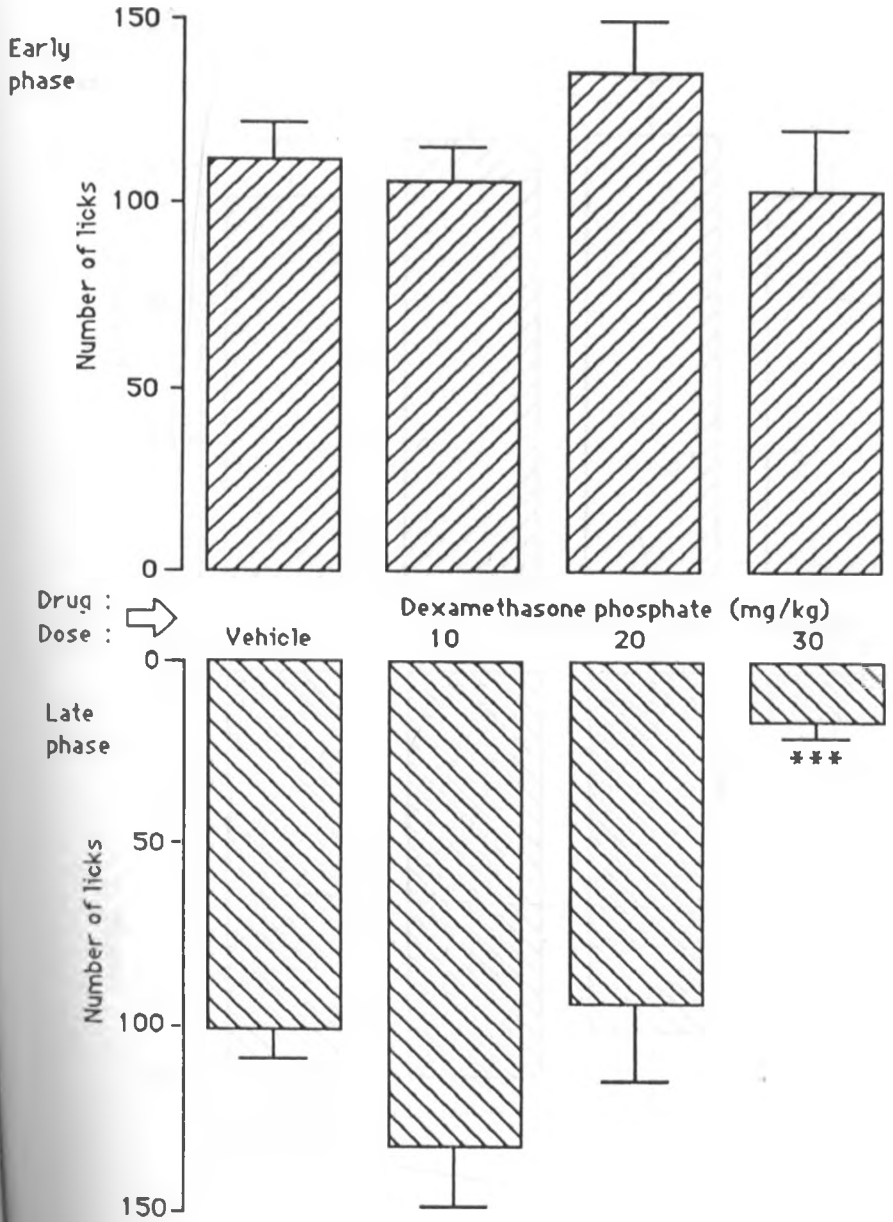

Fig. 14.: Effect of intraperitoneally administered dexamethasone (10, 20, 30 mg/kg) or vehicle on licking activity in the early and late phase of the formalin test (mean ± s.e.m.; n = 10; $F_{(1,18)}$ = 0.82, 1.95, 0.17 and 2.59, 0.1, 81.98 for the early and late phase respectively; ***P< 0.001, Student's t-test subsequent to ANOVA).

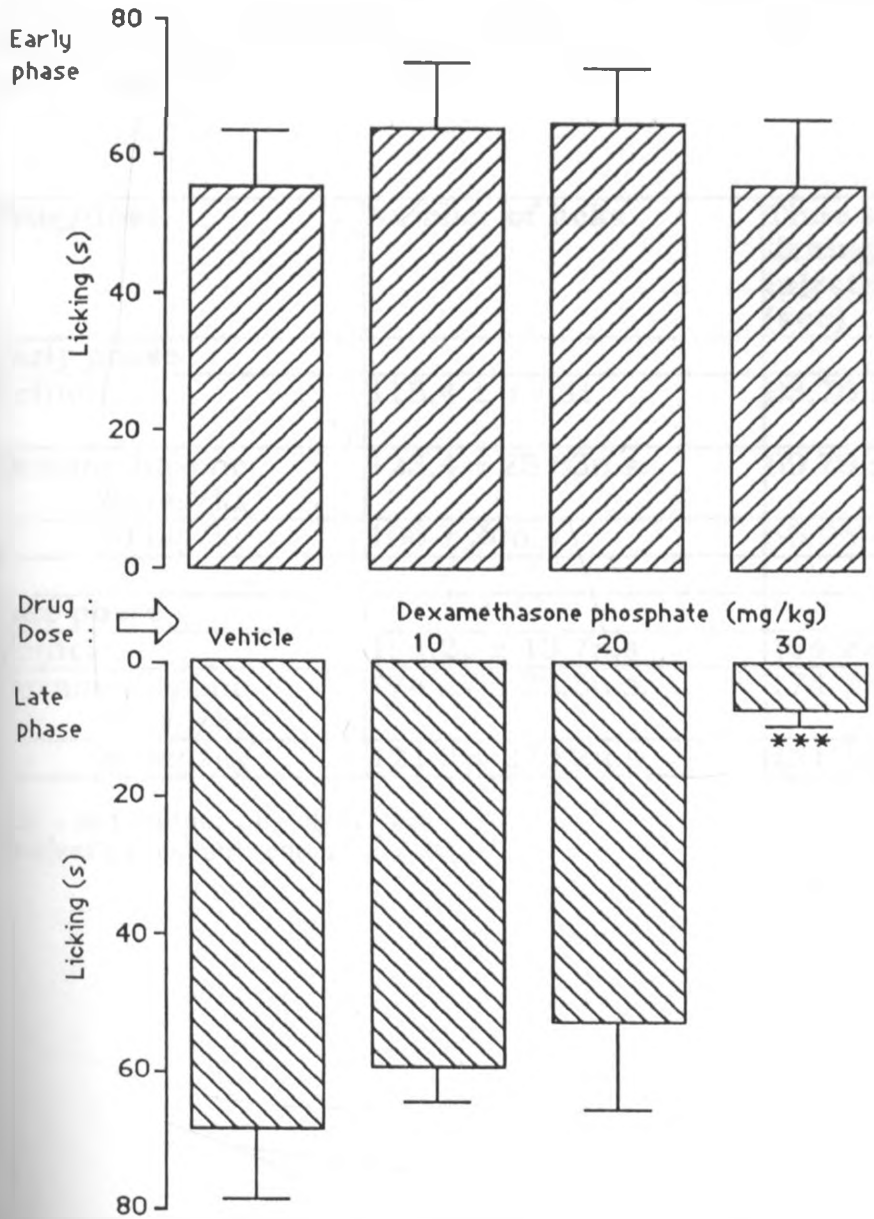

Fig. 15.: Effect of intraperitoneally administered dexamethasone (10, 20, 30 mg/kg) or vehicle on time spent licking the injected hind paw in the early and late phase of the formalin test (mean ± s.e.m.; n = 10; $F_{(1,18)}$ = 1.1, 8.23, 35.22 and 0.69, 0.95, 36.39 for the early and late phase respectively; ***P< 0.001, Student's t-test subsequent to ANOVA).

Table 7: Number of licks and time spent licking the injected hind paw (mean ± s.e.m.) after administration of dexamethasone (20, 30 mg/kg) in the early and late phase of the formalin test.

Drug/dose	**Number of licks**	**Time spent licking the injected hind paw (sec)**
Early phase		
Vehicle	115.4 ± 19.81	63.76 ± 12.49
Dexamethasone 20 mg/kg	123.4 ± 25.83n.s.	69.79 ± 13.9n.s.
30 mg/kg	106 ± 30n.s.	56.95 ± 17.03n.s
Late phase		
Vehicle	154.22 ± 13.7n.s.	154.22 ± 13.77n.s
Dexamethasone 20 mg/kg	174.27 ± 22.5n.s.	174.27 ± 2.58n.s
30 mg/kg	121.2 ± 22.82n.s.	121.18 ± 22.81n.s

n.s. - Not statistically significant.
Student's t-test subsequent to ANOVA.

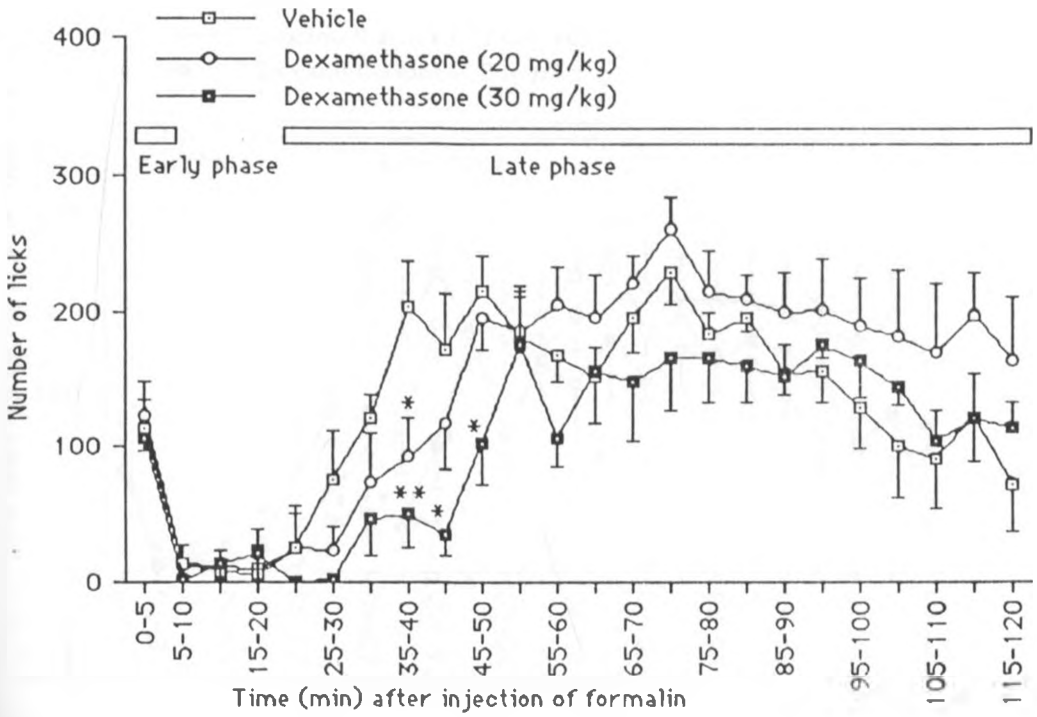

Fig. 16.: Effect of intraperitoneally administered dexamethasone (20, 30 mg/kg) on licking activity in the formalin test (mean ± s.e.m.; n = 5; *P< 0.05,**P <0.01; Student's t-test subsequent to ANOVA). Each point represents the total number of licks during a 5 minute observation period. The animals were observed for 2 h.

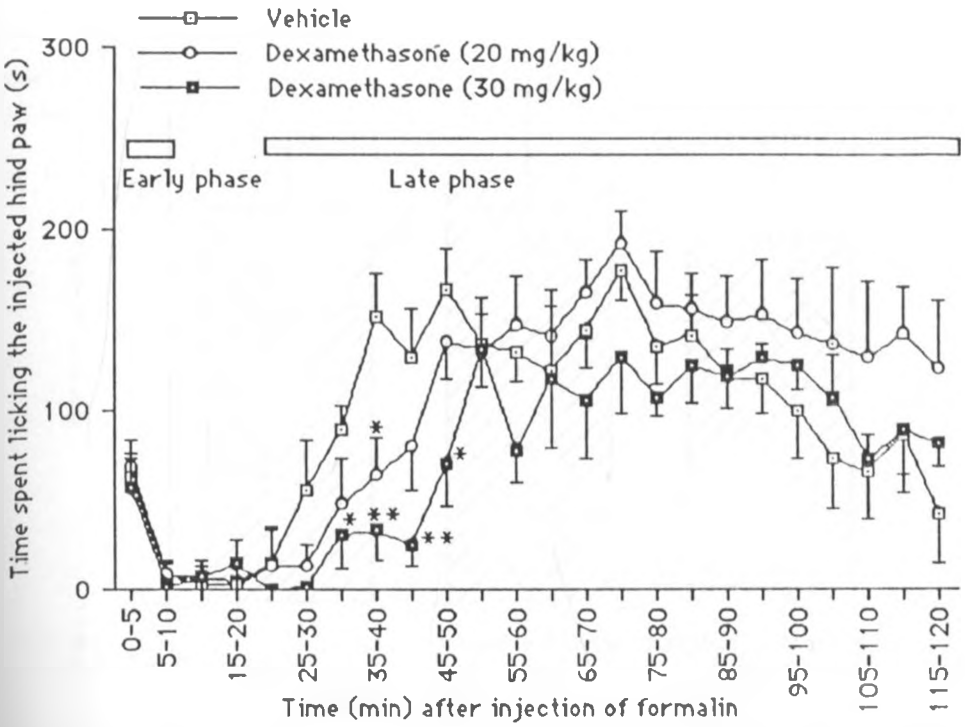

Fig. 17.: Time-course of paw-licking after a subcutaneous injection of 20 µl of 10% formalin into the dorsal right hind paw (mean ± s.e.m.; n = 5; *P< 0.05,**P <0.01; Student's t-test subsequent to ANOVA). Each point represents the amount of time the animals spent licking the injected hind paw during a 5 minute observation period. The animals were observed for 2 h.

