EFFECTS OF SELECTED DIRECT ACTING CHOLINERGIC DRUGS ON PAIN BEHAVIOUR IN THE NAKED MOLE-RAT (*Heterocephalus glaber*)

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR MASTERS OF SCIENCE DEGREE OF UNIVERSITY OF NAIROBI (COMPARATIVE MAMMALIAN PHYSIOLOGY).

BY

THOMAS DAIDO DULU, BVM, (UNIVERSITY OF NAIROBI),

DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

2010

DECLARATION

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

Signature: _____ Date: 06-08-2010

THOMAS DAIDO DULU, (B.V.M)

This thesis has been submitted for examination with our approval as University supervisors.

Signature: _____ Date: 13/8/2010.

TITUS I. KANUI, Ph.D.

Professor, Department of Veterinary Anatomy and Physiology,

University of Nairobi

Signature: _____ Date: 20th August 2010

PHILEMON K. TOWETT, Ph. D.

Lecturer, Department of Veterinary Anatomy and Physiology,

University of Nairobi.

Signature: _____ Date: Aug 2nd 2010

KLAS S.P. ABELSON, Ph. D.

Assistant Professor, Department of Neuroscience, Division of Comparative Medicine,

University of Uppsala, Sweden.

DEDICATION

To God, for making the study possible,

my wife (Diana Nanetia Daido) and children (Terian, Namwenye, Abawama, Mpoke) for their patience, love and encouragement during my studies.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to the following persons:

Prof. Titus Ikusya Kanui, Ph.D., my teacher and supervisor, for welcoming me to his laboratory and introducing the fascinating field of neurophysiology to me. His profound knowledge and experience, as well as his endless encouragement, optimism and support, enabled me to successfully complete this project. I will always appreciate his hospitality and kindness during the preparation of this thesis.

Prof. Klas S. P. Abelson, Ph.D., for his immeasurable support and kindness, having sent all the drugs used during the study, from his laboratory based in Uppsala, Sweden. I am particularly indebted to his guidance during the preliminary experiments that generated data to trigger off the rest of animal treatments and evaluations. His guidance has been invaluable particularly in reviewing the thesis and forwarding his encouraging comments that enabled its improvement.

Dr. Philemon Kipkemoi Towett, Ph.D., for his confidence in me during the finalization of this project and thesis. His inputs gave me focused direction in development of experimental design, data capture and analysis. I wish to express my sincere thanks for his contribution to the nociceptive behavioural studies and for valuable discussions during preparation of this thesis.

All the academic staff in the Department of Veterinary Anatomy and Physiology for creating a pleasant atmosphere to work in and for all their support including scientific and non-scientific discussions during my two year study. Special tribute goes to Prof. Geoffrey Moriaso ole Maloiy, Prof. Seth M. Kisia, Prof. Okello Oduor, Dr. Stephen G. Kiama, Dr. Mathiu Mbaabu,

Dr. Jemimah A. Oduma, Dr. Rodi O. Ojoo, Dr. Stanley N. Wambugu and the Chief Technologist, Mr. Jackson Mugweru.

All my dear friends, particularly Drs. Allan Njogu, Ben Kavoi, Kisipan Mosiany, Catherine K. Kaluwa, Dominic Ochwang'i and Mr. Christopher Musembi for their interest and friendship, not to mention their endless confidence that I would pull through. At the animal house my appreciation goes to Mr. Kamonde and Mr. Marete who helped me in care and handling of the naked mole-rats at Chiromo campus.

Finally, I am immensely indebted to my employer the Government of the Republic of Kenya, through Dr. Peter Maina Ithondeka, the Director of Veterinary Services, Mr. Kenneth Lusaka, Permanent Secretary, Ministry of Livestock Development and Mr. Titus Ndambuki, Permanent Secretary, Public Services and/or Director, Directorate of Personnel Management for granting me approval to pursue the studies from October 2007 to finalization.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

ABSTRACT

Various endogenous substances are involved in the control of nociception both at the segmental and at the higher levels of central nervous system. These substances include acetylcholine, which modify pain processing in a wide variety of experimentally induced or clinically related pain states by interacting with specific receptors. Acetylcholine is the endogenous ligand for the cholinergic receptor system with muscarinic and nicotinic receptors, and systemic or intrathecal stimulation of these receptors results in modulation of pain responses in animals and humans.

In this study, the role of cholinergic system in nociception in the naked mole-rat (*Heterocephalus glaber*) was evaluated. The study explored the antinociceptive effects of the muscarinic receptor agonist, oxotremorine (10, 20, 50 and 100 μg/kg body weight) and the nicotinic receptor agonist, epibatidine (0.5, 1, 2 and 3 μg/kg body weight) using three commonly used nociceptive tests. These were the formalin (20μl, 10%), the hot plate (60 °C) and the tail flick (56 °C) tests. To elucidate possible interaction with opioidergic system, a general opioid receptor antagonist, naloxone, was simultaneously administered with the cholinergic agonists. Muscarinic (atropine) and nicotinic (mecamylamine) blockers were used for antagonistic reactions.

In the formalin test, the duration the animal took licking/biting the injected paw was scored in blocks of 5 minutes for a duration of 60 minutes, whereas in the hot plate test the latency (s) the animal took to react to the thermal pain was recorded. In the tail flick test, tail-flick withdrawal latency (s) was scored. The selected high doses (20, 50 or 100 μg/kg) of oxotremorine induced a statistically significant ($P<0.05$) dose-dependent reduction in the mean

time spent licking/biting the injected paw in both the first and second phases of the formalin test. In both early and late phases of formalin test, the effect of oxotremorine on the mean time spent licking/biting the injected paw was reversed by atropine.

The animals treated with epibatidine (1, 2, or 3 μg/kg) showed a statistically significant ($P<0.05$) reduction in the mean time spent in licking/biting the injected paw in both early and late phases of the formalin test. When mecamylamine was administered together with epibatidine (2 μg /kg), it significantly increased the mean time spent in licking/biting the injected paw in both phases of the formalin test. In the hot-plate test, oxotremorine (20, 30 or 50 μg /kg) and epibatidine (1, 2 or 3μg/kg) caused a significantly different dose-dependant increase in the hot-plate response latency. The effects of oxotremorine and epibatidine were blocked by atropine and mecamylamine, respectively. In the tail-flick test, oxotremorine (20, 30 or 50 μg /kg) and epibatidine (1, 2 or 3μg/kg) caused a significantly different dose-dependant increase in the tail-flick response latency. In the same test, the effects of oxotremorine and epibatidine were blocked by atropine and mecamylamine, respectively. In all the three nociceptive tests, naloxone in combination with oxotremorine or epibatidine exhibited synergism of their effects. Administration of atropine, mecamylamine or naloxone alone did not show any significant effect on the nociceptive behaviour in any of the three tests.

In conclusion, the study showed that oxotremorine and epibatidine are effective antinociceptive drugs in the naked mole-rat and that naloxone is able to potentiate their antinociceptive effects. The data further reveals that the cholinergic system is crucial in pain regulation in the naked mole-rat.

CHAPTER 1

1:0 INTRODUCTION

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage (IASP, 1979; Merskey *et al.*, 1986). Animals are sometimes subjected to stimuli that disrupt the physical well-being of their body. The ability to detect such stimuli and take action to minimize their effects revolves around nociception which is a basic feature of animals (Kavaliers, 1988). Animals with such capability possess nociceptors and effectors, which respond to the sensory input to induce either reflex and/or non-reflex behavioural responses. The capacity to detect noxious events is vital for the survival of all animals. Animals that have various forms of neuropathological conditions like congenital insensitivity to intense stimuli (Dennis and Melzack, 1983) have greatly reduced life expectancies. One common feature is that all animal species posses distinctive behavioural responses to noxious stimulation (Dennis and Melzack, 1983).

Different species of animals respond differently to noxious stimuli. Such differences also exist in members of the same species. There is evidence indicating that sex (Bodman *et al.*, 1988), age (Kavaliers and Hirst, 1983), developmental changes (Hamm and Kinsley, 1988), and geographical location (Innes and Kavaliers, 1987) influence nociception in animals.

Pain is one of the foremost causes of suffering in humans as well as in animals. Several antinociceptive agents for pain suppression are available, but most of them are not always adequate and are often associated with several adverse effects. The nociceptive system has been investigated in a number of animals including mice and rats (Hunskaar, 1987a; Abram and

O'Connor, 1995; Abelson, 2005), in Fish (Olson *et al.*, 1978; Kavaliers, 1984), in amphibia (Pezalla, 1983; Pezalla and Stevens, 1984), in lizards (Mauk *et al.*, 1981), in aves (Gentle and Hill, 1987; Hughes and Sufka, 1991), and in wild rodents (Kanui and Hole, 1990; Towett and Kanui, 1993,1995; Park *et al.*, 2008). The use of different types of animal species is useful for shedding light on evaluation of nociception, and the pharmacological and physiological mechanisms that regulate pain behaviour.