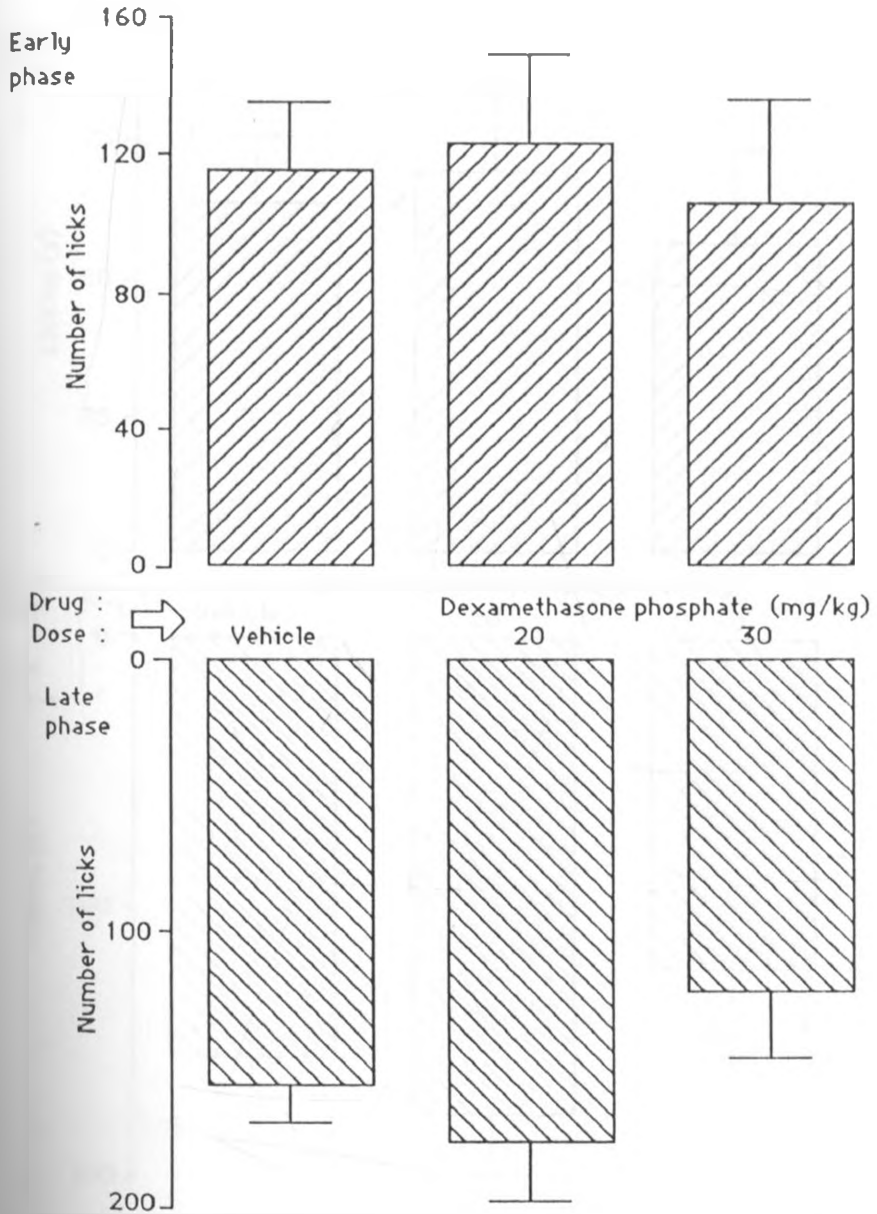

Fig. 18.: Effect of intraperitoneally administered dexamethasone (20, 30 mg/kg) or vehicle on licking activity in the early and late phase of the formalin test. In this and Fig. 19, the animals were observed for 2 h.

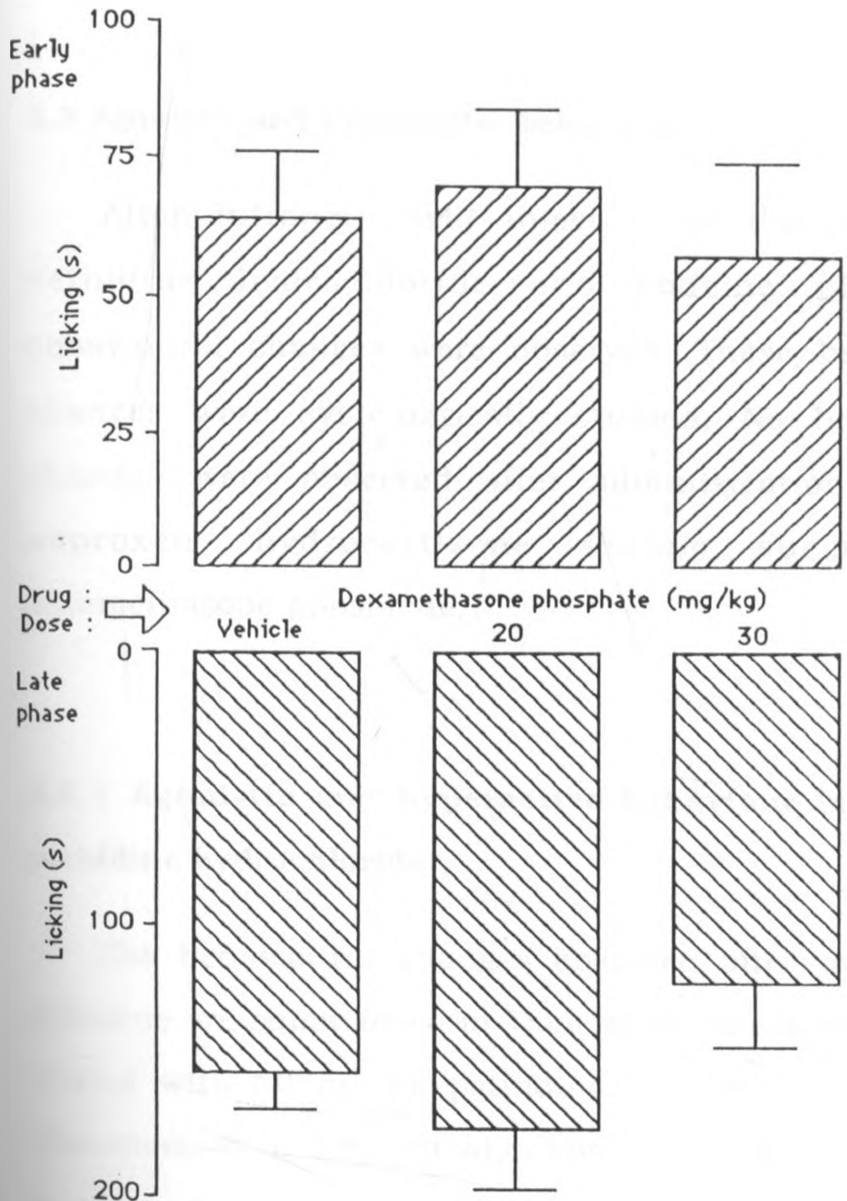

Fig. 19.: Effect of intraperitoneally administered dexamethasone (20, 30 mg/kg) or vehicle on time spent licking the injected hind paw in the early and late phase of the formalin test.

and 30 mg/kg) and the controls in both the early and the late phase over the 2 hour observation period (Table7, Figs. 18 and 19).

3.2 Agonistic and hyperactive behaviour

After intraperitoneal injection of the 2 opioids, pethidine hydrochloride and codeine phosphate, behavioural changes were observed. These behavioural changes were systematically studied. No behavioural changes were observed after administration of ASA, naproxen, hydrocortisone sodium succinate or dexamethasone phosphate.

3.2.1 Agonistic and hyperactive behaviour induced by pethidine hydrochloride

The behavioural changes observed after injection of pethidine are summarized in Table 8. In all naked mole-rats treated with 30 mg/kg pethidine, an initial depression, characterized by hypoactivity, was observed. It started 10 minutes after the intraperitoneal injection of pethidine and lasted for 15-30 minutes. The period of hypoactivity was followed by excitation that was observed up to the end of the 60 min observation period.

Table 8: Effect of intraperitoneal pethidine alone or pethidine + naloxone on behaviour and mortality in the naked mole-rat.

Drug	No.	Cage	Fighting	Wounded	Dead	Behaviour
Vehicle	10	colony	0	0	0	normal
Pethidine						
10mg/kg	10	colony	0	0	0	excited hyperactive hypersensitive
10mg/kg	10	single	-	-	0	excited hyperactive hypersensitive
20mg/kg	10	colony	4	4	0	excited hyperactive hypersensitive aggressive motor impairment
20mg/kg	10	single	-	-	0	excited hyperactive hypersensitive motor impairment
30mg/kg	10	colony	10	10	7	excited hyperactive hypersensitive aggressive motor impairment
30mg/kg	10	single	-	-	0	excited hyperactive hypersensitive motor impairment
Pethidine (10,20/30) mg/kg + naloxone (2mg/kg)	10x3	colony	0	0	0	normal

In colony cages, the animals were hyperactive and this was characterized by increased mobility and vocalization. Hypersensitivity to any kind of mild stimulation was also observed and was characterized by jumping, running, vocalization and aggression. The animals were aggressive and most of the time, faced each other in a threatening position, and attacked each other with their teeth, causing small wounds. Table 9 and Fig. 20 show the number of skin lesions counted on the bodies of these naked mole-rats at the end of the 18 h observation period. Animals treated with pethidine (30 mg/kg) had more skin lesions (32.8 lesions) than those injected with 20 mg/kg pethidine (7.3 lesions). The animals injected with pethidine (20 or 30 mg/kg) + naloxone (2 mg/kg) had no lesions at all. All naked mole-rats treated with pethidine 30 mg/kg participated in vigorous fighting while only 4 in those injected with 20 mg/kg fought. 7 mole-rats in the colony cages receiving pethidine (30 mg/kg) were dead 18 h after the injection.

Naked mole-rats injected with pethidine (10, 20 or 30 mg/kg) and housed in single cages, showed hypersensitivity and motor hyperkinesis. None of these animals died during a further 14 day observation period.

Injection of 20 or 30 mg/kg of pethidine also induced extensor rigidity. The rigidity started 3-11 minutes after drug administration and lasted for 1/2-1 min. This extensor

Table 9: Number of skin lesions counted from colony caged naked mole-rats, 18 h after injection of pethidine alone or pethidine + naloxone.

Mole-rat no.	Drug/dose (mg/kg)	Alive/dead	No. of skin lesions
1	Pethidine 20	Alive	2
2	Pethidine 20	Alive	8
3	Pethidine 20	Alive	17
4	Pethidine 20	Alive	8
5	Pethidine 20	Alive	3
6	Pethidine 20	Alive	1
7	Pethidine 20	Alive	12
8	Pethidine 20	Alive	2
9	Pethidine 20	Alive	4
10	Pethidine 20	Alive	16
1	Pethidine 30	Dead	46
2	Pethidine 30	Dead	42
3	Pethidine 30	Dead	30
4	Pethidine 30	Dead	35
5	Pethidine 30	Dead	41
6	Pethidine 30	Dead	53
7	Pethidine 30	Dead	45
8	Pethidine 30	Alive	14
9	Pethidine 30	Alive	13
10	Pethidine 30	Alive	9
1 - 10	Pethidine 20 or 30 + naloxone 2	Alive	0

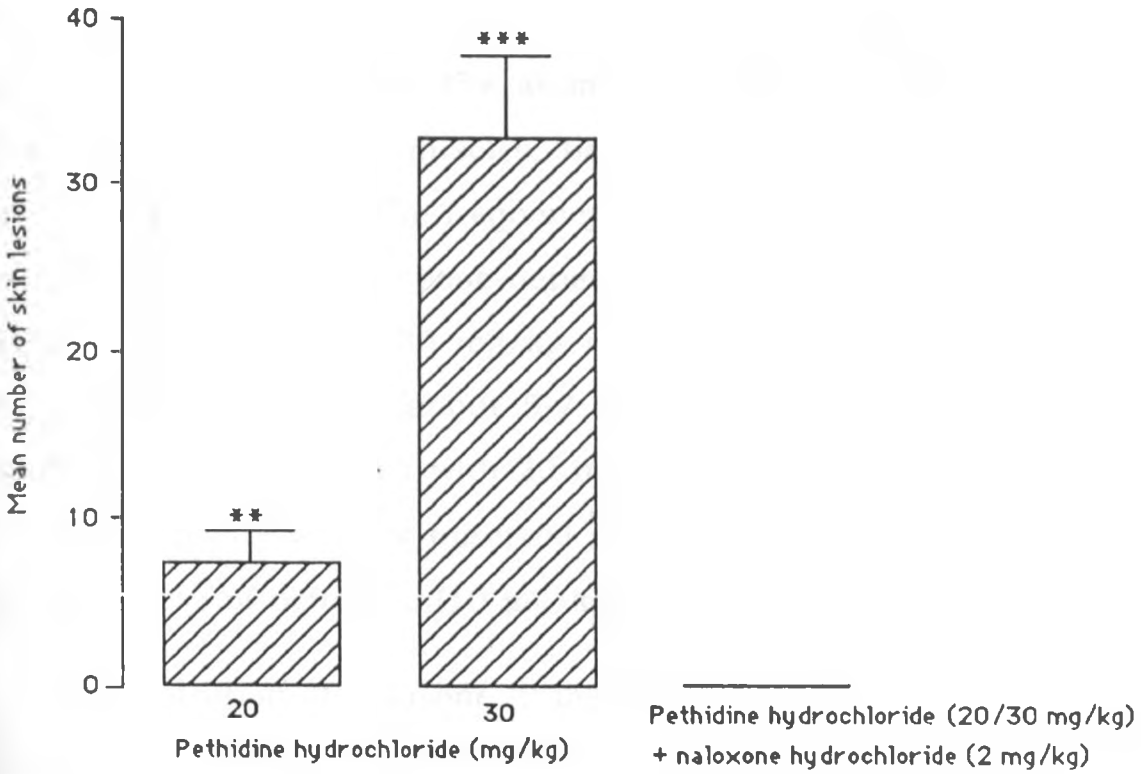

Fig. 20.: Number of skin lesions counted from colony caged naked mole-rats 18 h after injection of pethidine alone (20 or 30 mg/kg) or pethidine + naloxone. (mean ± s.e.m.; n = 10; $F_{(1,18)}$ = 15.08 and 43.86 respectively; **P <0.01,***P < 0.001, Student's t-test subsequent to ANOVA).

rigidity was followed by a period of muscle flaccidity which lasted for 1/2-1 min and was characterized by immobility and sprawling of the animal on the floor of the cage. Thereafter, complete recovery was attained. During the period of extensor rigidity, the animals also had tremors particularly of the neck muscles, backward treading and finally, loss of balance. The frequency and duration of this motor impairment was dose-dependent. It was more frequent at a dosage of 30 mg/kg where it recurred 3-4 times at short intervals (about 5-7 minutes), whereas in animals that were injected with 20 mg/kg, it occured only 1-2 times. Rigidity was not observed after administration of the lower dose of pethidine (10 mg/kg).