The naked mole-rat is a good model for studying pain mechanisms in animals. Most of the reported work on pain research in the naked mole rat involved the opioidergic systems (Kanui and Hole, 1990; Kanui *et al.*, 1993; Towett *et al.*, 2006, 2009). However, there is ample evidence indicating the importance of other systems such as the serotonergic, adrenergic and cholinergic in pain regulation in other rodents (Prado and Gonçalves, 1997; Hai-Chun *et al.*, 2001; Abelson and Höglund, 2002a, 2002b, 2004; Abelson *et al.*, 2004, 2006). Acetylcholine appears to be an essential neurotransmitter in spinal pain modulation (Fürst, 1999; Abelson *et al.*, 2006; Cervero, 2009). Thus it is important to investigate the role of cholinergic system in pain modulation in different species of animals. This study was performed to provide such information. Such a study has never been reported in the naked mole-rat, a fossorial rodent having unique anatomy and physiology.

1:1 OBJECTIVES

1:1.1 THE GENERAL OBJECTIVE

The main objective of this study was to evaluate, using common behavioural nociceptive tests, the antinociceptive effects of cholinergic agonists and antagonists, and to determine any existence of interactions with opioidergic system in the naked mole-rat.

1:1.2 SPECIFIC OBJECTIVES

The specific objectives of this study were:-

- To investigate the effects of oxotremorine (a muscarinic receptor agonist) on pain induced behaviour in the formalin, the hot-plate and the tail-flick tests.
- To investigate the effects of co-administration of oxotremorine and atropine or naloxone on pain induced behaviour using the formalin, the hot-plate and the tail-flick tests.
- To investigate the effects of epibatidine (a nicotinic receptor agonist) on pain induced behaviour in the formalin, the hot-plate and the tail-flick tests.
- To investigate the effects of co-administration of epibatidine and mecamylamine or naloxone on pain induced behaviour in the formalin, the hot-plate and the tail-flick tests.

1:2 HYPOTHESIS

1.2.1 Null Hypothesis

The cholinergic system does not play any role in pain modulation and antinociception, and neither have any interaction with the opioidergic system in the naked mole-rat.

CHAPTER 2

2:0 LITERATURE REVIEW

2:1 THE NAKED MOLE-RAT (*Heterocephalus glaber)*

The naked mole-rat (*Heterocephalus glaber*), a subterranean rodent belongs to the family *Bathyergidae*. The animal inhabits the hot, dry regions of Kenya, Somalia and Ethiopia. The rodent has a eusocial structure that resembles that of termites, and lives in colonies of 75-250 individuals with only one breeding female (Jarvis, 1981; Sherman *et al*., 1992; Faulkes *et al*.; 2004). They live in an environment characterised by high humidity (>70%), high temperature (30-32°C), high carbon dioxide concentration (0.5-2%) and low oxygen concentration (15-20%) (Sherman *et al*., 1992). The naked mole-rat shows peculiar and interesting physiology. The metabolic rate is low (McNab, 1966, 1968; Jarvis, 1978; Park *et al*., 2008) and its thermoregulatory capacity is very poor, and it has been classified as a poikilotherm mammal (Jarvis, 1978). Furthermore, the mole-rat lacks subcutaneous fat and sweat glands, and conserves water by excreting dry fecal pellets, and by producing concentrated urine (Sherman *et al*., 1992; Towett and Kanui, 1993). The mole-rat has a comparatively long lifespan; in captivity some animals have survived over 26 years (Sherman and Jarvis, 2002). It is an appropriate animal model for human aging research (Buffenstein and Jarvis, 2002; Buffenstein, 2005).

The naked mole-rat has an interesting and unique nociceptive system. It has been reported that the animal completely lacks cutaneous C-fibre immunoreactive to substance P and calcitonin gene-related peptide (Park *et al*., 2003, 2008). When treated with morphine or pethidine, they show aggressive behaviour and hyperalgesia instead of analgesia in the hot-plate test (Kanui and Hole, 1990; Towett and Kanui, 1993). This appears to be related to different properties of μ-, δ-

and κ-receptors in mole-rats, compared to other rodents (Towett *et al.*, 2006). Analgesic effects of non-steroidal and steroidal anti-inflammatory drugs have been investigated in the animal (Kanui *et al.*, 1993; Karim *et al.*, 1993). Other analgesiometric tests, such as formalin-test, have been used to demonstrate analgesic effects of opioids in the naked mole-rat (Kanui *et al.*, 1993; Karim *et al.*, 1993; Towett *et al.*, 2009). In view of the cited data, the naked mole-rat appears to be a very interesting animal model for pain research.

2:2 PAIN PHYSIOLOGY

2:2.1 Definition of Pain

Pain is a complex physiological phenomenon that often has a physical cause, associated to injury to the body outside of the nervous system (Beecher, 1957). It is also extremely difficult to recognize and interpret in animals. Pain has been defined by the International Association for the study of Pain (IASP) as "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (IASP, 1979). Pain perception is a subjective experience in that it differs not only in different subjects, but also in the same subject at different times and in different situations. In the case of injury to the body outside the nervous system, pain is initiated by mechanical, thermal or chemical changes in non-nervous tissues; this causes activation of specific nerves which relay to spinal centres concerned with the detection of injury, and thence to the thalamus and cortex. The animal's reaction to pain that arises from the threat of tissue damage may prevent or greatly reduce further damage (Dennis and Melzack, 1983). This depends on the time interval between the initial contact of a potentially damaging stimulus and the onset of tissue damage. Such a threat-related, damage-minimizing system causes pain sensation.

2:2.2 Pain Terminologies

The pain terminologies defined by a group of researchers led by Merskey (1986) for use in pain research are as follows:

A noxious stimulus is one which is damaging to normal tissue. A **nociceptor** is a specialized receptor or nerve ending that detects pain in the body. In animals, **pain threshold** is the reflex signs of reaction to noxious stimuli. These include actions that are visualized as lameness or biting and scratching at an irritation site, or obscure signs, such as loss of appetite, lassititude, and dysuria. The primary pain-related responses are autonomic, somatic motor, and motivational affective (Melzack and Casey, 1968).

Analgesia is the absence of pain in response to stimulation which would normally be painful, while **hypoalgesia** is diminished pain response to normally painful stimulus. **Hyperaesthesia** is an increased sensitivity to stimulation, excluding the special senses. In some conditions, excitation of pain fibers becomes greater as pain stimulus continues, leading to a condition called **hyperalgesia**.

Hyperalgesia is therefore defined as an increased response to a stimulus which is normally painful. The state of increased response to stimuli can be induced by heat, exposure to ultraviolet radiation, or injection of hyperalgesic agents such as prostaglandins, histamines, bradykinins, and capsaicin, into the skin (Nakamura-Craig and Smith, 1989). Hyperalgesia may also result from opioid tolerance or in some cases following acute opioid administration (Towett and Kanui, 1993, 1995; Towett *et al.*, 2006, 2009).

Cutaneous injury causes hyperalgesia to heat and mechanical stimuli. The hyperalgesia that occurs at the site of injury is referred to as primary hyperalgesia while hyperalgesia felt in

the area surrounding the injury is known as secondary hyperalgesia (Hardy *et al.*, 1950; LaMotte *et al.*, 1982, 1991; Raja *et al.*, 1984). It has been demonstrated that primary hyperalgesia to heat stimuli is mediated by sensitization of peripheral Aδ - and C - fibres (Meyer and Campbell, 1981; LaMotte *et al.*, 1982; Torebjörk *et al.*, 1992). Secondary hyperalgesia is due to the sensitization of neurons in the central nervous system caused by discharges of nociceptive afferents (Aδ - and C – fibres) (Simone *et al.*, 1991; LaMotte *et al.*, 1991; 1992). When nociceptors are stimulated they release excitatory amino acids and other peptides like substance P, neurokinin-A, vasoactive intestinal peptide and calcitonin gene-related peptide (CGRP) in the central nervous system (Gamse *et al.*, 1979; Sorkin and McAdoo, 1993). The agents have a sensitizing effect on nociceptors and can cause hyperalgesia.

The N-methyl-D-aspartate (NMDA) receptors are essential for development of central sensitization (Haley *et al.*, 1990; Coderre *et al.*, 1993; Woolf and Chong, 1983; Dolan *et al.*, 2000; Warncle *et al.*, 2000). NMDA receptor activation results in the production of a number of intracellular second messengers, including nitric oxide and prostaglandins, which are also closely associated with the development of hyperalgesia (Malmberg and Yaksh, 1992; Meller *et al.*, 1994; Dolan and Nolan, 1999).

Allodynia is pain from normally non-painful stimuli. It is due to activity in non-nociceptive, myelinated, fast conducting, Aβ tactile afferents, which evoke pain in the event of inflammation or after nerve injury (Price *et al.*, 1989; 1992; Torebjörk *et al.*, 1992). Nociceptor activity originating in the area of the injury triggers and maintains a state of central sensitization that amplifies the sensory effects of Aβ tactile input, rendering it painful (Price *et al.*, 1989; 1992; Kajander *et al.*, 1992; Torebjörk *et al.*, 1995).

Pain threshold can be influenced by a number of factors, including diurnal variation, race, sex, age, circulatory change, skin temperature, trauma, anxiety and fear (Beecher, 1957; Rosland, 1991, Hole and Tjølsen, 1993). Analgesic agents are capable of altering pain threshold in animals and humans. Hyperalgesia, sweating, fatigue and high partial pressure of carbon dioxide do also influence pain threshold in both humans and animals (Beecher, 1957). Finally, **Pain tolerance** level is the greatest level of pain which a subject is prepared to tolerate.

2:3 PERIPHERAL MECHANISMS OF NOCICEPTION

Peripheral mechanisms of nociception are among the multiple mechanisms that can produce pain, identified as peripheral sensitization or nociceptor activation and structural organization.

2:3.1 Pain Transmission

Nociception is the sole mechanism underlying physiological activities that lead to a painful sensation. Nociception comprises the processes of detection, transduction, conduction and perception. Transduction is the conversion of a noxious thermal, mechanical or chemical stimulus into electrical activity that comprises action potentials in the peripheral terminals of Aδ - and C - fibres. Transduction is mediated by nociceptors and nociceptive afferents (Beital and Dubner, 1976; LaMotte *et al.*, 1982).

The transducer ion channels are non-selective cation or sodium channels that are gated by temperature, chemical ligands and mechanical shearing forces (Ephrem and Dennis, 1994). Once they are activated, the channels open and sodium or calcium ions flow into the nociceptor peripheral terminal producing an inward current that depolarizes the membrane. If the depolarizing current is sufficient to activate voltage-gated sodium channels, they will open,

further depolarizing the membrane and initiating a burst of action potentials (Ephrem and Dennis, 1994).

The conduction of the action potentials is the passage from the peripheral terminals along axons to the central terminal of nociceptors in the central nervous system. Transmission of action potentials occurs through synaptic transfer and modulation of input from one neuron to the other. The nociceptive pathways can be described as a three-neuron chain that transmits nociceptive information from the periphery to the cerebral cortex. The first-order neurons, which constitute the nociceptive afferents, have their cell bodies in the dorsal root ganglion from where two axons project, one to the peripheral tissues and the other to the dorsal horn of the spinal cord. In the spinal cord, the signal is switched over to the second order neurons and ascends to the thalamus or other regions of the brainstem. From the thalamus, the third-order neurons project to the cerebrocortex, (Cross, 1994; Millan, 1999).

2:3.2 Nociceptors

Peripheral neural mechanisms of pain sensitivity in various species of animals have been extensively investigated (Iggo, 1959, 1960; Burgess and Perl, 1967; Burgess et al., 1968; Perl, 1968; Bessou and Perl, 1969; Iggo and Ogawa, 1971; Beital and Dubner, 1976; Georgopoulos, 1976). In most peripheral tissues throughout the body, such as the skin, the muscles, joints and viscera, the presence of nociceptors has been described. The nociceptors are free nerve-endings that have their cell bodies outside the spinal column in the dorsal root ganglion. Nociceptors can be directly activated by strong mechanical, thermal or chemical stimuli, or activated after being sensitized during tissue injury, inflammation, ischemia or low pH (Cross, 1994; Willis and Westlund, 1997; Riedel and Neeck, 2001). One of the fundamental influences on nociceptive

sensitivity is the pH of the surrounding tissue. High local proton concentrations occur in many inflammatory states (Steen *et al.*, 1992; Steen and Reeh, 1993). Decreased pH contributes to sensitization of polymodal nociceptors (Handwerker *et al.*, 1991, 1992; Reeh and Steen, 1996).

Altered pH of the local chemical environment of peripheral nociceptors is a particularly important factor in inducing mechanical sensitization and ischemic pain (Steen *et al.*, 1992; Steen and Reeh, 1993; Dray, 1995). A combination of inflammatory and chemical mediators with altered tissue pH appears to be more effective in inducing sensitization than individual chemical mediators alone (Handwerker *et al.*, 1992).

The presence, specificity and threshold of the nociceptor transducers are the most important filters in the activation of nociceptors and define the different classes of primary sensory neurons as unimodal, which react only to one type of stimulus (e.g. noxious heat) or polymodal, which react to several kinds of stimuli. Polymodal primary sensory neurons are more common than the unimodal variety. Some nociceptor neurons are effectively silent; they fail to react under normal circumstances to any non-damaging stimulus because the basal threshold of their transducers is high (Almeida *et al.*, 2004).

The nociceptors are associated with the first-order neurons. There are two types of first-order afferent nerve fibres; Aδ-and C-fibres. The Aδ-fibres are thinly myelinated, 2-6 μm in diameter and conduct nerve signals with a velocity of about 30-100 m/s. The C-fibres are unmyelinated and thereby thinner (0.4-1.2 μm) than the Aδ-fibres. The C-fibres are also slower in conducting nerve signals with a velocity of 12-30 m/s (Besson and Chaouch, 1987; Almeida *et al.*, 2004). Both the Aδ-and C-fibres are abundant in the skin, muscular and articular tissues.

Noxious stimuli when applied to the skin, excites nociceptors resulting in the generation of impulses and the perception of pain that can be divided into two components, referred to as sharp rapidly conducted pain and the slow, dull longer lasting pain (Campbell and LaMotte, 1983). There is evidence that these two types of pain are mediated by two different afferent fibres. The Aδ-fibres mediate the sharp pain, while C-fibres mediate the dull or burning pain (Zotterman, 1939; Iggo and Ogawa, 1971; Torebjörk and Hallin, 1973; Tania *et al.*, 2004). The dual sensation of pain is attributed to the different nerve fibre conduction velocity.

The Aδ- and the C-fibres can transmit both innocuous and noxious stimuli. However, the threshold for the response of these receptors to noxious stimuli is variable, with the activity increasing with the intensity of stimulation, and in all cases, the maximal response is only produced by frankly noxious stimuli (Bessou and Perl, 1969; Beital and Dubner, 1976; Georgopoulos, 1976). The majority of nociceptors so far examined are excited by a number of stimuli i.e. strong mechanical stimuli, noxious heat and chemical irritants (Armstrong *et al.*, 1953; Iggo, 1959; Fjalibrant and Iggo, 1961; Burgess and Perl, 1967; Bessou and Perl, 1969; Iggo and Ogawa, 1971). These receptors are thus referred to as polymodal nociceptors and are connected to unmyelinated afferents (Bessou and Perl, 1969).

The C-fibres transmit pain more slowly than the A-fibres do because the C-fibres are smaller and lack a myelin sheath. (Chakour *et al.*, 1996). The C-fibres polymodal nociceptors that have been demonstrated using electrophysiological studies in several mammalian species, including the cat (Bessou and Perl, 1969; Beck *et al.*, 1974), the monkey (Burgess and Perl, 1973; Beital and Dubner, 1976; Georgopoulos, 1976) and man (Torebjörk and Hallin, 1974). Thermal threshold of C-polymodal nociceptors in the monkey is in the range of 45-55° C (Beital

and Dubner, 1976). When the temperature is raised from the threshold level to 50-53° C, the C-polymodal nociceptors become sensitized on further application of heat, and also other chemical irritant stimuli (Bessou and Perl, 1969; Beital and Dubner, 1976). Sensitization is manifested by a reduction in the threshold temperature, an increase in the frequency of discharge, a reduction in the response latency, and the presence of after-discharge (Beital and Dubner, 1976). Therefore, hyperalgesia and hyperesthesia caused by nociceptive stimulation can be explained on the basis of sensitization (Besson and Chauoch, 1987).

Application of injurious stimuli such as scratching or ultraviolet light, or injection of irritant substances to the skin may also cause sensitization (Lynn, 1977; Reeh *et al.*, 1987; Clarke and Harris, 2004). The studies that have been undertaken so far on mammalian nociceptors, demonstrated that sensitization may be produced using heat, mechanical, adenosine triphosphate (ATP) and acid pH stimulation (Reeh and Steen, 1996). Chemical mediators released into the tissues following an injury promote sensitization of peripheral nociceptors. The key mediators that have been identified include histamine, bradykinins, serotonin, potassium ions, ATP, protons, prostaglandins, nitric oxide, leukotrienes and cytokines (Handwerker, 1976; King *et al.*, 1976; Dray, 1995; Ji *et al.*, 2003).

The effects of these mediators involve binding to specific receptors, activation of ion channels for depolarization, release of a range of neuropeptides to promote neurogenic inflammation, activation of intracellular second messenger systems and alteration of neuronal properties by modifying gene transcription (Dray, 1995; Bevan, 1996). A number of receptors and second messenger systems may be activated following the release of different inflammatory mediators (Mizamura and Kumazawa, 1996).