Administration of naloxone (2 mg/kg) clearly reversed aggressive behaviour, hyperactivity, hypersensitivity and motor impairment observed after injection of 10, 20 and 30 mg/kg pethidine. The animals appeared more normal than those recieving pethidine alone.

3.2.2 Agonistic and hyperactive behaviour induced by codeine phosphate

The behavioural changes observed after administration of codeine are summarized in Table 10. These behavioural changes are similar to those observed after administration of pethidine (10, 20 or 30 mg/kg). In colony cages, after injection of codeine phosphate (25 or 50 mg/kg) the

Table 10: Effect of codeine alone or codeine + naloxone on behaviour and mortality in the naked mole-rat.

Drug	No.	Cage	Fighting	Wounded	Dead	Behaviour
Vehicle	10	colony	0	0	0	Normal
Codeine						
10mg/kg	10	colony	0	0	0	excited hyperactive hypersensitive
10mg/kg	10	single	-	-	0	excited hyperactive hypersensitive
25mg/kg	10	colony	10	7	0	excited hyperactive hypersensitive aggressive motor impairment
25mg/kg	10	single	-	-	0	excited hyperactive hypersensitive
50mg/kg	10	colony	10	10	1	excited hyperactive hypersensitive aggressive motor impairment
50mg/kg	10	single	-	-	0	excited hyperactive hypersensitive motor impairment
codeine (10,20/30) mg/kg + naloxone (2mg/kg)	10x3	colony	0	0	0	normal

animals were hyperkinetic and hypersensitive and most of the time, faced each other in a threatening position. They also inflicted small wounds particularly on the muzzles of others with their teeth. Table 11 and Fig. 21 show the number of skin lesions counted on the bodies of naked mole-rats at the end of the 18 h observation period. The average count (9.4 lesions) was higher in the animals receiving codeine (50 mg/kg) than in those receiving codeine (25 mg/kg) (1.6 lesions). The animals injected with codeine (25 or 50 mg/kg) + naloxone (2 mg/kg) had no lesions at all. All naked mole-rats treated with codeine (25 or 50 mg/kg) in the colony cages participated in moderate fighting. 18 h after codeine administration, only 1 mole-rat in the colony cage receiving codeine (50 mg/kg) was dead. The animals injected with codeine (10 mg/kg) did not participate in fighting at all.

Naked mole-rats injected with codeine (10, 25 or 50 mg/kg) and housed in single cages, were hypersensitive and hyperactive. None of the animals died during a further 14-day observation period.

Injection of codeine (50 mg/kg) also induced extensor rigidity but in only 4 animals. The rigidity started 25-50 min after drug administration and lasted for 1/2-1 min. The rigidity was followed by a period of muscle flaccidity which lasted for 1/2-1 min and thereafter, complete recovery. During the period of extensor rigidity, the animal also had tremors particularly of the neck muscles, backward

Table 11: Number of skin lesions counted from colony caged naked mole-rats 18 h after injection of codeine alone or codeine + naloxone.

Mole-rat no.	Drug/dose (mg/kg)	Alive/dead	No. of skin lesions
1	Codeine 25	Alive	2
2	Codeine 25	Alive	0
3	Codeine 25	Alive	2
4	Codeine 25	Alive	1
5	Codeine 25	Alive	4
6	Codeine 25	Alive	1
7	Codeine 25	Alive	2
8	Codeine 25	Alive	0
9	Codeine 25	Alive	0
10	Codeine 25	Alive	4
1	Codeine 50	Alive	9
2	Codeine 50	Alive	1
3	Codeine 50	Alive	18
4	Codeine 50	Alive	7
5	Codeine 50	Alive	4
6	Codeine 50	Alive	6
7	Codeine 50	Alive	11
8	Codeine 50	Alive	12
9	Codeine 50	Dead	23
10	Codeine 50	Alive	3
1 - 10	Codeine 25 or 50 + naloxone 2	Alive	0

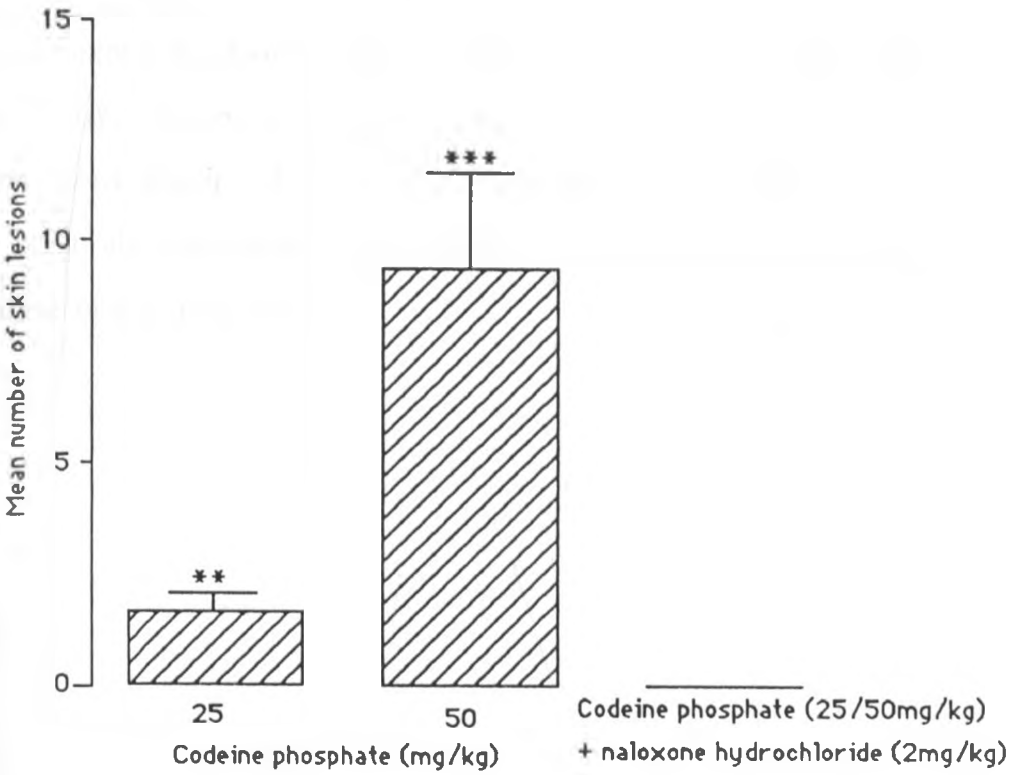

Fig. 21.: Number of skin lesions counted from colony caged naked mole-rats 18 h after injection of codeine alone (25 or 50 mg/kg) or codeine + naloxone (mean ± s.e.m.; n = 10; $F_{(1,18)}$ = 11.29 and 18.65 respectively; **P <0.01,***P < 0.001, Student's t-test subsequent to ANOVA).

treading and finally, loss of balance. The rigidity occured only once in each of the affected animals and was not observed in animals receiving codeine (10 and 25 mg/kg).

Naloxone hydrochloride (2 mg/kg) reversed aggressive behaviour, hypersensitivity, hyperkinesis and motor impairment observed after codeine (10, 25 or 50 mg/kg). The animals appeared more normal than those receiving codeine phosphate alone.

CHAPTER 4

4.0 Discussion

4.1 The formalin test

The present study demonstrates that the formalin test is a valid and reliable model of pain in the naked mole-rat. The test is easy to perform, though some precautions are necessary in order to obtain satisfactory results. Silence in the test room is necessary to prevent stress-induced analgesia (an increase in pain threshold following exposure to stressful events). Since these animals are almost blind, their other senses may be very well developed and so, even minimal disturbances could cause stress-induced analgesia. Stress-induced analgesia has been reported to occur in rats under many stressful conditions like exposure to non-noxious stimuli (cold water) (Bodner *et al.*, 1978), food deprivation (Spiaggia *et al.*, 1977) and noxious stimuli like electric foot shocks (Madden *et al.*, 1977), and i.p. injection of hypertonic saline (Hayes *et al.*, 1978). Stress-induced analgesia is thought to occur due to activation of endogenous pain-control mechanisms (Bodner *et al.*, 1978; Madden *et al.*, 1977). It is therefore important to minimize stress in the naked mole-rats as this may interfere with the experimental results and increase the intra- and inter-individual variability.

Minimal restraint was used during drug injections. The animals moved freely during the observation period. Perhaps, increasing the frequency of handling of these animals during the acclimatization period, and increasing the duration of adaptation to

the observation chamber for about an hour would also minimize variability of the results.

Only one response was monitored in the formalin test; licking of the injected right hind paw. During preliminary studies, hind paw licking gave more consistent results than licking of the fore paw, because of less interference by the normal grooming behaviour. Scoring fore paw licking (Dubuisson and Dennis, 1977) is less reliable than hind paw licking (Hunskaar *et al.*, 1985a; 1986; Hunskaar and Hole, 1987; Hunskaar, 1987b; Shibata *et al.*, 1989). Hind paw licking was chosen for use in the naked mole-rat during these experiments.

In preliminary experiments, concentrations less than 10% were found to induce the early phase only. In the present study, a formalin concentration of 10% in 0.9% NaCl was used. This was the lowest concentration that induced both the early and the late phase. No adverse effects were observed on the animals when 10% formalin was used. In other rodents, substantially lower concentrations of formalin have been used to induce both the early and the late phase; in mice, 1% and 0.5% (Hunskaar *et al.*, 1986; Hunskaar and Hole, 1987; Shibata *et al.*, 1989) in rats, 5% (Dubuisson and Dennis, 1977).

Recently, Rosland *et al.*, (1990) examined the effect of different formalin concentrations on the pain response in mice and recommended concentrations of 0.05-0.2% for inducing the early phase and concentrations of 1% or higher for inducing the late phase. In this study, a 10% concentration induced both the early and late phase with negligible suffering. The naked mole-rat may

have a much higher pain threshold than the other rodents, since a much stronger noxious chemical stimulus was needed to induce the licking response. Perhaps the receptors are more inaccessible.

Subcutaneous injection of 20 µl of formalin into the dorsal right hind paw produced two periods of high pain behaviour, the early and the late phase. This is in agreement with that reported in earlier studies, but using different concentrations and volumes of formalin, in rats and cats (Dubuisson and Dennis, 1977), mice (Hunskaar *et al.*, 1985a; Hunskaar *et al.*, 1986; Hunskaar and Hole, 1987; Hunskaar, 1987b; Shibata *et al.*, 1989) and in monkeys (Alreja *et al.*, 1984).

The early phase lasted from 0-5 min in the naked mole-rats and this is similar to that reported in other species (Dubuisson and Dennis, 1977; Alreja *et al.*, 1984; Hunskaar *et al.*, 1985a; Hunskaar *et al.*, 1986; Hunskaar and Hole, 1987; Hunskaar, 1987b; Shibata *et al.*, 1989). The onset of the late phase was delayed and the duration longer than that described in the rat and cat (Dubuisson and Dennis, 1977), mouse (Hunskaar *et al.*, 1985a; Hunskaar *et al.*, 1986; Hunskaar and Hole, 1987; Hunskaar, 1987b; Shibata *et al.*, 1989) and in the monkey (Alreja *et al.*, 1984). The late phase in the naked mole-rat started 25 min after the injection of formalin whereas in the other species it started after 15-20 min. Furthermore, the late phase in the naked mole-rat could be demonstrated upto 120 min whereas in the other species it lasted 60 min. This suggests that in the naked mole-rat, the inflammatory process (acute inflammation) is slightly delayed and is of a longer duration.

The early phase may be due to a direct excitation of nociceptors (Dubuisson and Dennis, 1977) while the late phase may be due to inflammation (Dubuisson and Dennis, 1977; Hunskaar *et al.*, 1985a; Hunskaar *et al.*, 1986; Hunskaar and Hole, 1987; Hunskaar, 1987b; Shibata *et al.*, 1989). Substance P and bradykinin may participate in the initiation of the early phase response while the inflammatory mediators such as histamine, serotonin, prostaglandins and bradykinin may be involved in the late phase (Shibata *et al.*, 1989). However, the mechanisms are still not clear.

The delay in inflammation may be due to slow inflammatory processes in these naked mole-rats. Since inflammatory pain is thought to be due to stimulation of chemoreceptors by inflammatory mediators such as PGs (Ferreira, 1982), the delay may be due to a slow release of PGs and other mediators, e.g., bradykinin and histamine. The effects of PGE_1 and PGE_2 are cumulative and sustained (Ferreira, 1982). Thus, continuous generation of minute amounts of PGs at the site of injury will sensitize the nerves so that mechanical stimulation and mediators of inflammation such as bradykinin and histamine cause pruritus or pain (Ferreira, 1982). The mechanisms of inflammation in the naked mole-rat need to be further investigated, particularly using microscopy to understand the cellular changes that take place during the inflammatory process.

An increase in ambient temperature has been demonstrated to influence the late phase of the formalin test. The intensity and duration of pain behaviour increased as ambient temperature rose from 20 to 28^{0}C (Rosland, 1991). It is not known to what extent

changes in ambient temperature influence pain behaviour in the naked mole-rat. This needs to be investigated.

4.2 Effects of narcotic analgesics and antiinflammatory drugs in the formalin test

In the present study, pethidine and codeine induced significant analgesia during both the early and the late phase. These results are in agreement to those published earlier (Dubuisson and Dennis, 1977; Hunskaar *et al.*, 1985a; Hunskaar *et al.*, 1986; Hunskaar and Hole, 1987; Hunskaar, 1987b; Shibata *et al.*, 1989). The doses required for effect in the naked mole-rat were much higher than those used in the other species. A faster biotransformation and excretion rate of these drugs may explain the higher doses required in the naked mole-rat. The pharmacodynamic and pharmacokinetic properties of drugs in the naked mole-rat need to be further investigated.

In an earlier study using the hot-plate test in the naked mole-rat, analgesia by morphine could not be demonstrated (Kanui and Hole, 1990). It was suggested that the opioid system of these animals was not involved in the regulation of nociception. However, during this study, very significant analgesia was demonstrated using the formalin test. This suggests that the opioid system of the naked mole-rat is indeed involved in the regulation of both acute and chronic pain. These results also further suggest that the formalin test is more sensitive and superior to the hot-plate test as a test of nociception. The analgesic effects could be mediated by central opiate receptors,

probably the μ and δ (Yaksh, 1987; Cotton *et al.*, 1984; Portoghese and Takemori, 1985). It is not as yet clear why anti-nociception by morphine could not be observed in the earlier study (Kanui and Hole, 1990).

The two non-steroidal antiinflammatory drugs, ASA and naproxen, significantly reduced pain behaviour in the late phase only, in the naked mole-rat. These results are as expected since these drugs inhibit inflammation by inhibiting the synthesis of prostaglandins (Vane, 1971), an important mediator of inflammation. Thus, in the naked-mole rat, ASA only seems to have an antiinflammatory effect. In other rodents, however, the effect of ASA in the early phase of the formalin test are contradictory. Shibata *et al.*, (1989) reported no effect of ASA in the early phase in mice while Hunskaar *et al.*, (1985a; 1986; 1987b) described inhibitory effects in the early phase in mice, suggesting a central site of action of ASA. A central site of action by ASA has indeed been shown in several studies (Ferreira *et al.*, 1978; Yaksh and Hammond, 1982; Ferreira, 1983; Shyu *et al.*, 1984; Chiu and Richardson, 1985). Like pethidine and codeine the doses used were much higher than those used in other rodent species. This could be explained also by an increase in biotransformation and excretion of these drugs, in the naked mole-rat. This also needs further investigation.

The two steroidal antiinflammatory drugs, hydrocortisone and dexamethasone significantly inhibited the late phase only. This is again as was expected since these drugs inhibit the enzyme phopholipase A_2 and thus inhibit synthesis of multiple inflammatory mediators (Nijkamp *et al.*, 1976; Blackwell *et al.*,

1978). The results described are in agreement with earlier results published for other rodent species. The effect of dexamethasone on pain-induced behaviour in the late phase persisted upto 90 min. This was however not statistically significant. The doses used in this study are much higher than in other species. Biotransformation and excretion is probably much faster in the naked mole-rat. When the two steroidal antiinflammatory drugs were compared, the analgesic effect of hydrocortisone was more potent than that of dexamethasone in the naked mole-rat contrary to what has been reported in the literature for other species (Goth, 1974). Perhaps this can be explained by differences in biotransformation in the different animals for these steroids.

4.3 Agonistic and hyperactive behaviour

Administration of ASA, naproxen, hydrocortisone and dexamethasone did not induce agonistic or hyperactive behaviour nor did they induce motor impairment in the naked mole-rat. Pethidine and codeine, however, induced agonistic behaviour, hypersensitivity, hyperactivity and motor impairment. This is in agreement with an earlier study in which morphine was administered (Kanui and Hole, 1990).

The higher doses of pethidine (20 and 30 mg/kg) and codeine (50 mg/kg) initially induced depression characterized by hypoactivity, which disappeared with time and was followed by hyperactive behaviour. The low doses of these drugs produced motor hyperkinesis and hypersensitivity to mild stimulation. These biphasic responses are comparable to those reported in other

animal species (Martin *et al.*, 1963; Vasko and Domino, 1978; Mucha *et al.*, 1981; Numan and Lal, 1981; Bartoletti *et al.*, 1983; Jorenby *et al.*, 1988).

The basis of the initial depression observed is not clear. Hyperactivity, hyperkinesis and hypersensitivity may be due to activation of an excitatory opiate receptor subtype (Geller *et al.*, 1983; Jorenby *et al.*, 1988).

Perhaps, the agonistic behaviour observed, characterized by fighting of colony caged mole-rats, after pethidine and codeine administration was due to a memory impairment caused by these opiates (Gaungher and Kapp, 1978; Castellano *et al.*, 1984). The animals probably failed to recognize one another and appeared strangers, hence the fights. A decrease in emotionality has been thought to account for morphine-induced memory impairment in rodents (Gaungher and Kapp, 1978; Castellano *et al.*, 1984).

It is difficult to pin-point, at this juncture, the specific opiate receptor that is involved in the behavioral effects observed. Probably, the μ and δ receptors could be involved (Lord *et al.*, 1977; Frenk *et al.*, 1978), since the behaviour was naloxone reversible. However, the μ opiate receptor subtype is the most likely to be the receptor involved since low doses of naloxone were used, and δ receptors are more difficult to antagonize with naloxone (Frenk *et al.*, 1978). Extensor rigidity only occured after high doses of pethidine and codeine. These seizures could also be mediated through the μ opioid receptor since they were easily reversed by naloxone (Frenk *et al.*, 1978). Thus, the behavioral effects observed could be mediated by the μ opiate receptor

subtype, probably the μ_2. Studies using more specific receptor antagonists are needed to reveal the specific receptor subtype involved.

The sites of action of pethidine and codeine in the naked mole-rat are not known. More studies are required to determine the exact sites of action of these drugs. Opiate receptors are widely distributed in the brain (Kuhar *et al.*, 1973; Kuhar and Atweh, 1977) but are very highly concentrated in the limbic system (Simon and Hiller, 1978). The limbic system has been strongly implicated in the control of emotional behaviour. A direct excitatory action of pethidine and codeine on opiate receptors, located in the limbic system of these animals may induce agonistic behaviour. It is also likely that other brain areas may be involved in mediating the behavioral effects observed. The effect of the opiates could have been mediated via other neurotransmitters (Deakin and Dostrovsky, 1978; Yaksh, 1979; Proudfit and Hammond, 1981; Berge *et al.*, 1983; Kuraishi *et al.*, 1983). Specifically, acetylcholine (Vasko and Domino, 1978), serotonin (Basbaum *et al.*, 1973), dopamine (Stewart and Vezina, 1987) or noradrenaline (Booth, 1988) neurotransmission could have been affected by the opioids.

The deaths observed in the colony cages could not have been due to toxicity of the drugs used, since quite low doses as compared to those that are known to cause toxicity in mice (221-311 mg/kg) (Booth, 1988) were used. Furthermore, all animals in single cages survived. Deaths were probably due to haemorrhage, asphyxia, and fatigue following the fights.

4.4 Conclusions

This study investigated in the naked mole-rat, the effects of pethidine hydrochloride, codeine phosphate, acetylsalicylic acid, naproxen, hydrocortisone sodium succinate and dexamethasone phosphate, using the formalin test. The results have led to the following conclusions:

1. Subcutaneous injection of 20 µl of 10% formalin into the dorsal right hind paw induced two phases of high pain behaviour (licking and biting), the early and late phase. The early phase lasted 0-5 min whereas the late phase started 25-30 min after formalin injection and was demonstrated upto 120 min.

2. The onset of the late phase was slightly delayed (25-30 min after formalin injection) and the duration was long (upto 120 min). The delay in onset may have been due to a slow inflammatory process in the naked mole-rat. More investigations are needed to elucidate further the inflammatory process in the naked mole-rat.

3. The centrally acting narcotic analgesics, pethidine and codeine, induced strong, dose-dependent analgesia in both the early and late phase of the formalin test.

4. In the naked mole-rat, pethidine and codeine administration induced hyperkinesis, hypersensitivity, motor impairment and agonistic behaviour, perhaps via the activation of excitatory opioid receptor subtypes.

5. The non-steroidal antiinflammatory drugs (ASA and naproxen) and the steroidal antiinflammatory drugs

(hydrocortisone and dexamethasone) supressed licking activity in only the late phase of the formalin test. These drugs are effective against inflammatory pain in the naked mole-rat.

6. The naked mole-rat has anti-nociceptive systems that can be stimulated by the analgesic and antiinflammatory drugs used in this study.

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APPENDIX

Appendix 1: Shows time (sec) spent licking the injected hind paw, in blocks of 5 min after subcutaneous injection of 10% formalin or vehicle in the hind paw in the naked mole-rat during a 1 h observation period.

Treatment	Animal number	0 - 5 min	5 - 10 min	10 - 15 min	15 - 20 min	20 - 25 min	25 - 30 min	30 - 35 min	35 - 40 min	40 - 45 min	45 - 50 min	50 - 55 min	55 - 60 min
formalin 10%	1	44.05	0	3.29	3.55	9.35	10.29	40.18	100.83	85.59	140.25	131.58	162.49
formalin 10%	2	42.98	0	0	0	.98	4.2	11.39	12.2	87.35	21.2	75.89	166.23
formalin 10%	3	82.87	38.58	28.54	0	0	0	0	10.93	87.18	84.62	101.45	83.83
formalin 10%	4	82.58	0	13.03	.45	0	5.64	53.98	47.84	42.03	76.2	72.15	87.98
formalin 10%	5	54.01	0	0	0	.72	0	8.83	6.38	87.33	53.41	78.03	79.15
formalin 10%	6	89.53	15.05	38.65	1	100.01	38.05	85.81	58.58	98.84	89.03	88.37	127.4
formalin 10%	7	92.08	0	48.9	100.88	9.08	42.89	82.95	88.87	125.82	159.45	151.55	158.45
formalin 10%	8	85.25	.72	0	0	13.01	33.03	48.98	40.09	89.41	83.81	88.95	112.92
formalin 10%	9	30.54	0	0	0	8.88	0	0	2.09	48.08	32.72	21.28	42.16
formalin 10%	10	52.81	1.08	0	39.62	0	0	21.88	0	13.11	89.37	71.11	58.14
vehicle	1	0	0	0	0	0	0	0	0	0	0	0	0
vehicle	2	0	0	0	0	0	0	0	0	0	0	0	0
vehicle	3	0	0	0	0	0	0	0	0	0	0	0	0
vehicle	4	2.3	0	0	0	0	3.48	3.7	5.4	.45	1.08	4.09	4.03
vehicle	5	14	0	0	0	0	0	0	0	18.99	4.88	8.52	17.85
vehicle	6	0	0	0	0	0	0	0	0	0	0	0	0
vehicle	7	0	0	0	0	0	0	0	0	0	0	0	0
vehicle	8	1.08	0	0	0	0	0	11.48	3.01	0	0	0	0
vehicle	9	0	0	0	0	0	0	0	0	0	0	0	0
vehicle	10	4.98	0	0	0	0	0	4.31	0	0	5.97	0	0

Appendix 2: Time (sec) spent licking the injected hind paw, in blocks of 5 min after subcutaneous injection of 10% formalin or vehicle in the hind paw of the naked mole-rat during a 2 h observation period.

Treatment	Animal number	0 - 5 min	5 - 10 min	10 - 15 min	15 - 20 min	20 - 25 min	25 - 30 min	30 - 35 min	35 - 40 min	40 - 45 min	45 - 50 min	50 - 55 min	55-60 min	60 - 65 min	65 - 70 min	70 - 75 min	75 - 80 min	80 - 85 min	85 - 90 min	90 - 95 min	95 - 100 min	100 - 105 min	105 - 110 min	110 - 115 min	115 - 120 min
formalin 10%	1	25.2	0	16.2	0	22.91	9.46	117.44	69.46	121.25	109.5	117.86	86.52	98.05	110.83	171.86	158.75	144.87	82.23	79.12	116.13	69.36	64.96	85.93	27.33
formalin 10%	2	48.05	0	6.93	2.4	2.81	40.17	87.57	140.96	150.8	155.83	164.54	127.21	142.02	108.1	123.89	69.13	94.43	89.47	78.46	56.61	26.34	61.76	0	0
formalin 10%	3	82.89	35.29	52.22	87.09	30.21	158.82	103.82	169.29	140.13	159.83	176.49	185.59	157.39	124.15	175.67	101.26	103.48	141.99	170.53	96.76	160.64	165.54	172.12	150.91
formalin 10%	4	67.2	0	14.39	0	0	76.36	104.23	165.13	195.17	170.12	180.2	120.54	25.13	170.19	252.73	180.38	199.65	112.02	102.43	39.43	0	0	45.2	28.02
formalin 10%	5	95.64	0	0	0	105.06	0	38.48	213.73	31.88	243.17	48.1	144.83	193.99	207.58	165.09	172.93	177.17	164.91	159.89	193.26	116.07	41.5	154.83	8.06
vehicle	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vehicle	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vehicle	3	4.87	0	0	7.98	0	0	0	0	0	0	9.87	2.33	1.32	4.76	0	0	0	2.67	9.54	0	5.4	1.85	2.09	0
vehicle	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vehicle	5	3.23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.43	0	2.09	1.06	0	0	1.63	0	0

Appendix 3: Shows number of licks recorded in blocks of 5 min of after i.p. injection of vehicle or pethidine (pet) 10, 20 and 30 mg/kg in the naked mole-rat during a 1 h observation period.