The chemical mediators have been found to play a major role for both the peripheral and central sensitization mechanisms. As opposed to central sensitization, peripheral sensitization mechanisms produce increases in pain sensitivity restricted to the site of inflammation. The intracellular contents release is further augmented by inflammatory cells recruited to the site of damage (Levine and Reichling, 1999). Central sensitization requires brief but intense nociceptor activity produced by sensitized nociceptors during inflammation and by spontaneous ectopic activity generated in sensory neurons after nerve injury. The C-polymodal nociceptors can also be sensitized by prolonged intense thermal stimuli above 55° C (Bessou and Perl, 1969; Beital and Dubner, 1976; Georgopoulos, 1976), transient contact with a very hot object (>75° C) or when the stimulation is repeated at short intervals. The inactivation is due to gross destruction of tissue, presumably including the nerve terminal.

The A-polymodal nociceptors are associated with Aδ fibres and are activated by high threshold mechanical and thermal stimuli, and their characteristics are almost like those of C-polymodal nociceptors (Georgopoulos, 1976). The receptors are also capable of being sensitized by thermal stimuli. Receptors that are exclusively excited by thermal nociceptive stimuli (heat or cold) have been rarely reported (Iggo, 1959; Georgopoulos, 1976). These receptors do not seem to form a large population, compared to polymodal Aδ and C-nociceptors (Bessou and Chauoch, 1987).

Nociceptors that are excited best by strong mechanical stimulation, unimodal mechanoreceptors, have been described (Iggo, 1960; Burgess and Perl, 1967; Burgess *et al.*, 1968; Perl, 1968; Bessou and Perl, 1969; Georgopoulos, 1976). These receptors driven by Aδ- and C-fibres are not excited by noxious heat, intense cold or algesic chemicals. Repeated

applications of noxious heat stimuli may however sensitize these receptors to heat (Fitzgerald and Lynn, 1977).

2:3.3 Mechanisms of Nociceptor Activation

The mechanisms of activation of nociceptors by natural stimuli are not clearly known. However, a direct effect of the stimuli and an indirect effect involving algogenic agents have been suggested (Besson and Chauoch, 1987). The short response latency observed following noxious stimulation of C-polymodal fibres (Iggo, 1959; Hensel and Boman, 1960) suggests acute peripheral sensitization on the receptor. Severe nociceptive stimuli can also cause tissue injury resulting in the release of biologically active agents (Lim, 1970). These algogens in turn excite the nociceptor. Administration of such algogens to intact or inflammed skin has been shown to produce pain of different qualities (Armstrong *et al.*, 1953). Capsaicin is the pungent ingredient of peppers that activates transient potential vanniloid-1 (TRPV1) receptor and produces an intense but transient pain owing to activation of TRPV1-expressing nociceptors (Ji, *et al.*, 2002). Capsaicin causes both sensitization and desensitization of nociceptive fibres to noxious stimuli (Schmelz *et al.*, 2003). Repeated administration of capsaicin produces desensitization due to depletion of substance P and CGRP.

While capsaicin always evokes pain but never itching, histamine always provokes itching but rarely pain (Schmelz *et al.*, 2003). The pruritic potency of inflammatory mediators is characterized by their ability to activate histamine positive mechano-insensitive C-fibres (Schmelz *et al.*, 2003). However, concomitant activation of mechano-sensitive and mechano-insensitive histamine negative nociceptors will decrease the itch. Therefore the itch sensation is based on both, activity in the "itch-pathway" and absence of activity in the "pain-pathway"

(Schmelz *et al.*, 2003). This may therefore indicate that the mechanism of actions of different algogens is at least in part different. The role of the algogens in the mechanism of nociceptor stimulation has also been investigated using electrophysiological methods (Dash and Deshpande, 1975; Handwerker, 1976; King *et al.*, 1976).

The algogens that have received a lot of emphasis are prostaglandins of the E series which tend to sensitize nociceptors to other algogens (Ferreira, 1972) and also to mechanical stimulation (Ferreira *et al.*, 1973; Moncada *et al.*, 1975; Handwerker, 1976). Corticosteroids are potent anti-inflammatory agents and capable of depressing the sensitizing effect of algogenic endogenous substances (King *et al.*, 1976). The possible role in peripheral neural mechanisms and sites of action of algogenic agents have been suggested to be either at the receptor or terminal portions of the sensory fibres (Dash and Deshpande, 1975). Peripheral nociceptive function may be modified by substances released by the peripheral terminals of primary nociceptive afferent when activated (Lynn, 1977).

Since polymodal receptors respond to a wide range of stimuli, it is apparent that different molecular receptors and second messenger systems are involved in excitation and sensitization for different stimulation modalities (Mizamura and Kumazawa, 1996). It is therefore important to note that nociceptors may become differentially sensitized to thermal, mechanical and/or chemical stimuli. An individual nociceptor can potentially exhibit sensitization to thermal stimuli for example, while retaining normal sensitivity to mechanical or chemical stimuli.

2:4 SPINAL AND SUPRASPINAL CONTROL OF NOCICEPTION

About 70% of nociceptive fibres enter the spinal cord through the dorsal root, but the rest double back and enter the ventral root. The grey matter of the spinal cord has ten layers or

laminae. The important ones that are directly involved in pain modulation are lamina I (marginal zone), lamina II (substantia gelatinosa), lamina V (part of "nucleus proprius of dorsal horn" with IV, VI), laminae VII and VIII (intermediate spinal grey matter with X). Unmyelinated C-fibres synapse in laminae I to V, while Aδ-fibres synapse in laminae I, V and X (Willis and Westlund, 1997; Beydoun and Misha-Miroslav, 2003).

2:4.1 Organization and Physiology of the Dorsal Horn of the Spinal Cord

The spinal cord laminae reflect neuronal groupings as seen in cytoarchitectonic studies (using Nissl's stains) based on shapes, sizes, density and distribution of neuronal cell bodies (Rexed, 1952, 1954). Such lamination has been noted in the rat (Steiner and Turner, 1972), and in the monkey (Scheibel and Scheibel, 1968; Light and Perl, 1979a, 1979b; Ralston and Ralston, 1979). Laminae I-VI altogether makes 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). The large lamina I (LI) neurons also known as marginal cells occupy the dorsal margin of lamina I (Waldeyer, 1888). 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 two groups. The LI cells comprise pyramidal and multipolar cells that can be further subdivided into two subgroups respectively. 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) but occasionally, dip down into

LII. Marginal cells of LI have axons that project for long distances but also have local connections with other LI neurons via short axons or collaterals via Lissauer's tract (Szentagothai, 1964).

Christensen and Perl (1970) reported that the marginal cells in LI respond to peripheral stimulation in either one of the three ways: the first group of cells responded only to mechanical nociceptive stimulation. The second group was responsive to mechanical and thermal nociceptive fibres. The presence of nociceptive neurons in LI has also been confirmed in the rat (Menetrey *et al.*, 1977; Kumazawa and Perl, 1978; Cervero *et al.*, 1979).

Lamina II (Rexed, 1952, 1954) is a well-defined layer running from the medial side of the dorsal horn, across it, around the apex and down the lateral side. The layer also referred to as substantia gelatinosa (SG) is covered dorsally and laterally by Lamina I and consists of small closely packed cells with radial orientation with respect to the surface of the cord (Rexed, 1952, 1954; Szentagothai, 1964). Two distinct cell types were described by Cajal (1909), the central cells and the border cells, also called the islet and stalked cells respectively (Gobel, 1978; Light and Perl, 1979b; Ralston and Ralston, 1979). The dendrites of Lamina II cells remain largely within Lamina II and are extensively branched (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 substantia gelatinosa (SG) is made up of neurons that respond exclusively to noxious stimuli (Light *et al.*, 1979; Wall *et al.*, 1979; Bennet *et al.*, 1980; Fürst,

1999). It is mainly at the SG where the afferent Aδ-and C-fibres terminate and where the switch-over to second order neurons occurs.

It has been observed that pain from the viscera is sometimes referred to the skin. This is due to a viscero-somatic convergence. Viscero-somatic convergence occurs in Lamina V and VIII of the spinal cord (Selzer and Spencer, 1969). Milne *et al.*, (1981) demonstrated that in the monkey viscero-somatic convergence of both visceral or testicular and cutaneous nociceptors occurred on spinothalamic neurons. Thus some of the neurons showing convergence project to the thalamus.

2:4.2 Spinal Cord Pathways

From the dorsal horn, the nociceptive information is transmitted to the brain via the second-order neurons. These neurons have their cell bodies in the dorsal horn and their axon terminations in the brain, and are mainly of two types, wide-dynamic-range (WDR) and nociceptive-specific (NS) neurons. The WDR neurons respond to non-noxious and noxious stimuli, while the NS neurons respond solely to noxious stimuli. The second-order neurons reach the brain via afferent pathways (Cross, 1994; Almeida *et al.*, 2004).

There are five main ascending spinal pathways involved in nociception. These are the spinothalamic tract, the spinoreticular tract, the dorsal column sub-pathway, the spinocervical tract and spinomesencephalic tract. The spinothalamocortical connections are the major ascending central pathways for pain in the antero-lateral quadrant of the spinal cord (Willis, 1985; Palecek et al., 2002). The pathways terminate in the contralateral thalamus via two projections. In the lateral projection, the spinothalamic axons mainly originate in laminae I and V and terminate in the ventral posterior lateral nucleus and the ventral posterior inferior part of the

lateral thalamus. In the medial projection, axons originate from deeper parts of the dorsal horn, and from the ventral horn, and terminate in the central lateral locus. The thalamus is considered the most important relay for reception and processing of nociceptive information at the supraspinal level. The lateral part of the thalamus is thought to be involved in the sensory-discriminative component of pain, while the medial part is involved in motivational-affective aspects of pain (Hodge and Apkarian, 1990; Cross, 1994; Willis and Westlund, 1997; Almeida *et al.*, 2004; Abelson, 2005). Spinothalamic neurons respond well to cutaneous noxious, mechanical and heat stimuli (Kenshalo *et al.*, 1979; Surmeier *et al.*, 1986). Responses of spinothalamic neurons to mechanical stimuli can be enhanced after strong stimulation of C-fibres, as shown following capsaicin intradermal treatment (Dougherty and Willis, 1992).

The spinoreticular tract is a major secondary pathway that is important for nociceptive signaling (Kevetter *et al.*, 1982; Willis, 1985). The neurons of this tract originate from the same areas as for the spinothalamic tract. On reaching, the dorsal and ventral horn the spinoreticular tract axons run as far as the nuclei of reticular formation. These neurons have both nociceptive and non-nociceptive inputs. The tract projects into the nucleus gigantocellularis and nucleus parvocellularis of the medulla (Willis, 1985). The spinoreticular tract is thought to be concerned with generalized cortical activation that includes arousal, autonomic, and somatomotor escape and orientating responses associated with pain (Price, 1999).

The spinocervical tract is predominantly associated with transmission of tactile stimuli but may also act as a minor pathway for noxious information. Its neurons originate in layers III and IV of dorsal horn and ascend in the postero-lateral cord and end in the lateral cervical nucleus where most of its neurons cross over and project to the thalamus.

The dorsal column sub-pathway originates from neurons of layers III and IV of the spinal cord and project to the dorsal column nuclei. The sub-pathway is known to conduct information from low threshold mechanoreceptors (Willis and Coggeshall, 1991) and transmission of visceral nociceptive signals in the rat (Palecek *et al.*, 2002).

The spinomesencephalic tract includes projections to different areas in the midbrain. Most axons originate in laminae I and IV-VI, but some have their origin in lamina X or in the ventral horn. The tract terminates in regions such as periaqueductal gray (PAG), nucleus cuneiformis, intercolliculus nucleus, deep layers of the superior colliculus, and anterior and posterior pretectal nuclei. The projections to the PAG seem to contribute to arousal, autonomic, aversive behaviour and orientating responses associated with pain (Willis and Westlund, 1997; Almeida *et al.*, 2004; Abelson, 2005).

In addition, several other ascending nociceptive pathways have been described. The spino-limbic tracts consist of the spinoreticulothalamic, the spinoamygdalar, and the spinohypothalamic pathways (Burstein *et al.*, 1987, 1990). Neurons of the spinohypothalamic tract originate from similar areas as those of spinothalamic tract and terminate into the hypothalamus, a structure that contributes to responses associated with painful stimulation (Almeida *et al.*, 2004).

The nociceptive information is transmitted from the thalamus to the cerebral cortex via third-order neurons. Depending on their origin, the neurons terminate in different parts of the cortex. Neurons from the lateral thalamic nuclei project to the primary somatosensory cortex, where a conscious localization and characterization of the pain occurs. Neurons from the medial nuclei are projected to the Anterior Cingulate Gyrus, which has been suggested to be involved in

perception of suffering, and emotional reactions to pain. Several other areas of the cerebral cortex have also been described as important for processing of nociceptive information and the experience of pain. The secondary somatosensory cortex, regions of the interior and anterior parietal cortex, the insular cortex and the medial prefrontal cortex have all been identified as regions activated by noxious stimuli from cutaneous and intramuscular tissue (Cross, 1994; Davis *et al.*, 1997; Casey, 1999; Riedel and Neeck, 2001; Timmermann *et al.*, 2001, Abelson, 2005).

2:4.3 Gate Control Theory of Pain

The gate control theory of Melzack and Wall (1965) is the basis of most research work on pain mechanisms. At the dorsal horn of the spinal cord three components of the gate control interact. They are the sensory afferents, the segmental cells, and the descending controls. The sensory afferents include the nociceptive and the low-threshold large-diameter afferents. The segmental cells are cells within the central nervous system whose role is to select and compute combinations that terminate on them (Wall, 1983).

Melzack and Wall (1965) in their gate control system model proposed that the substantia gelatinosa (SG) acts as a gating mechanism to control afferent input before it affects the spinal nociceptive neurons (T cells) located in the dorsal horn. The theory suggests that the inhibitory action of SG on nociceptive transmission is influenced by the activity of primary afferents. Stimulation of large fibres enhances the inhibitory effect of SG neurons, while stimulation of small fibres (Aδ- and C-fibres) reduces it. For the gate to operate properly, it was suggested that an on-going activity in Aδ- and C-fibres, a stimulus-evoked activity, and a relative balance of activity in large verses small fibres need to be present. It was postulated that tonic activity in small fibres would keep the gate partly open, while an input over large fibres would close the

gate, thus limiting the output from the nociceptive neurons. Descending pathways are also capable of altering the gate, by probably setting the excitability level of both the pre-and postsynaptic mechanisms.

One of the main features of this theory is that output from the SG neurons is determined by the activity in low and high thresholds afferents that converge onto them. The dorsal horn neurons, including those receiving inputs from nociceptors receive convergent inputs from low and high thresholds afferents (Wall, 1967; 1973; Pomeranz *et al.*, 1968; Wagman and Price, 1969; Gregor and Zimmerman, 1972). Some studies have demonstrated the gate control system activity when large diameter afferents inhibits nociceptive transmission and cutaneous afferents induce analgesia (Wall and Sweet, 1967; Gregor and Zimmerman, 1972; Cervero *et al.*, 1976). Spinal cord stimulation has also been shown to inhibit nociceptive responses in Lamina V cells (Hillman and Wall, 1969; Foreman *et al.*, 1976). Evidence supporting the role of descending control systems in the gate theory has been well demonstrated (Wall, 1967; Hillman and Wall, 1969; Reynolds, 1969; Schmidt, 1973; Cervero *et al.*, 1976).

2:4.4 Dorsal Horn Neurochemicals

There are endogenous substances involved in the control of nociception both at the segmental and at the higher levels of central nervous system. These substances include several amino acids, opioids, acetylcholine, noradrenaline, serotonin (5-hydroxytryptamine or 5-HT), gamma-amino butyric acid (GABA) and dopamine (Yaksh, 1991). The substances modify pain processing in a wide variety of experimentally induced or clinically related pain states by interacting with specific receptors (Pert, 1987; Nicoll *et al.*, 1990; Yaksh, 1991). Most of these substances are released to the spinal cord from the terminals of descending systems originating

from supraspinal structures. These include opioidergic, GABAergic, noradrenergic, serotonergic and cholinergic systems.

In the superficial dorsal horn, a large variety of receptor classes and neurotransmitters exist. Peripheral noxious stimuli lead to nociceptor activation followed by release of neurotransmitters in the dorsal horn. The most important neurotransmitter classes for nociceptive transmission are neuropeptides and excitatory amino acids such as glutamate and aspartate. The major neurotransmitter released from primary afferents is substance P (SP), an 11-amino acid polypeptide and a neuromediator for thin nociceptive afferent fibres. SP is found in great concentrations at the level of the dorsal roots and dorsal horn (Takahashi and Otsuka, 1975; Otsuka and Konishi, 1976). Thus, the primary afferent neuron transmits neural stimuli centrally and releases neuromediators of inflammation peripherally into surrounding tissue. SP is released from peripheral nerve endings at the site of injury (Lynn, 1977; Lembeck and Holzer, 1979) where it induces neurogenic inflammation. SP also sensitizes nociceptors to further challenges with its sub-threshold doses and also to other analgesic agents such as dopamine or prostacyclin (Nakamura and Smith, 1989). This shows that the effects of SP on nociceptors are exerted via peripheral receptors. SP present in primary afferent fibres is involved in excitatory transmission processes related to the passage of nociceptive information in the spinal cord. SP modulates the excitability of dorsal horn neurons. It usually co-exists with calcitonin-gene-related-peptide (CGRP) in the same sensory neurons (Wieselfield-Hallin *et al.*, 1984).

Other substances that act as neurotransmitters in primary afferent fibres are excitatory amino acids and adenosine 5'-triphosphate. The nociceptive transmission by excitatory amino acids, such as glutamate and aspartate, is mediated by ionotropic and metabotropic glutamate

receptors. The ionotropic receptors can be divided into three sub-categories; N-methyl-D-aspartic acid (NMDA), α-amino-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and kainate receptors (Fürst, 1999. Hao-Jun *et al.*, 2002, 2003; Spivak *et al.*, 2004; Chu and Moenter, 2005). The metabotropic glutamate receptors (mGluRs) consist of at least eight subtypes and are present in the spinal cord (Coggeshall and Carlton, 1997; Fürst, 1999; Millan, 1999; Riedel and Neeck, 2001).