Treatment	Animal number	0 - 5 min	5 - 10 min	10 - 15 min	15 - 20 min	20 - 25 min	25 - 30 min	30 - 35 min	35 - 40 min	40 - 45 min	45 - 50 min	50 - 55 min	55 - 60 min
vehicle	1	13	0	13	0	4	113	23	40	126	79	109	190
vehicle	2	70	0	2	1	0	0	0	0	5	21	10	32
vehicle	3	34	0	0	0	4	7	131	19	44	46	66	94
vehicle	4	114	0	0	0	0	25	13	48	45	179	123	128
vehicle	5	39	0	17	1	0	0	0	32	45	6	145	100
vehicle	6	86	4	0	2	0	0	0	0	0	0	15	39
vehicle	7	69	6	7	0	25	81	29	112	58	161	123	171
vehicle	8	43	0	0	0	19	31	77	158	146	210	218	288
vehicle	9	141	63	4	4	0	37	29	72	101	137	149	174
vehicle	10	177	0	30	1	0	11	131	118	114	174	176	150
Pet 10	1	64	0	0	0	0	104	25	55	42	74	115	65
Pet 10	2	149	0	0	0	0	0	0	0	9	16	72	0
Pet 10	3	107	0	0	0	0	0	0	21	13	35	154	240
Pet 10	4	95	0	0	0	0	0	0	0	0	0	189	13
Pet 10	5	43	0	0	0	0	0	0	0	0	41	105	128
Pet 10	6	205	0	0	0	0	0	0	0	0	32	245	49
Pet 10	7	150	93	0	0	0	0	0	43	105	189	60	106
Pet 10	8	55	0	228	49	98	282	41	147	63	110	191	423
Pet 10	9	82	0	0	0	0	0	0	0	0	0	278	7
Pet 10	10	146	35	0	71	0	0	2	65	173	282	286	228
Pet 20	1	29	0	0	12	0	0	0	0	0	18	38	89
Pet 20	2	11	0	0	0	0	0	0	0	0	0	0	0
pet 20	3	17	0	0	0	0	0	0	0	0	0	163	154
pet 20	4	0	0	0	0	0	0	0	0	0	0	0	0
Pet 20	5	32	0	0	0	0	0	0	0	0	0	0	0
Pet 20	6	95	0	0	0	0	0	0	0	0	0	0	0
Pet 20	7	4	0	2	5	3	4	0	0	0	0	18	14
Pet 20	8	0	0	0	0	0	0	0	0	0	0	0	0
Pet 20	9	71	14	0	0	0	0	0	0	0	4	0	38
Pet 20	10	0	0	0	0	0	0	0	0	0	0	0	0
pet 30	1	51	2	0	0	0	0	0	0	0	0	9	0
pet 30	2	15	0	0	0	0	0	0	0	0	0	0	0
pet 30	3	49	0	0	0	0	0	0	0	0	0	0	0
pet 30	4	56	0	0	0	0	0	0	0	0	0	0	0
pet 30	5	38	0	0	0	0	0	0	0	0	0	0	0
pet 30	6	8	0	0	0	0	0	0	0	0	0	0	0
pet 30	7	0	0	0	0	0	0	0	0	0	0	0	0
pet 30	8	38	0	0	0	0	0	0	0	0	46	91	164
pet 30	9	59	39	13	0	0	0	0	0	0	0	0	102
pet 30	10	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 4: Shows time (sec) spent licking the injected hind paw, in blocks of 5 min after i.p. injection of vehicle or pethidine (pet) (10, 20 and 30 mg/kg) in the naked mole-rat during a 1 h observation period.

Treatment	Animal number	0 - 5 min	5 - 10 min	10 - 15 min	15 - 20 min	20 - 25 min	25 - 30 min	30 - 35 min	35 - 40 min	40 - 45 min	45 - 50 min	50 - 55 min	55 - 60 min
vehicle	1	19.31	0	14.15	0	1.44	62.89	16.73	20.74	90.8	51.08	59.53	101.41
vehicle	2	42.24	0	.96	.96	0	0	0	0	2.84	10.18	5.9	17.11
vehicle	3	17.69	0	0	0	1.93	2.93	54.17	8.34	15.98	15.46	23.53	35.52
vehicle	4	46.26	0	0	0	0	11.12	8.27	20.42	18.58	84	55.42	81.26
vehicle	5	20.26	0	8.68	.96	0	0	0	13.13	17.93	2.56	56.8	39.66
vehicle	6	37	2.44	0	1.63	0	0	0	0	0	5.90	11.99	34.93
vehicle	7	25.34	3.1	2.69	0	13.87	26.81	12.36	50.69	25.06	69.93	54.63	77.95
vehicle	8	14.29	0	0	0	7.59	11.46	27.26	60.48	58.05	85.87	90.87	121.31
vehicle	9	50.4	24.92	1.94	1.9	0	15.72	12.12	26.13	39.76	58.66	64.18	75.13
vehicle	10	79.84	0	21.23	.5	0	4.84	54.99	51.93	46.84	72.58	76.1	66.68
Pet 10	1	27.53	0	0	0	0	50.72	16.18	21.93	19.99	32.82	50.83	28.13
Pet 10	2	67.97	0	0	0	0	0	0	0	3.83	7.49	33.9	0
Pet 10	3	7.48	0	0	0	0	0	0	0	0	0	0	0
Pet 10	4	43.63	0	0	0	0	0	0	0	0	0	88.43	6.56
Pet 10	5	22.23	0	0	0	0	0	0	0	0	21.14	49.06	82.61
Pet 10	6	105.53	0	0	0	0	0	0	0	0	15.56	128.36	23.47
Pet 10	7	73.13	53.69	0	0	0	0	0	25.43	74.38	120.19	37.88	79.12
Pet 10	8	26.63	0	104.32	25.69	52.38	143.15	20.94	66.18	28.18	52.3	104.51	247.35
Pet 10	9	35.71	0	0	0	0	0	0	0	0	0	123.96	3.14
Pet 10	10	83.54	15.96	0	37.25	0	0	1.48	30.83	88.41	132.08	125.59	95.42
Pet 20	1	12.28	0	0	5.36	0	0	0	0	0	6.69	18.1	38.56
Pet 20	2	5.84	0	0	0	0	0	0	0	0	0	0	0
pet 20	3	7.33	0	0	0	0	0	0	0	0	0	97.9	75.53
pet 20	4	50.06	0	0	0	0	0	8.49	46.63	147.23	137.34	115.08	146.76
Pet 20	5	10.2	0	0	0	0	0	0	0	0	0	0	0
Pet 20	6	44.79	0	0	0	0	0	0	0	0	0	0	0
Pet 20	7	1.83	0	1.66	2.36	1.08	2.87	0	0	0	0	12.98	10.08
Pet 20	8	0	0	0	0	0	0	0	0	0	0	0	0
Pet 20	9	19.93	0	0	0	0	0	0	.92	82.71	28.66	150.81	109.66
Pet 20	10	0	0	0	0	0	0	0	0	0	0	0	0
pet 30	1	20.34	1.63	0	0	0	0	0	0	0	0	4	0
pet 30	2	6.85	0	0	0	0	0	0	0	0	0	0	0
pet 30	3	24.98	0	0	0	0	0	0	0	0	0	0	0
pet 30	4	30.4	0	0	0	0	0	0	0	0	0	0	0
pet 30	5	18.49	0	0	0	0	0	0	0	0	0	0	0
pet 30	6	3.8	0	0	0	0	0	0	0	0	0	0	0
pet 30	7	0	0	0	0	0	0	0	0	0	0	0	0
pet 30	8	21.13	0	0	0	0	0	0	0	0	20.08	35.38	75.09
pet 30	9	29.98	19.17	8.48	0	0	0	0	0	0	0	0	45.03
pet 30	10	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 5: Number of licks recorded in blocks of 5 min after i.p. injection of vehicle or codeine (cod) (10, 25 and 50 mg/kg) in the naked mole-rat during a 1 h observation period.

Treatment	Animal number	0 - 5 min	5 - 10 min	10 - 15 min	15 - 20 min	20 - 25 min	25 - 30 min	30 - 35 min	35 - 40 min	40 - 45 min	45 - 50 min	50 - 55 min	55 - 60 min
vehicle	1	83	0	7	7	19	22	78	173	161	254	230	274
vehicle	2	75	0	0	0	2	11	25	27	159	34	172	186
vehicle	3	201	90	64	0	0	0	0	49	159	120	198	204
vehicle	4	181	0	29	1	0	15	129	115	110	179	170	162
vehicle	5	120	0	0	0	1	0	16	14	130	120	165	164
vehicle	6	190	25	69	2	165	75	127	117	173	135	170	238
vehicle	7	183	0	90	181	17	72	134	151	259	293	246	298
vehicle	8	161	1	0	0	29	68	118	107	138	197	121	243
vehicle	9	66	0	0	0	10	0	0	6	97	67	45	91
vehicle	10	71	5	0	76	0	0	43	0	27	183	125	75
cod 10	1	24	0	0	15	0	0	0	0	0	0	68	2
cod 10	2	17	0	0	0	0	0	0	0	0	0	10	0
cod 10	3	108	0	0	0	0	0	0	0	0	0	132	242
cod 10	4	90	0	0	0	0	0	0	0	112	153	79	108
cod 10	5	103	16	39	0	20	0	0	18	34	59	117	102
cod 10	6	135	29	9	3	0	0	0	0	5	191	185	157
cod 10	7	103	0	0	0	0	0	29	210	89	42	180	153
cod 10	8	60	0	0	0	0	0	0	0	41	72	86	184
cod 10	9	84	12	0	0	0	20	28	27	136	202	182	78
cod 10	10	139	0	0	0	0	0	0	0	55	127	122	174
cod 25	1	39	0	0	0	0	0	27	30	146	92	86	178
cod 25	2	73	0	4	0	0	0	41	22	126	109	116	128
cod 25	3	82	44	38	0	0	0	0	33	150	13	158	86
cod 25	4	147	0	0	0	0	0	0	91	85	141	180	182
cod 25	5	57	0	0	0	0	0	0	0	98	134	111	129
cod 25	6	89	0	0	0	0	0	0	140	78	150	126	92
cod 25	7	95	0	0	0	0	0	0	35	49	82	101	132
cod 25	8	67	0	0	0	0	0	0	0	0	0	52	107
cod 25	9	65	0	0	0	0	0	0	0	21	63	97	142
cod 25	10	53	0	0	0	0	0	0	29	52	103	101	97
cod 50	1	5	0	0	0	0	0	0	0	0	0	0	0
cod 50	2	20	0	0	0	0	0	0	0	0	0	0	0
cod 50	3	0	0	0	0	0	0	0	0	0	0	0	0
cod 50	4	5	0	0	0	0	0	0	0	0	0	0	0
cod 50	5	0	0	0	0	0	0	0	0	0	0	0	0
cod 50	6	14	0	0	0	0	0	0	0	0	0	0	0
cod 50	7	38	0	0	0	0	0	0	0	0	0	0	0
cod 50	8	24	0	0	0	0	0	0	0	0	0	0	0
cod 50	9	30	0	0	0	0	0	0	0	0	0	0	0
cod 50	10	64	0	0	0	0	0	0	0	0	0	0	0

Appendix 6: Time (sec) spent licking the injected hind paw, in blocks of 5 min after i.p. injection of vehicle or codeine (cod) (10, 25 and 50 mg/kg) in the naked mole-rat during a 1 h observation period.

Treatment	Animal number	0 - 5 min	5 - 10 min	10 - 15 min	15 - 20 min	20 - 25 min	25 - 30 min	30 - 35 min	35 - 40 min	40 - 45 min	45 - 50 min	50 - 55 min	55 - 60 min
vehicle	1	44.05	0	3.29	3.55	9.36	10.29	40.16	100.83	85.59	140.24	131.58	182.49
vehicle	2	42.96	0	0	0	.96	4.2	11.39	12.2	67.36	21.2	75.89	106.23
vehicle	3	82.67	38.55	26.54	0	0	0	0	19.03	67.18	54.82	101.43	85.63
vehicle	4	82.56	0	12.63	.45	0	5.64	53.58	47.64	42.93	76.2	72.15	87.98
vehicle	5	54.01	0	0	0	.72	0	8.83	8.58	87.33	53.41	76.03	79.13
vehicle	6	99.53	13.05	38.85	1	100.01	39.05	65.61	59.58	98.54	69.03	86.37	127.4
vehicle	7	92.06	0	45.9	100.88	9.06	42.99	82.96	66.87	125.82	159.45	131.55	159.45
vehicle	8	65.23	.72	0	0	13.01	33.05	48.96	40.09	69.41	83.54	56.95	112.92
vehicle	9	30.54	0	0	0	5.85	0	0	2.99	45.06	32.72	21.25	42.16
vehicle	10	32.81	1.98	0	39.02	0	0	21.89	0	13.11	99.27	71.11	38.14
cod 10	1	12.3	0	0	6.39	0	0	0	0	0	0	36.33	.85
cod 10	2	8.21	0	0	0	0	0	0	0	0	0	3.35	0
cod 10	3	52.05	0	0	0	0	0	0	0	0	0	79.66	149.52
cod 10	4	47.51	0	0	0	0	0	0	0	73.29	89.64	45.74	61.54
cod 10	5	58.88	9.01	17	0	9.78	0	0	7.2	18.82	32.82	73.09	88.42
cod 10	6	50.75	16.62	4.95	1.09	0	0	0	0	2.07	105.29	110.31	91.13
cod 10	7	56.3	0	0	0	0	0	13.09	114.03	44.83	21.21	102.48	86.06
cod 10	8	33.62	0	0	0	0	0	0	0	21.63	41.34	50.66	109.48
cod 10	9	45.17	5.03	0	0	0	11.21	14.8	15.98	72.37	114.52	107.17	39.74
cod 10	10	50.26	0	0	0	0	0	0	0	25.29	81.12	80.06	91.89
cod 25	1	19.79	0	0	0	0	0	14.12	14.9	74.28	57.78	52.17	85.01
cod 25	2	35.43	0	2.28	0	0	0	20.87	12.17	69.15	66.28	71.89	80.41
cod 25	3	51.67	29.13	25.74	0	0	0	0	20.34	97.49	7.82	81.69	39.14
cod 25	4	85.21	0	0	0	0	0	0	44.32	41.97	78.33	104.29	101.24
cod 25	5	30.35	0	0	0	0	0	0	0	43.82	69.75	49.5	69.05
cod 25	6	42.18	0	0	0	0	0	0	85.14	32.01	101.23	76.02	41.22
cod 25	7	42.67	0	0	0	0	0	0	17.44	27.41	31.38	48.45	76.25
cod 25	8	31.08	0	0	0	0	0	0	0	0	0	22.65	51.02
cod 25	9	20.19	0	0	0	0	0	0	0	9.36	32.98	47.62	63.68
cod 25	10	27.96	0	0	0	0	0	0	13.78	26.06	55.29	53.1	50.48
cod 50	1	3.59	0	0	0	0	0	0	0	0	0	0	0
cod 50	2	11.09	0	0	0	0	0	0	0	0	0	0	0
cod 50	3	0	0	0	0	0	0	0	0	0	0	0	0
cod 50	4	2.7	0	0	0	0	0	0	0	0	0	0	0
cod 50	5	0	0	0	0	0	0	0	0	0	0	0	0
cod 50	6	4.8	0	0	0	0	0	0	0	0	0	0	0
cod 50	7	20.8	0	0	0	0	0	0	0	0	0	0	0
cod 50	8	14.11	0	0	0	0	0	0	0	0	0	0	0
cod 50	9	15.33	0	0	0	0	0	0	0	0	0	0	0
cod 50	10	35.3	0	0	0	0	0	0	0	0	0	0	0

Appendix 7: Number of licks recorded in blocks of 5 min after i.p. injection of vehicle or ASA (200, 400 and 600 mg/kg) in the naked mole-rat during a 1 h observation period.

Treatment	Animal number	0 - 5 min	5 - 10 min	10 - 15 min	15 - 20 min	20 - 25 min	25 - 30 min	30 - 35 min	35 - 40 min	40 - 45 min	45 - 50 min	50 - 55 min	55 - 60 min
vehicle	1	88	0	0	55	81	103	180	220	140	178	188	218
vehicle	2	166	10	32	15	53	203	186	167	213	194	203	218
vehicle	3	117	31	18	0	39	12	187	83	73	139	151	82
vehicle	4	58	18	90	158	49	73	4	115	240	250	180	172
vehicle	5	162	0	0	0	0	46	180	85	40	256	107	108
vehicle	6	225	84	90	61	0	0	0	122	18	120	205	178
vehicle	7	218	0	118	130	0	0	0	0	114	174	180	103
vehicle	8	152	0	0	0	0	46	159	88	47	249	107	108
vehicle	9	118	0	0	0	32	1	0	2	18	101	72	59
vehicle	10	189	15	90	158	49	73	3	118	235	258	182	170
ASA 200	1	144	29	0	0	0	19	82	54	143	124	129	137
ASA 200	2	134	0	10	2	36	110	82	145	153	187	179	170
ASA 200	3	110	48	0	0	0	23	0	0	22	88	117	20
ASA 200	4	43	0	8	5	9	9	8	14	160	83	77	101
ASA 200	5	119	5	0	8	0	0	0	0	58	112	121	121
ASA 200	6	171	98	82	81	24	0	44	159	138	209	164	198
ASA 200	7	57	0	0	0	0	9	0	166	40	83	140	183
ASA 200	8	132	2	22	11	21	2	0	57	17	86	60	32
ASA 200	9	177	99	97	0	0	0	127	284	158	33	205	118
ASA 200	10	140	32	80	0	45	0	9	15	81	0	73	67
ASA 400	1	90	8	0	2	0	21	18	48	75	98	137	124
ASA 400	2	89	0	0	0	0	0	0	58	42	118	123	129
ASA 400	3	148	0	0	0	0	0	0	97	57	0	138	0
ASA 400	4	31	13	0	11	60	70	69	88	157	127	161	115
ASA 400	5	145	3	28	0	0	0	141	194	61	179	188	175
ASA 400	6	154	55	0	0	0	0	141	34	84	52	117	95
ASA 400	7	165	128	5	0	0	0	0	0	0	242	111	93
ASA 400	8	154	74	0	14	0	20	181	229	22	135	101	141
ASA 400	9	107	48	0	0	104	183	0	4	129	32	96	107
ASA 400	10	62	0	0	0	14	0	0	2	7	78	95	182
ASA 600	1	222	82	0	10	0	0	0	0	0	0	0	204
ASA 600	2	134	0	0	0	0	0	0	37	0	0	188	38
ASA 600	3	144	3	22	30	0	0	0	0	0	0	0	0
ASA 600	4	103	35	80	0	0	0	0	0	7	27	188	114
ASA 600	5	70	3	0	0	1	0	0	0	6	0	55	30
ASA 600	6	108	0	0	0	0	0	0	67	108	84	133	45
ASA 600	7	114	0	0	0	0	0	0	20	0	100	120	40
ASA 600	8	65	13	0	7	7	15	0	0	108	91	47	80
ASA 600	9	79	37	61	0	0	0	0	0	0	0	100	117
ASA 600	10	69	27	52	0	28	30	0	0	0	70	68	138

Appendix 8: Time (sec) spent licking the injected hind paw, in blocks of 5 min after i.p. injection of vehicle or ASA (200, 400 and 600 mg/kg) in the naked mole-rat during a 1 h observation period.

Treatment	Animal number	0 - 5 min	5 - 10 min	10 - 15 min	15 - 20 min	20 - 25 min	25 - 30 min	30 - 35 min	35 - 40 min	40 - 45 min	45 - 50 min	50 - 55 min	55 - 60 min
vehicle	1	46.92	0	0	39.39	58.21	152.44	140.28	169.88	109.42	144.93	152.3	186.59
vehicle	2	72.78	4.94	14.78	8.54	26.62	158.95	126.56	114.56	157.07	148.49	162.48	171.78
vehicle	3	56.05	16.32	7.63	0	18.01	8.21	104.93	41.45	46.34	94.76	108.08	83.73
vehicle	4	34.4	7.15	45.63	93.01	26.28	36.65	1.59	85.32	106.55	149.84	151.95	135.89
vehicle	5	68.05	0	0	0	0	27.56	113.53	48.59	20.05	105.62	79.19	78.62
vehicle	6	111.71	0	63.1	87.32	0	0	0	0	43.97	74.42	96.31	41.26
vehicle	7	27.9	0	31.06	0	12.39	15.63	22.62	132.37	107.8	63.05	136.11	140.94
vehicle	8	97.04	20.57	47.23	25.73	0	0	0	57.35	8.03	55.26	99.23	91.20
vehicle	9	84.24	7	45.64	93.01	28.28	36.65	1.45	59.59	122.48	137.07	63.91	91.88
vehicle	10	62.19	0	0	0	19.93	19	0	1.56	8.5	84.98	36.33	28.2
ASA 200	1	74.03	16.58	0	0	0	12.06	58.58	39.17	107.8	98.5	67.92	110.77
ASA 200	2	78.06	0	5.23	1.13	22.81	80.06	56.54	101.77	107.12	139.01	144.07	122.99
ASA 200	3	82.35	27.79	0	0	0	16.87	0	0	13.99	86.16	93.58	10.76
ASA 200	4	22.18	0	4.12	2.83	4.69	5.09	4.72	8.24	112.24	57.09	81.59	86.31
ASA 200	5	57.59	2.13	0	3.58	0	0	0	0	41.48	76.79	84.25	84.96
ASA 200	6	68.07	0	0	0	0	16.73	78.33	29.82	21.3	132.82	58.54	41.06
ASA 200	7	75.53	46.97	33.14	31.15	12.48	0	20.58	101.28	73.59	94.24	49.35	93.73
ASA 200	8	57.37	95	10.41	4.2	10.18	1	0	34.12	8.17	29	23.46	14.16
ASA 200	9	84.46	51.21	51.78	0	0	0	67.29	131.5	89.69	16.75	91.23	85.72
ASA 200	10	24.07	0	0	0	0	3.65	0	74.07	17.42	28.07	88.86	84.76
ASA 400	1	26.39	0	0	0	5.89	0	0	97	2.81	35.26	41.28	143.89
ASA 400	2	51.35	25.53	0	0	51.56	98.06	0	1.83	62.68	14.26	46.25	60.01
ASA 400	3	88.89	71.83	1.86	0	0	0	0	0	0	130.04	60.00	46.02
ASA 400	4	86.25	26.66	0	0	0	0	75.23	20.38	45.23	26.14	50.80	45.78
ASA 400	5	82.42	42.64	0	4.53	0	7.59	81.17	106.24	12.36	71.06	50.41	72.72
ASA 400	6	43.72	2.57	0	1.28	0	15.22	7.42	32.92	51.58	66.48	99.1	99.72
ASA 400	7	49.48	0	0	0	0	0	0	46.72	31.22	97.56	102.74	97.75
ASA 400	8	75.45	0	0	0	0	0	0	72.83	42.26	0	109.58	0
ASA 400	9	14.62	6.67	0	5.58	40.96	48.74	46.58	86.85	114.94	66.73	126.18	80.03
ASA 400	10	60.09	1.25	18.48	0	0	0	68.72	94.29	28.09	82.15	79.03	73.52
ASA 600	1	51.63	0	0	0	0	0	0	43.57	83.95	59.7	113.77	35.98
ASA 600	2	65.37	0	0	0	0	0	0	11.14	0	76.48	63.9	26.43
ASA 600	3	30.21	6.51	3.66	3.11	3.8	10.18	0	0	80.23	85.62	34.33	85.78
ASA 600	4	39.63	25.78	47.04	0	0	0	0	0	0	0	82.67	97.02
ASA 600	5	32.92	16.33	26.21	0	17.19	17.51	0	0	0	48.88	47.43	95.81
ASA 600	6	63.53	0	0	0	0	0	0	21.21	0	0	91.34	18.69
ASA 600	7	117.09	46.9	0	4.36	0	0	0	0	0	0	0	103.32
ASA 600	8	82.92	1.34	15.18	17.57	0	0	0	0	0	0	0	0
ASA 600	9	44.55	18.09	25.13	0	0	0	0	0	3.45	12.08	108.54	60.91
ASA 600	10	41.43	1.32	0	0	92	0	0	0	4.01	0	28.41	12.71

Appendix 9: Number of licks recorded in blocks of 5 min after i.p. injection of vehicle or naproxen (nap) (50, 100 and 200 mg/kg) in the naked mole-rat during a 1 h observation period.

Treatment	Animal number	0 - 5 min	5 - 10 min	10 - 15 min	15 - 20 min	20 - 25 min	25 - 30 min	30 - 35 min	35 - 40 min	40 - 45 min	45 - 50 min	50 - 55 min	55 - 60 min
vehicle	1	58	0	0	0	4	8	129	21	49	98	102	93
vehicle	2	179	0	0	0	1	10	131	118	118	174	178	153
vehicle	3	200	30	13	14	0	170	78	29	135	38	288	132
vehicle	4	187	9	29	180	45	63	13	118	135	170	182	202
vehicle	5	78	0	0	0	0	0	38	27	180	38	178	180
vehicle	6	119	0	0	0	0	0	15	18	132	116	170	161
vehicle	7	117	2	0	0	0	0	0	179	62	129	191	236
vehicle	8	121	8	12	0	0	0	0	0	88	70	141	150
vehicle	9	50	1	0	0	0	0	29	62	90	18	29	80
vehicle	10	89	22	0	0	7	8	29	80	80	141	98	217
nap 50	1	183	17	15	10	14	0	10	90	37	139	91	99
nap 50	2	122	76	59	0	33	0	38	140	37	78	55	183
nap 50	3	184	154	19	24	132	11	3	0	42	44	110	88
nap 50	4	223	115	99	80	0	41	88	25	101	111	113	172
nap 50	5	104	26	29	89	11	18	89	0	129	98	167	196
nap 50	6	225	24	102	22	47	71	150	83	109	120	171	186
nap 50	7	128	18	0	0	0	0	61	147	135	177	201	188
nap 50	8	98	8	0	0	0	0	18	0	108	115	182	71
nap 50	9	60	15	0	0	0	0	29	81	129	72	29	18
nap 50	10	73	0	0	0	0	0	0	115	82	17	22	39
nap 100	1	172	19	51	17	0	73	37	141	185	159	155	215
nap 100	2	138	8	17	35	15	8	11	0	24	48	31	131
nap 100	3	122	69	95	8	5	35	148	5	0	0	77	5
nap 100	4	104	0	0	34	74	0	0	0	0	0	0	0
nap 100	5	226	60	30	91	59	104	28	93	38	121	104	198
nap 100	6	76	11	0	0	8	7	13	88	81	94	71	88
nap 100	7	78	30	18	88	15	28	9	18	30	31	40	61
nap 100	8	244	112	2	28	0	25	44	84	18	240	112	90
nap 100	9	87	33	142	88	8	18	107	0	5	180	80	53
nap 100	10	120	57	44	11	18	141	101	153	109	113	174	123
nap 200	1	65	31	0	0	0	0	0	0	70	53	153	61
nap 200	2	102	94	22	0	0	0	0	0	0	115	80	120
nap 200	3	236	100	143	9	0	0	0	0	114	166	33	108
nap 200	4	150	43	32	98	24	90	87	11	85	85	32	82
nap 200	5	153	98	43	20	9	5	28	87	81	134	218	101
nap 200	6	62	0	0	8	0	0	0	4	0	0	0	0
nap 200	7	88	17	62	40	0	0	0	31	211	116	138	183
nap 200	8	73	4	48	28	0	22	0	0	0	0	0	0
nap 200	9	74	0	7	0	0	0	0	0	0	0	0	0
nap 200	10	89	20	18	22	0	0	0	0	0	0	86	29

Appendix 10: Time (sec) spent licking the injected hind paw, in blocks of 5 min after i.p. injection of vehicle or naproxen (nap) (50, 100 and 200 mg/kg) in the naked mole-rat during a 1 h observation period.