Nitric oxide also plays an important role in nociceptive transmission. Nitric oxide has been shown to contribute to the antinociceptive actions of opioids as well as of adrenergic and cholinergic agonists, at both spinal and supraspinal levels (Iwamoto and Marion, 1994a; Iwamoto and Marion, 1994b; Xu and Tseng, 1994; Xu *et al.*, 1997). Nitric oxide production plays a pivotal role in the mechanisms of central sensitization and has been proposed to initiate presynaptic glutamate release, (Fürst, 1999). It has been proposed that the activation of the NMDA receptor leads to an influx of calcium ion, which activates the enzyme nitric oxide synthetase. Intracellular nitric oxide release stimulates transduction of protein kinase C, increases the effects of glutamate, and may interfere with release of inhibitory neurotransmitters from inhibitory neurons (Carr and Cousins, 1998; Basbaum, 1999). The nitric oxide antagonists prevent central sensitization and enhance the antinociceptive effect of oxotremorine (Machelska *et al.*, 1999). The stimulation of muscarinic and nicotinic receptors by cholinergic agonists is pivotal in the modulation of spinal antinociceptive mechanisms by nitric oxide (Xu *et al.*, 1996, 2000).

2:4.5 Opioidergic system

The opioidergic system is important in supraspinal as well as spinal antinociceptive mechanisms. Opioidergic mechanisms are mediated by three types of opioid receptors i.e. μ-, κ-,

and δ- receptors. The μ-receptor is generally considered the most essential in antinociceptive actions, but κ-, and δ- receptors have also been shown to mediate antinociception. In addition, there is a fourth type of opioid receptor the ε-receptor, which is thought to mediate β-endorphin-induced analgesia, but the existence of such a receptor is still controversial. Activation of opioid receptors can inhibit Ca^{2+} channels specifically on afferent C-fibres and thereby inhibit their spinal activity. Opioid receptors are also present on interneurons and cell bodies of second-order neurons, where the nociceptive information can be blocked (Taddese *et al.*, 1995; Ossipov *et al.*, 2004). The various subtypes of opioid receptors have high affinity for naloxone (Yaksh, 1987a). Opioid receptors in the spinal cord are found throughout the spinal gray matter, with a higher density in the dorsal horn (Fields *et al.*, 1980; Slater and Patel, 1983; Morris and Herz, 1987). Opioid receptors are also found pre- and postsynaptic to small primary afferent terminals (LaMotte *et al.*, 1976; Gamse *et al.*, 1979; Fields *et al.*, 1980; Yaksh, 1987b). In addition, the opioidergic system has been found to interact with NMDA receptors, which might contribute to the antinociceptive actions of opioids, but also to development of tolerance to and dependence of opioid agonists. The opioids may act by modulating the NMDA receptor-mediated electrophysiological events or by interacting at an intracellular level (Mao, 1999).

2:4.6 GABAergic system

The GABAergic system is one of the descending systems implicated in modulation of nociception at the spinal cord level (Hole and Berge, 1981; Berge, 1986; Hunskaar, 1987a). Studies have shown that GABA is an inhibitory neurotransmitter in the central nervous system of vertebrates. (Schmidt, 1973; Roberts, 1984). There are at least two subclasses of GABA

receptors, GABA-A and GABA-B (Algiers and Nicoll, 1982; Bowery *et al.*, 1984). These receptors are widely distributed in the central nervous system (Nicoll *et al.*, 1990).

Intrathecal injections of GABA-B agonists have been shown to produce a measurable effect on thermal nociception in the rat (Wilson and Yaksh, 1978). Activation of GABA-B receptors can also depress substance P release from peripheral sensory neurons. Krogsgaard-Larsen, (1984) reported potent antinociceptive effect by specific GABA-A agonists in animals and man. GABAergic receptors are Cl- channel whose activation leads to Cl- influx, hyparpolarisation leading to analgesia.

Administration of GABA agonist, muscimol, can potentiate morphine analgesia in the hot-plate and tail-flick tests in rodents (Biggio *et al.*, 1977). Intensification of morphine analgesia in the hot-plate test in both naïve and tolerant mice has also been documented after injection of GABA-transaminase inhibitors (Contreras *et al.*, 1979).

2:4.7 Noradrenergic system

The Noradrenergic system is one of the diffusely organized systems in the central nervous system, but mainly associated to nucleus locus coeruleus (Nicoll *et al.*, 1990). Pharmacophysiological studies in the peripheral and the central nervous systems indicate that noradrenaline can act on four distinct receptor subtypes: alpha-1 (α_1), alpha-2 (α_2), beta-1 (β_1) and beta-2 (β_2).

Activation of bulbospinal noradrenergic pathways by brainstem manipulations strongly inhibits the activity of dorsal horn neurons (McCreery *et al.*, 1979) and produces a behavioural analgesia in animals (Segal and Sandberg, 1977), and iontophoretic application of noradrenaline depresses neuronal responses in dorsal horn to noxious stimulation (Engberg and Ryall, 1966;

Belcher *et al.*, 1978). These reports suggest that the descending noradrenergic systems exercise a tonic inhibitory influence on spinal mechanisms mediating nociception and that modulation of pain transmission may differ with the analgesic tests.

2:4.8 Serotonergic system

The involvement of central serotonergic systems in the regulation of nociceptive information is well documented (Belcher *et al.*, 1978; Yaksh. 1979; Yaksh and Wilson, 1979; Kuraishi *et al.*, 1983, 1985; Roberts, 1984; Berge, 1986). Most of the afferents utilizing serotonin (5-hydoxytryptamine) originate from the nuclei of the median raphe. Diffuse afferents from these nuclei innervate virtually all levels of the central nervous system from sacral spinal cord up through telencephalon (Nicoll *et al.*, 1990). This discrete distribution of 5-hydoxytryptamine (5-HT) provides the basis for an influence on numerous central nervous system functions such as endocrine activity, appetite, sleep mechanisms, sexual behaviour, temperature regulation, motor activity and some cognitive functions including memory and learning (Slater and Blundell, 1980; Cox *et al.*, 1981; Steinbusch, 1981; Lin *et al.*, 1983; Nicoll *et al.*, 1990).

The 5-HT receptors are the receptors for serotonin. They are located on the cell membrane of nerve cells and other cell types in animals and mediate the effects of serotonin as the endogenous ligand and of a broad range of pharmaceutical and hallucinogenic drugs. 5-HT receptors mediate the pre- and postsynaptic actions of 5-HT. They are classified into seven groups (5-HT $_{1-7}$), comprising a total of at least 14 structurally and pharmacologically distinct mammalian receptor subtypes (Hoyer *et al.*, 1994). With the exception of the 5-HT_3 receptor, a ligand gated ion channel, all other 5-HT receptors are G protein coupled seven transmembrane

(or heptahelical) receptors that activate an intracellular second messenger cascade (Hannon and Hoyer, 2008). Serotonergic action is terminated primarily via uptake of 5-HT from the synapse.

It has been shown that serotonergic systems may enhance or leave unaltered the behavioural responses, depending on the type of noxious input (Fasmer *et al.*, 1983, 1985; Berge *et al.*, 1985). Further, different serotonergic mechanisms modulate complex and reflex responses to noxious stimulation (Ogren and Berge, 1984).

2:4.9 Cholinergic system

The cholinergic system is extremely diffuse, innervating most regions of the central nervous system (Nicoll *et al.*, 1990). The effects of this system are mediated through muscarinic and nicotinic receptors. Muscarinic actions can be excitatory or inhibitory while nicotinic actions are usually excitatory. There is evidence that cholinergic system is involved in the modulation of nociception (Pert, 1987).

Activation of muscarinic receptors may cause inhibition or enhancement of nociceptive reactions in experimental animals (Pert, 1987). However, activation of nicotinic receptors has been reported to cause an increase in hot-plate latencies in mice and also in tail-flick latencies to radiant heat in rats and mice (Sahley and Bernston, 1979). Muscarinic receptors are G-protein coupled receptors with seven trans-membrane domains. The muscarinic receptors were defined as receptors activated by muscarine and blocked by atropine (Dale, 1914). It was later demonstrated that the effects mediated by muscarinic receptors were associated to four subtypes of receptors (Waelbroeck *et al.*, 1990, Abelson *et al.*, 2006). The well defined subtypes discovered through pharmacological studies were termed M_1, M_2, M_3 and M_4 (Caulifield and Birdsall, 1998). However, genetic and molecular biological studies have characterised five genetic subtypes

termed as m1, m2, m3, m4 and m5. The first four code for pharmacologic types M_1-M_4. The fifth, m5, corresponds to a subtype of receptor which has not been detected pharmacologically. m1 and m2 were determined based upon partial sequencing of M_1 and M_2 receptor proteins, the others were found by searching for homology, using bioinformatic techniques. The receptor subtypes differ in function with regard to their specific G-protein coupling and second messenger activation. Muscarinic receptors have been found in the spinal grey matter, including the superficial laminae, in several species such as humans and rats (Gilberg and Aquilonius, 1985; Gilberg *et al.*, 1989; Höglund and Baghdoyan, 1997, Abelson, 2005).

Nicotinic receptors are ligand-gated ion-channels transmembrane proteins and defined as, receptors that were activated by nicotine and blocked by curare (Dale, 1914). The receptor consists of five subunits arranged symmetrically in the cell membrane to form a central pore. The existing subunits types can be divided into two amine categories: the α subunits (α1-9) that possess adjacent cysteines for acetylcholine binding, and the non- α subunits (β1-4, γ, δ and ε) that lack the cysteines (Sargent, 1993; Corringer *et al.*, 2000; Le Novere and Changeux, 2001). Various nicotinic receptor subtypes are present in the spinal cord, also in the superficial laminae. However, the nicotinic receptors appear to exist in lower quantities than muscarinic receptors (Abelson, 2005).

The acetylcholinergic receptor system has been found to interact with most other receptor systems in the spinal cord. Muscarinic receptors have been shown to be involved in spinal antinociceptive mechanisms mediated by the GABAergic (Baba, *et al.*, 1998; Chen and Pan, 2003), opioidergic (Harris *et al.*, 1969; Pert, 1975; Chen and Pan, 2001), and adrenergic (Detweiler *et al.*, 1993; Klimscha *et al.*, 1997; Pan *et al.*, 1999; Honda *et al.*, 2002, 2003)

receptor systems. Nicotinic receptors are also involved in modulation of nociceptive signals by other receptor systems. In addition, both muscarinic and nicotinic receptors have been suggested to play an important role in the antinociceptive mechanism of nitric oxide in the spinal cord (Xu *et al.*, 1996, 2000).

2:5 BRAINSTEM NUCLEI INVOLVED IN PAIN MODULATION

It has been demonstrated by electrophysiological studies that a number of brainstem sites are involved in the induction of analgesia. The nucleus raphe magnus, the nucleus reticularis paragigantocellularis of the ventral medulla, the periaqueductal gray matter of the mesencenphalon, the nucleus locus coeruleus and basal ganglia are the five main supraspinal areas known to be involved in pain modulation (Reynolds, 1969; Mayer *et al.*, 1971; Liebeskind *et al.*, 1973; Hayes *et al.*, 1979; Hole and Berge, 1981; Berge, 1986; Baker, 1988; Chudler and Dong, 1995).

The Nucleus Raphe Magnus (NRM) contains serotonergic and noradrenergic neurons that project to the spinal dorsal horn. Electrical stimulation of the nucleus produces behaviourally defined analgesia, and also reduces the response of spinal dorsal horn neurons to noxious peripheral stimulation (Guilbaud *et al.*, 1977). The findings from earlier studies suggest that NRM exercises tonic inhibition on spinal nociceptive reflexes via the descending serotonergic neurons (Willis and Coggeshall, 1978; Hole and Berge, 1981; Berge, 1986) and bulbospinal noradrenergic neurons (Sagen and Proudfit, 1981; Hammond and Yaksh, 1984).

The Nucleus Reticularis Paragigantocellularis (NRPG) is one of the brainstem nuclei involved in nociceptive regulation (Takagi, 1980). Electrical stimulation of this centre is reported

to readily elicit behavioural analgesia (Akaike *et al.*, 1978). The NRPG neurons form part of the negative feedback loop mediating analgesia (Azami *et al.*, 1981).

Electrical stimulation of Periaqueductal Gray Matter (PAG) nuclei is documented to produce analgesia (Reynolds, 1969), and also to reduce responses of dorsal horn neurons to noxious peripheral stimuli (Liebeskind *et al.*, 1973). Aversive behavioural responses, or behavioural analgesia with or without the autonomic changes, usually associated with stress and pain, may be elicited following stimulation of PAG (Lovick, 1985; Sheng and Gary, 1995).

The Nucleus Locus Coeruleus (LC) consists predominantly of noradrenaline-containing cell bodies and is suggested to be the major source of noradrenergic nerves to the ventral and dorsal columns of the spinal cord (Nygren and Oslon, 1977). Activation of LC has been shown to produce inhibition of spinal dorsal horn nociceptors (Hodge *et al.*, 1983) and antinociception in behavioural nociceptive tests (Segal and Sandberg, 1977; Jones and Gebhart, 1986). The antinociceptive effect is mediated in part by postsynaptic α_2-adrenoreceptors (Jones and Gebhart, 1986).

The basal ganglia have been shown to be important for processing of nociceptive somatosensory information. The basal ganglia are involved in the sensory-discriminative dimension of pain, the affective dimension of pain, modulation of nociceptive information, and sensory gating of nociceptive information to higher motor areas (Baker, 1988; Chudler and Dong, 1995, Abelson, 2005).

2:6 NOCICEPTIVE BEHAVIOURAL TESTS

The behavioural measurement of antinociception is an indirect method of gaining access into the sensorium of the subject. Several nociceptive tests have been developed to facilitate the

assessment of nociceptive behaviours in animals. The tests differ in the intensity, duration and avoidance of the noxious stimulus. The different kinds of pain induced by different pain tests may be mediated and modulated in varying degrees in the central nervous system (Dennis and Melzack, 1980). An ideal nociceptive test is one where the noxious input exclusively activates pain fibres, i.e. small diameter Aδ- and/or C-fibres and that the behavioural output assessed by the investigator should likewise occur only as a result of a specific noxious input.

The hot plate and tail flick tests are two of the most common thermal analgesiometric tests that are widely used in mammals (D'Amour and Smith, 1941; Woolfe and MacDonald, 1944). Another nociceptive test that has become very popular for investigating chronic pain mechanisms is the formalin test (Dubuisson and Dennis, 1977). The three tests elicit motor responses that are used to assess pain threshold.

2:6.1 The Hot-plate test

The hot-plate test measures the response to a brief noxious heat stimulus. This test was initially used for measuring the ability of drugs to inhibit the reflex responses of mice placed in contact with a hot surface maintained at constant temperatures between 55 and 70^0 C (Woolfe and MacDonald, 1944). Eddy, (1950, 1953) and his associates described what is known customarily as a constant temperature hot-plate test, where the temperature was maintained at 55 ± 0.5^0 C. The constant temperature hot-plate test has since been used extensively and with some success. It has been claimed, however, by some authors that the method may demonstrate none or only weak analgesia of non-narcotics and narcotic antagonists (Ankier, 1974; Taber, 1974; Hunskaar *et al.*, 1986b).

Ankier (1974) was able to demonstrate the antinociceptive activity of narcotics, and narcotic antagonists, when he performed a hot-plate test at 50, 55, and 59^0 C in mice. Later, Hunskaar *et al.*, (1986a) described an increasing temperature hot-plate test in mice and rats. The antinociceptive effects of weak narcotic and non-narcotic analgesics were demonstrated when the hot-plate temperature was increased in steps of 2^0 C from 42^0 C to 52^0 C. It was therefore concluded that the increasing temperature hot-plate test was a useful test in both mice and rats for evaluating and screening both narcotic and non-narcotic analgesics.

Some of the pain-related behavioural responses observed when an animal is put on a hot plate include jumping, kicking and dancing, thumping of the foot, lifting the foot off the plate, and licking the forepaws, the hindpaws or both (Hunskaar *et al.*, 1986a). Licking of the forepaws, hindpaws or both have been the main criteria for determining the endpoint (Woolfe and MacDonald, 1944; Eddy *et al.*, 1950; Eddy and Leimbuch, 1953; Ankier, 1974; O'Callaghan and Holtzman, 1975; Yaksh and Rudy, 1977; Berge *et al.*, 1983; Hunskaar *et al.*, 1986a). However, most researchers have used the latency to the licking or stepping of the hindpaw rather than the licking of the forepaws because the latter has a relatively shorter latency and may not be elicited by noxious stimuli (Hunskaar *et al.*, 1986a). The hot-plate test has been used to evaluate pain behaviour in the naked mole-rat, using an IITC Inc., Woodland Hills, CA, model 35D analgesiometer (Kanui and Hole, 1990; Towett and Kanui, 1993). The hot plate temperature was set at 60^0 C because the animal appeared to have a higher pain-threshold than other rodents (Kanui and Hole, 1990). The response to the hot-plate test is complex due to involvement of supraspinal functions.

2:6.2 The Tail-flick test

The tail-flick test in rodents developed by D'Amour and Smith, (1941) has been a standard method for investigating nociception and analgesia. The test has been applied in mice and rats (Woolfe and Macdonald, 1944; Gamble and Milne, 1989; Mogil *et al.*, 1996, 1997). The success of the tail-flick assay is dependent on gentle restraint of the animal. Animal is placed in a restraining tube with its tail protruding out. Thermal stimulus is applied to the tail and the latency to a flick of the tail is recorded. The test has a number of limitations despite its regular use for screening drugs and studying pain mechanisms in animals. The test employs a transient stimulus which may not reflect clinical pain. Studies have also been undertaken to evaluate the effect of the animal restraint during the tail-flick test believed to induce fear and discomfort which may distort nociception (Hargreaves *et al.*, 1988). Tail-skin temperature has also been reported to influence tail-flick latency in this test (Tjølsen *et al.*, 1988). Despite these drawbacks, the tail-flick test is useful for studying the tail-flick reflex, a spinally integrated nociceptive reflex, and also for evaluating efficacy of new drugs, especially opiates. Prior to this study, the tail-flick test had not been used in the naked mole-rat.