Treatment	Animal number	0 - 5 min	5 - 10 min	10 - 15 min	15 - 20 min	20 - 25 min	25 - 30 min	30 - 35 min	35 - 40 min	40 - 45 min	45 - 50 min	50 - 55 min	55 - 60 min
vehicle	1	30.98	0	0	0	1.96	2.99	55.23	10.41	25.48	52.89	58.52	49.87
vehicle	2	89.04	0	0	0	.42	4.54	54.98	51.51	47.69	73.61	78.25	87.2
vehicle	3	89.94	11.17	6.2	6.19	0	88.75	39.13	15.53	78.22	21.52	139.24	81.97
vehicle	4	85.01	4.58	15.52	89.11	25.19	33.45	6.21	60.59	71.09	91.88	93.94	105.81
vehicle	5	43.96	0	0	0	0	0	15.59	12.21	88.38	22	81.89	100.22
vehicle	6	53.21	0	0	0	0	0	6.82	8.98	67.63	80.41	79.08	79.12
vehicle	7	52.95	1.98	0	0	0	0	0	91.3	29.58	86.38	106.38	145.34
vehicle	8	70.40	2.5	6.18	0	0	0	0	0	45.83	44.07	91.58	96.39
vehicle	9	28.01	.41	0	0	0	0	15.85	33.54	50.01	7.59	15.89	45.91
vehicle	10	48.81	12.81	0	0	4.2	5.98	19.04	45.03	38.8	89.13	84.78	122.82
nap 50	1	109.48	8.35	9.9	8.28	9.35	0	7.01	84.99	28.99	88.32	80.17	66.99
nap 50	2	83.24	51.89	39.08	0	21.38	0	22.83	101.3	23.04	40.86	29.73	110.26
nap 50	3	108.36	102.89	12.05	14.79	90.23	6.99	1.49	0	24.72	22.8	60.96	42.91
nap 50	4	139.83	67.81	65.38	39.09	0	28.83	84.83	11.77	82.91	83.84	72.29	107.84
nap 50	5	59.37	15.69	15.68	84.85	6.97	10.96	58.6	0	85.69	84.8	81.98	114.83
nap 50	6	135.94	9.93	58.8	11.33	28.87	43.68	85.88	49.13	60.5	69.08	107.7	99.24
nap 50	7	81.11	8.33	0	0	0	0	30.3	91.83	82.77	117.01	144.83	125.07
nap 50	8	46.29	5.01	0	0	0	0	10.25	0	80.69	65.89	92.02	45.84
nap 50	9	36.28	8.29	0	0	0	0	16.25	49.88	79.03	52.81	16.26	10.17
nap 50	10	45.21	0	0	0	0	0	0	66.98	50.19	9.13	12	23.87
nap 100	1	93.36	10.33	37	12.23	0	46.88	23.12	96.82	115.88	109.71	100.38	124.89
nap 100	2	81.14	5.47	10.68	19.6	9.26	2.68	8.73	0	15.55	26.49	18.48	86.01
nap 100	3	76.08	49.63	68.96	2.69	1.47	23.43	102.61	2.17	0	0	55.3	2.14
nap 100	4	66.09	0	0	23.36	52.92	0	0	0	0	0	0	0
nap 100	5	147.78	35.24	20.34	61.8	38.58	73.78	17.03	67.94	27.04	91.09	73.93	145.25
nap 100	6	48.02	4.93	0	0	5.05	4.5	7.4	42.26	41.52	58.78	44.68	55.65
nap 100	7	47.59	14.66	9.04	58.06	8.66	18.12	1.07	11.87	19.28	20.19	24.06	37.34
nap 100	8	159.49	86.78	.94	2.52	0	14.8	56.57	60.85	10.86	181.05	81.05	63.45
nap 100	9	58.3	20.31	108.69	45.99	4.88	10.81	81.42	8.18	2.25	189.89	40.2	35.91
nap 100	10	72.84	37.34	28.99	5.09	9.49	86.49	76.51	103.38	76.12	73.05	127.53	83.28
nap 200	1	37.93	23.16	0	0	0	0	0	0	48.98	38.99	91.41	38.53
nap 200	2	62.28	55.49	13.06	0	0	0	0	0	0	66.78	42.38	73.25
nap 200	3	184.32	142.62	104.42	6.29	0	0	0	0	79.27	124.83	28.31	127.84
nap 200	4	104.28	26.75	20.69	69	18.1	78.2	65.21	24.27	71.09	39.27	17.21	28.36
nap 200	5	99.33	84.29	28.98	12.15	4.52	5.01	17.79	84.07	52.65	91.06	159	72.4
nap 200	6	39.49	0	0	3.11	0	0	0	1.49	0	0	0	0
nap 200	7	63.21	11.28	42.75	29.06	0	0	0	20.81	159.2	86.79	100.62	126.28
nap 200	8	50.79	2.06	30.22	16.33	0	18.13	0	0	0	0	0	0
nap 200	9	45.27	0	2.76	0	0	0	0	0	0	0	0	0
nap 200	10	52.06	11.26	11.21	12.63	0	0	0	0	0	0	58.92	19.63

Appendix 11: Number of licks recorded in blocks of 5 min after i.p. injection of vehicle or hydrocortisone (hyd) (40, 75 and 150 mg/kg) in the naked mole-rat during a 1 h observation period.

Treatment	Animal number	0 - 5 min	5 - 10 min	10 - 15 min	15 - 20 min	20 - 25 min	25 - 30 min	30 - 35 min	35 - 40 min	40 - 45 min	45 - 50 min	50 - 55 min	55 - 60 min
vehicle	1	243	99	31	0	0	0	97	43	159	198	201	185
vehicle	2	128	15	14	85	18	22	12	131	133	165	172	132
vehicle	3	128	89	104	0	40	42	21	148	138	255	220	232
vehicle	4	72	2	0	4	0	12	19	78	124	171	115	111
vehicle	5	52	0	52	24	8	0	58	65	58	100	93	135
vehicle	6	180	0	0	0	0	0	0	0	0	0	142	111
vehicle	7	85	7	20	42	7	8	33	79	82	139	94	221
vehicle	8	115	1	0	0	0	12	18	136	101	73	111	129
vehicle	9	178	0	22	0	0	20	152	117	118	179	180	181
vehicle	10	100	18	88	128	12	0	3	123	208	228	170	163
hyd 40	1	137	0	11	80	0	50	11	0	95	112	24	91
hyd 40	2	115	78	0	0	0	0	0	0	0	0	0	0
hyd 40	3	147	4	0	0	0	25	21	44	60	138	185	119
hyd 40	4	240	71	88	0	0	0	0	5	118	180	80	199
hyd 40	5	93	3	0	0	0	0	0	0	129	178	199	209
hyd 40	6	208	74	0	0	5	0	98	44	178	125	198	210
hyd 40	7	200	99	35	5	5	9	0	9	0	0	34	26
hyd 40	8	125	26	105	24	13	3	48	125	200	50	88	279
hyd 40	9	158	13	9	8	0	0	102	122	113	60	110	215
hyd 40	10	122	18	1	9	0	0	0	0	103	112	98	121
hyd 75	1	90	8	12	25	0	0	0	0	84	0	7	18
hyd 75	2	133	62	25	65	18	0	41	0	247	35	0	0
hyd 75	3	163	0	0	0	0	0	0	0	40	0	62	20
hyd 75	4	168	2	51	0	0	0	0	18	21	223	14	99
hyd 75	5	97	0	0	0	7	5	2	0	5	8	0	4
hyd 75	6	58	0	0	0	0	0	0	0	0	98	1	155
hyd 75	7	108	18	19	14	8	20	0	45	74	18	138	40
hyd 75	8	44	0	0	0	0	0	0	0	18	118	61	48
hyd 75	9	121	0	0	0	0	0	0	0	8	12	78	98
hyd 75	10	152	5	0	0	0	0	0	29	121	78	94	28
hyd 150	1	125	25	5	0	0	52	24	30	81	28	89	0
hyd 150	2	158	0	0	0	0	0	0	0	0	0	0	0
hyd 150	3	191	182	43	0	0	20	0	0	0	0	0	0
hyd 150	4	94	50	0	0	0	0	0	0	0	0	20	33
hyd 150	5	233	10	0	0	0	0	0	0	0	0	0	13
hyd 150	6	126	7	13	16	0	0	0	44	88	89	142	148
hyd 150	7	133	88	0	0	0	0	0	0	0	3	114	17
hyd 150	8	112	13	0	0	0	0	0	0	0	20	88	41
hyd 150	9	78	0	0	0	0	0	0	0	0	0	98	22
hyd 150	10	68	0	0	3	0	0	0	0	0	0	0	12

Appendix 12: Time (sec) spent licking the injected hind paw, in blocks of 5 min after i.p. injection of vehicle or hydrocortisone (hyd) (40, 75 and 150 mg/kg) in the naked mole-rat during a 1 h observation period.

Treatment	Animal number	0 - 5 min	5 - 10 min	10 - 15 min	15 - 20 min	20 - 25 min	25 - 30 min	30 - 35 min	35 - 40 min	40 - 45 min	45 - 50 min	50 - 55 min	55 - 60 min
vehicle	1	143.18	66.84	18.21	0	0	0	56.26	22.32	89.06	99.06	121.06	103.95
vehicle	2	82.96	7.42	7.9	58.83	10.23	10.9	7.26	79.37	85.09	136.17	119.61	93.89
vehicle	3	80.21	42.23	63.98	0	24.66	26.94	13.09	98.88	90.18	167.56	151.92	161.47
vehicle	4	46.23	.89	0	1.5	0	5.82	0.3	43.37	71.26	105.65	88.18	69.16
vehicle	5	26.58	0	35.58	12.98	5.06	0	34.93	45.33	30.82	55.71	54.23	82.16
vehicle	6	109.89	0	0	0	0	0	0	0	0	0	87.36	68.76
vehicle	7	46.84	4.53	12.53	24.06	2.97	4.23	20.03	42.91	36.64	88.17	52.73	124.26
vehicle	8	51.29	.43	0	0	0	6.09	6.69	74.57	60.07	40.14	82.92	78.23
vehicle	9	78.54	0	10.14	0	0	12.46	54.69	52.38	47.84	73.58	76.27	68.56
vehicle	10	85.36	7.68	45.15	76.98	7.12	0	.80	76.4	102.45	153.25	93.63	93.61
hyd 40	1	71.97	0	6.46	51.24	0	33.43	8.08	0	57.29	77.53	11.22	46.86
hyd 40	2	59.03	46.42	0	0	0	0	0	0	0	0	0	0
hyd 40	3	81.29	1.58	0	0	0	9.99	11.96	24.73	31.79	81.1	122.42	67.12
hyd 40	4	145.69	42.96	46.96	0	0	0	0	2.38	77.45	100.45	44.06	116.08
hyd 40	5	48.73	.94	0	0	0	0	0	0	83.43	92.58	120.11	121.38
hyd 40	6	112.33	45.29	0	0	2.33	0	57.71	23.8	108.06	87.27	104.18	125.01
hyd 40	7	107.55	100.27	20.96	2.06	2.06	8.29	0	5.15	0	0	19.21	13.8
hyd 40	8	70.85	15.35	73.81	16.12	6.05	1.06	22.93	79.9	134.53	27.85	81.84	184.33
hyd 40	9	80.38	6.46	5.29	3.82	0	0	60.49	72.07	61.49	28.36	61.19	125.34
hyd 40	10	56.02	8.05	.42	8.2	0	0	0	0	62.45	65.49	50.39	70.12
hyd 75	1	43.7	3.18	6.44	14.21	0	0	0	0	52.45	0	2.85	7.63
hyd 75	2	78.7	27.83	15.33	36.9	8.92	0	24.32	0	161.06	21.25	0	0
hyd 75	3	90.48	0	0	0	0	0	0	0	20.49	0	35.72	9.63
hyd 75	4	97.84	.98	28.3	0	0	0	0	6.89	11.87	137.85	8.89	56.88
hyd 75	5	51.31	0	0	0	3.19	1.81	.66	0	1.83	2.53	0	1.81
hyd 75	6	31.67	0	0	0	0	0	0	0	0	81.03	.89	80.75
hyd 75	7	105.78	10.29	10.13	7.22	4.09	19.99	0	28.16	46.91	9.96	79.53	20.5
hyd 75	8	22.1	0	0	0	0	0	0	0	9.03	65.07	35.17	26.99
hyd 75	9	84.23	0	0	0	0	0	0	0	3.84	5.25	37.96	62.54
hyd 75	10	80.91	2.24	0	0	0	0	0	13.29	85.08	35.49	49.06	12.24
hyd 150	1	65.22	11.73	1.58	0	0	30.3	13.33	16.35	51.47	18.44	61.89	0
hyd 150	2	98.09	0	0	0	0	0	0	0	0	0	0	0
hyd 150	3	101.98	102.54	33.07	0	0	14.55	0	0	0	0	0	0
hyd 150	4	48.8	36.49	0	0	0	0	0	0	0	0	10.05	20.46
hyd 150	5	125.71	4.99	0	0	0	0	0	0	0	0	0	6.03
hyd 150	6	72.42	2.46	6.48	0.69	0	0	0	28.06	59.92	44.06	81.85	96.31
hyd 150	7	72.53	58.6	0	0	0	0	0	0	0	1.38	70.96	10.96
hyd 150	8	72.8	7.32	0	0	0	0	0	0	0	17.53	50.96	24.67
hyd 150	9	40.01	0	0	0	0	0	0	0	0	0	49.22	12.51
hyd 150	10	38.44	0	0	1.18	0	0	0	0	0	0	0	4.68

Appendix 13: Number of licks recorded in blocks of 5 min after i.p. injection of vehicle or dexamethasone (dex) (10, 20 and 30 mg/kg) in the naked mole-rat during a 1 h observation period.

Treatment	Animal number	0 - 5 min	5 - 10 min	10 - 15 min	15 - 20 min	20 - 25 min	25 - 30 min	30 - 35 min	35 - 40 min	40 - 45 min	45 - 50 min	50 - 55 min	55 - 60 min
vehicle	1	72	4	0	9	0	15	84	128	90	129	280	227
vehicle	2	90	51	72	0	0	0	0	4	77	97	132	118
vehicle	3	83	0	5	0	0	0	0	84	128	90	121	289
vehicle	4	123	0	10	4	0	0	0	0	38	190	180	101
vehicle	5	132	14	0	0	21	4	13	0	61	270	318	301
vehicle	6	117	143	11	0	0	0	0	173	130	8	132	201
vehicle	7	118	2	0	0	0	0	2	180	130	180	253	212
vehicle	8	114	31	2	0	0	1	0	0	128	108	178	108
vehicle	9	182	22	0	0	2	0	0	82	83	132	187	201
vehicle	10	89	2	0	0	0	0	0	181	81	102	192	178
dex 10	1	138	230	87	0	29	0	208	230	238	298	247	281
dex 10	2	100	0	78	0	185	184	158	227	273	307	228	299
dex 10	3	105	0	183	4	0	0	0	107	91	180	93	237
dex 10	4	70	0	7	0	0	2	141	88	50	128	41	243
dex 10	5	56	171	37	5	233	99	72	148	81	201	204	69
dex 10	6	116	0	0	00	50	8	4	0	107	59	229	217
dex 10	7	104	0	0	28	103	98	29	197	122	198	205	267
dex 10	8	80	0	0	0	28	2	141	88	50	0	22	217
dex 10	9	133	07	18	49	0	14	50	07	113	192	128	85
dex 10	10	91	28	58	7	15	39	28	122	127	133	172	238
dex 20	1	73	0	2	0	0	0	0	0	0	0	0	0
dex 20	2	213	25	01	58	14	1	0	47	78	34	20	103
dex 20	3	77	01	42	104	0	115	114	130	144	47	143	120
dex 20	4	108	229	117	129	201	213	235	167	104	105	205	210
dex 20	5	122	128	63	37	58	24	31	0	48	88	108	118
dex 20	6	166	23	3	0	0	0	0	0	98	133	140	187
dex 20	7	178	47	25	0	0	33	15	137	148	180	142	210
dex 20	8	124	30	02	8	11	12	140	24	140	237	175	234
dex 20	9	144	117	52	125	71	0	00	271	122	256	280	298
dex 20	10	158	32	22	2	0	0	30	0	12	8	0	0
dex 30	1	115	0	2	2	0	0	0	0	0	0	1	0
dex 30	2	80	0	4	0	0	0	0	0	8	0	7	8
dex 30	3	110	0	0	0	0	0	12	0	0	15	0	34
dex 30	4	70	0	0	0	0	0	0	0	0	30	21	13
dex 30	5	210	0	15	4	0	0	8	0	47	80	52	91
dex 30	6	107	0	2	0	0	0	0	0	0	84	03	22
dex 30	7	70	0	0	3	0	1	0	0	0	0	71	52
dex 30	8	42	3	0	0	1	0	0	0	2	28	07	00
dex 30	0	58	7	11	0	0	0	0	0	0	0	12	8
dex 30	10	150	12	7	0	0	0	0	8	0	0	31	42

Appendix 14: Time (sec) spent licking the injected hind paw, in blocks of 5 min after i.p. injection of vehicle or dexamethasone (dex) (10, 20 and 30 mg/kg) in the naked mole-rat during a 1 h observation period.

Treatment	Animal number	0 - 5 min	5 - 10 min	10 - 15 min	15 - 20 min	20 - 25 min	25 - 30 min	30 - 35 min	35 - 40 min	40 - 45 min	45 - 50 min	50 - 55 min	55 - 60 min
vehicle	1	33.7	2.05	0	4.82	0	7.04	30.83	75.11	49.59	71.33	158.31	126.22
vehicle	2	42.89	26.6	42.56	0	0	0	0	2.83	57.03	49.24	62.66	80.35
vehicle	3	10.7	0	1.76	0	0	0	0	39.18	89.58	40.44	65.72	156.69
vehicle	4	108.22	0	5.04	2	0	0	0	0	21.7	109.03	96.00	58.07
vehicle	5	69.73	7.45	0	0	11.99	2.04	8.83	0	26.14	160.82	171.25	178.88
vehicle	6	52.58	84.08	4.9	0	0	0	0	93.26	66.94	5.4	56.62	116.83
vehicle	7	53.32	1.98	0	0	0	0	95	90.3	87.08	82.31	122.22	102.04
vehicle	8	53.71	16.69	1.93	0	0	.54	0	0	87.36	53.02	100.06	105.36
vehicle	9	86.82	10.1	0	0	2	0	0	37.29	29.52	85.73	105.99	120.29
vehicle	10	43.54	1.98	0	0	0	0	0	90.89	36.81	54.72	108.36	84.27
dex 10	1	68.09	134.12	51.35	0	16.87	0	110.63	109.33	129.32	181.25	126.33	156.78
dex 10	2	85.92	0	39.23	0	94.8	66.1	82.04	109.4	152.28	189.35	130.13	171.5
dex 10	3	51.09	0	100.26	2.25	0	0	0	59.9	53.78	98.83	50.87	147.86
dex 10	4	120.65	0	2.96	0	0	.64	66.6	45.51	24.93	59.64	21.8	173.53
dex 10	5	25.84	96.43	22.32	5.1	142.82	58.69	37.63	84.61	38.62	187.98	103.36	33.18
dex 10	6	79.99	0	0	51.18	26.05	2.03	1.23	0	85.55	54.83	150.69	132.59
dex 10	7	49.46	0	0	13.02	54.49	52.19	14.57	66.77	71.32	90.69	159.01	150.98
dex 10	8	25.77	0	0	0	17.31	1.01	65.31	41.04	24.03	0	11.86	108.31
dex 10	9	92.28	39.69	9.26	30.78	0	9.04	31	38.31	69.03	107.19	77.83	38.99
dex 10	10	42.37	12.87	30.73	2.86	7.13	20.81	13.34	71.13	76.74	82.84	108.26	145.53
dex 20	1	36.08	0	.94	0	0	0	0	0	0	0	0	0
dex 20	2	105.77	11.23	48.87	29.32	7.52	.45	0	26.08	37.05	17.7	7.16	102.98
dex 20	3	36.76	32.53	23.03	56.69	0	64.49	63.68	88	80.25	24.87	81.45	64.97
dex 20	4	36.58	108.53	59.26	72.16	80.13	121.75	132.1	100.16	82.58	66.08	122.93	151.58
dex 20	5	64.63	68.53	31	17.39	31.09	11.09	14.36	0	23.33	31.63	69.51	62.18
dex 20	6	82.43	10.29	1.25	0	0	0	0	0	42.64	59.1	66.66	73.11
dex 20	7	96.38	21.45	12.59	0	0	18.42	7.03	86.65	89.55	115.14	82.61	124.65
dex 20	8	70.09	22.69	36.63	2.06	6.61	4.66	66.47	13.22	80.33	143.78	106.97	132.33
dex 20	9	77.56	65.34	22.66	71.19	42.06	3.66	42.64	164.29	66.03	144.46	172.61	179.18
dex 20	10	36.99	16.47	12.61	.96	0	0	16.38	0	8.56	1.65	0	0
dex 30	1	54.53	0	.66	.66	0	0	0	0	0	0	.34	0
dex 30	2	89	0	1.53	0	0	0	0	0	4.47	0	3.4	2.19
dex 30	3	53.2	0	0	0	0	0	5.36	0	0	7.46	0	14.87
dex 30	4	36.29	0	0	0	0	0	0	0	0	18.04	11.36	5.33
dex 30	5	47.46	0	.98	0	0	4.5	0	0	0	51.76	41.96	10.36
dex 30	6	41.34	0	0	1.32	0	.1	0	0	0	0	25.02	18.91
dex 30	7	14.36	.98	0	0	.42	0	0	0	.98	7.98	48.63	48.25
dex 30	8	25.23	2.53	4.91	0	0	0	0	0	0	0	5.25	4.26
dex 30	9	93.39	4.99	4.15	4.92	0	0	0	4.31	0	0	14.82	15.01
dex 30	10	104.66	0	5.35	1.18	0	0	3.03	0	20.34	31.08	34.19	42.69

Appendix 15: Number of licks recorded in blocks of 5 min after i.p. injection of vehicle or dexamethasone (dex) (20 and 30 mg/kg) in the naked mole-rat during a 2 h observation period.

Treatment	Animal number	0 - 5 min	5 - 10 min	10 - 15 min	15 - 20 min	20 - 25 min	25 - 30 min	30 - 35 min	35 - 40 min	40 - 45 min	45 - 50 min	50 - 55 min	55 - 60 min	60 - 65 min	65 - 70 min	70 - 75 min	75 - 80 min	80 - 85 min	85 - 90 min	90 - 95 min	95 - 100 min	100 - 105 min	105 - 110 min	110 - 115 min	115 - 120 min
vehicle	1	50	0	27	0	38	17	145	92	146	135	144	112	119	141	211	195	178	113	107	142	95	88	91	45
vehicle	2	95	0	13	4	5	53	122	199	210	213	220	170	182	156	173	103	130	124	117	85	38	85	0	0
vehicle	3	150	68	76	92	40	199	130	200	172	211	228	231	184	166	255	147	144	187	228	132	223	226	251	201
vehicle	4	123	0	39	0	0	113	158	250	294	224	262	166	40	259	309	265	298	156	139	52	0	0	76	41
vehicle	5	159	0	0	0	140	0	59	280	40	297	60	164	242	256	201	211	228	201	198	238	150	85	190	68
dex 20	1	199	72	17	3	8	0	24	72	46	229	161	147	171	250	286	104	143	112	129	77	54	17	181	106
dex 20	2	85	0	0	10	61	95	97	23	173	205	130	246	281	217	260	247	248	247	194	256	99	177	240	132
dex 20	3	171	0	0	5	0	0	9	82	76	105	139	164	230	193	178	203	194	143	119	145	167	96	93	38
dex 20	4	69	0	18	0	156	3	207	193	231	236	277	291	205	274	295	272	255	241	291	222	298	282	192	290
dex 20	5	93	0	0	8	6	20	34	98	70	204	226	180	98	176	286	250	206	256	280	252	291	278	283	243
dex 30	1	43	8	14	0	0	7	16	29	55	132	187	122	254	105	177	164	169	180	176	191	169	77	105	38
dex 30	2	108	5	41	97	0	0	151	122	81	194	281	174	273	297	312	295	253	230	270	222	295	157	216	119
dex 30	3	90	0	48	0	0	0	61	98	40	54	181	65	108	196	116	139	107	149	125	215	104	105	18	127
dex 30	4	71	0	0	0	0	0	0	0	0	12	64	72	75	69	106	121	151	101	206	103	98	114	119	126
dex 30	5	218	0	14	5	0	0	9	0	0	127	173	97	79	76	121	116	121	109	103	95	61	72	153	162

Appendix 16: Time (sec) spent licking the injected hind paw, in blocks of 5 min after i.p. injection of vehicle or dexamethasone (dex) (20 and 30 mg/kg) in the naked mole-rat during a 2 h observation period.

Treatment	Animal number	0 - 5 min	5 - 10 min	10 -15 min	15 - 20 min	20 - 25 min	25 - 30 min	30 - 35 min	35 - 40 min	40 - 45 min	45 - 50 min	50 - 55 min	55 - 60 min	60 - 65 min	65 - 70 min	70 - 75 min	75 - 80 min	80 - 85 min	85 - 90 min	90 - 95 min	95 - 100 min	100 - 105 min	105 - 110 min	110 - 115 min	115 - 120 min
vehicle	1	25.2	0	16.2	0	22.91	9.46	117.44	69.46	121.25	109.5	117.86	86.52	98.06	110.83	171.36	158.75	144.87	82.23	79.12	116.13	69.36	64.96	65.93	27.33
vehicle	2	48.05	0	6.93	2.4	2.61	40.17	87.57	140.96	159.6	155.83	164.54	127.21	142.02	108.1	123.89	69.13	94.43	89.47	78.46	58.61	26.34	61.78	0	0
vehicle	3	82.69	35.29	52.22	67.09	30.21	158.82	103.82	169.29	140.13	159.63	176.49	185.59	157.39	124.15	175.67	101.26	103.48	141.99	170.53	96.76	160.64	165.54	172.12	150.91
vehicle	4	67.2	0	14.39	0	0	76.36	104.23	165.13	195.17	170.12	180.2	120.54	25.13	170.19	252.73	180.38	199.65	112.02	102.43	39.43	0	0	45.2	28.02
vehicle	5	95.64	0	0	0	105.06	0	38.48	213.73	31.88	243.17	48.1	144.63	193.99	207.58	163.09	172.93	177.17	164.91	159.89	193.26	116.07	41.5	154.83	8.06
dex 20	1	103.16	42.46	7.2	1.86	4.13	0	11.58	46.49	33.69	168.89	115.43	94.48	124.41	185.08	211.97	70.37	100.56	83.27	95.91	42.82	37.24	9.23	128.28	80.43
dex 20	2	53.36	0	0	4.22	38.46	58.74	66.23	12.64	114.71	141.03	98.07	177.56	201.45	156.96	180.7	179.43	184.81	182.3	150.87	190.36	68.89	126.59	171.91	103.56
dex 20	3	102.4	0	0	2.93	0	0	4.06	54.69	46.04	66.97	100.17	105.46	161.86	146.9	123.99	125.53	128.24	101.93	85.27	105.93	116.19	70.32	60.46	20.93
dex 20	4	35.25	0	10.23	0	117.36	1.58	140.93	140.52	168.57	182.6	204.66	235.78	168.37	223.16	234.31	233.3	196.35	172.49	239.22	173.51	240.66	212.28	143.73	222.19
dex 20	5	54.79	0	0	3.34	1.64	9.37	23.15	68.98	46.61	133.84	152.23	124.3	54.44	116.36	206.94	187.73	173.63	208.15	200.41	201.22	226.9	230.46	211.01	193.51
dex 30	1	21.53	3.4	5.86	0	0	4.16	7.79	19.59	43.54	92.56	161.51	96.46	200.5	76.55	131.54	129.41	149.32	140.32	141.54	145.33	127.5	54.34	73.09	27.4
dex 30	2	48.52	2.09	22.72	71.06	0	0	103.36	86.72	55.03	137.99	219.46	138.68	223.71	211.09	248.59	133.99	186.83	173.46	199.46	165.26	228.14	118.69	169.28	89
dex 30	3	53.77	0	34.32	0	0	0	42.3	65.26	27.06	28.01	119.9	45.16	76.36	155.64	87.6	103.73	73.73	112.1	90.73	164.37	73.73	77.33	10.83	84.53
dex 30	4	41.32	0	0	0	0	0	0	0	0	5.46	38.25	41.05	44.09	39.62	89.05	91.23	122.89	82.06	134.09	84.62	71.52	80.15	82.61	84.54
dex 30	5	121.82	0	6.34	1.29	0	0	4.01	0	0	91.71	132.35	70.53	50.15	51.29	92.71	80.55	94.7	89.13	84.59	70.43	40.68	41.34	116.38	129.44