2:6.3 The Formalin test

The formalin test was originally described for rats and mice by Dubuisson and Dennis, (1977). In this test a small amount of diluted formalin is injected into one of the paws of the animal. This induces a licking response whose time course has been divided into an "early phase" and a "late phase" (Hunskaar *et al.*, 1985b).

The formalin test has proved a useful model for studying tonic as well as phasic pain in animals such as mice (Hunskaar *et al.*, 1985a, 1986b; Hunskaar, 1987a; Hunskaar and Hole,

1987; Rosland *et al.*, 1990), monkeys (Alreja *et al.*, 1984), rats and cats (Dubuisson and Dennis, 1977; Dennis *et al.*, 1980; Dennis and Melzack, 1980,1983; Abbott *et al.*, 1982, Abbott, 1988; Gamble and Milne, 1990), rabbits, (Carli *et al.*, 1981), guinea-pigs, (Takahashi *et al.*, 1984), crocodiles (Kanui *et al.*, 1990), domestic fowls (Hughes and Sufka, 1991) and naked mole-rats (Kanui *et al.*, 1990,1993; Karim *et al.*, 1993; Towett *et al.*, 2009). It is also useful for demonstrating anti-inflammatory and analgesic effects of drugs. The formalin test is also reported to have a fair degree of objectivity, validity, reproducibility and quantifiability (Alreja *et al.*, 1984) besides being simple to perform. The test has been used for investigating analgesic effects of oxotremorine, the muscarinic receptor agonist (Yaksh *et al.*, 1985; Capone *et al.*, 1999; Abelson and Höglund, 2002b), epibatidine, the nicotinic receptor agonist in rats and mice (Qian *et al.*, 1993; Curzon *et al.*, 1998; Boyce *et al.*, 2000), morphine, nefopam and paracetamol (Kanui *et al.*, 1993), codeine, naproxen and dexamethasone (Karim *et al.*, 1993), opioid peptides (Towett *et al.*, 2009).

2:6.4 The Chemically Induced Writhing test

The chemically induced writhing test uses a chemical irritant for studying visceral pain although it has also been used to study cutaneous pain (Emilio and Eladio, 1998). It involves administering an irritant, for instance acetic acid, into the peritoneal cavity of an animal. The noxious agent causes a behavioural response in the experimental animal. In rats and mice, the behavioural response consists of a wave of constriction and elongation passing caudally along the abdominal wall. This is sometimes accompanied by twisting of the trunk and followed by extension of the hind limbs (Vander Wende and Margolin, 1956; Siegmund *et al.*, 1957; Collier *et al.*, 1968; Hayashi and Takemori, 1971; Bentley, *et al.*, 1981; Schmauss and Yaksh, 1984).

Besides acetic acid, the other irritants that have been used to induce the abdominal constriction response in rodents are acetylcholine (Collier *et al.*, 1968; Bentley *et al.*, 1981), sodium chloride, distilled water, phenylbenzoquinone, hypertonic saline and bradykinins (Siegmund *et al.*, 1957; Emele and Shanaman, 1963; Collier *et al.*, 1968). The conventional abdominal constriction test has been modified and used to study the antinociceptive effects of drugs (Bentley *et al.*, 1981). In the modified test, drugs to be tested are administered intraperitoneally six minutes after the acetic acid. The abdominal constriction test is commonly used in investigating the antinociceptive effects of drugs.

The main drawback for the test is that the animal is not able to terminate the noxious input and may be considered ethically unacceptable (Zimmerman, 1983). The test is time consuming and produces many false positives (Emele and Shanaman, 1963). Like other pain tests, the abdominal constriction test has been used in rodents and to a small extent in other small animals.

2:6.5 The Paw pressure test

This test is suitable for studying inflammatory processes. The test involves intradermal injection of yeast (Randall and Selitto, 1957) or any other irritant such as carrageenin (Vinegar *et al.*, 1976) into the hindpaw of an animal. In the injected animal vocalization or struggle may be elicited when the injected area is touched.

The test has put a lot of light on the mechanisms of hyperalgesia and the importance of various algogens such as prostaglandins (Ferreira, 1972) and substance P, (Nakamura and Smith, 1989) in pain mechanisms. The test is also good for differentiating the analgesics that predominantly act peripherally from those that mainly have central effects (Hunskaar, 1987a).

2:6.6 Adjuvant Induced Arthritis

The test provides a chronic pain model for studying the effects of analgesics. Sodium urate crystals or *Mycobacterium butyrium* with Freud's adjuvant (Pircio *et al.*, 1975) have been used to induce polyarthritis in rats. Oedema of the paw and vocalization following manipulation of the joint are two of the pain responses that have been used to study effects of analgesics in this test.

2:7 ANALGESIC DRUGS

Analgesic drugs are agents that cause analgesia by suppressing or controlling the development of pain mechanisms (Sindrup and Jensen, 1999). Some of the analgesic drugs used in pain management are opioids, steroidal anti-inflammatory agents and non-steroidal anti-inflammatory drugs. Cholinergic agents have not been readily applied for pain management due to the undesirable side effects that override the resultant antinociceptive effects.

2:7.1 Opioid Drugs

The endogenous opioid peptides as well as the exogenous opiates cause analgesia by acting on the same systems in the body of an animal and have been described in a wide range of species and phyla of vertebrates and invertebrates (Kavaliers, 1988). Activation of opioid systems results in the release of "endorphins" (Yaksh, 1987a). Four major pro-hormone families have been identified and sequenced. These are pro-enkephalin (enkephalins), pro-opiomelanocortin (endorphins), pro-dynorphin (dynorphins), and pro-nociceptin/orphanin FQ (Kakidani *et al.*, 1982; Noda *et al.* 1982; Yaksh, 1987a; Rossier, *et al.*, 1993).

Electrophysiological studies have demonstrated that opiates with an action in the spinal cord are capable of depressing the responses of spinal neurons excited by noxious stimuli (Le

Bars *et al.*, 1975; Duggan *et al.*, 1977; Einspahr and Piercey, 1980). Behavioural studies demonstrated that the distinct classes of μ, δ, and κ opioid receptors in the spinal dorsal horn are able to modulate nociceptive processing in a behaviourably relevant manner (Tyres, 1980; Upton *et al.*, 1982; Schmauss *et al.*, 1983; Ward and Takemori, 1983; Schmauss and Yaksh, 1984; Yaksh, 1987b).

Direct injection of opiates into the cerebral ventricles or several brainstem loci have been reported to produce analgesia as measured by behavioural models in animals (Dickenson *et al.*, 1979; Dennis and Melzack, 1980; Le Bars *et al.*, 1980; Azami *et al.*, 1981; Berge, 1986). This shows that opioids may also exert their analgesic effect supraspinally. The supraspinal sites where opiates can act are the periaqueductal gray (Lewis and Gebhart, 1977; Urca *et al.*, 1977), the nucleus raphe magnus (Proudfit and Anderson, 1975; Fields *et al.*, 1977; Yaksh, 1979; Yaksh and Wilson, 1979) and the reticular formation (Mayer and Hill, 1978; Satoh *et al.*, 1979).

Opiates can elicit both excitatory and depressive physiological effects in the animal (Klemm, 1981). The effects of opiate receptor activation are mediated by a G-protein, which couples the receptor directly to ion channels (Nicoll *et al.*, 1990; Sabbe and Yaksh, 1990). There is evidence that μ and δ opioid agonists inhibit neuronal activity by increasing potassium conductance, whereas κ agonists cause the same effect by inhibiting directly the entry of calcium (Sabbe and Yaksh, 1990).

2:7.2 Steroidal Anti-inflammatory Drugs

Anti-inflammatory steroids are so far the most potent anti-inflammatory agents. They are capable of suppressing the cardinal signs of inflammation regardless of the cause. It is clearly established that this class of drugs at least exert their anti-inflammatory properties by inhibiting

phospholipase A_2 and indirectly by causing the release of an inhibitory protein, which has been variously termed macrocortin, lipomodulin, renocortin or lipocortin (Vane and Botting, 1987). Steroids stimulate both the release and re-synthesis of lipocortin by binding onto specific membrane receptors. Lipocortin neutralizes both the cyclooxygenase and lipoxygenase pathways by inhibiting phospholipase A_2 activity.

Steroidal anti-inflammatory drugs cause analgesia in those conditions characterized by inflammation. For instance, the analgesic effect of hydrocortisone and dexamethasone has been demonstrated in the late phase of the formalin test (Hunskaar and Hole, 1987). It is suggested that the analgesic effect of steroids can be attributed to their anti-inflammatory effects and subsequently reduced symptoms of inflammation (Hunskaar and Hole, 1987).

2:7.3 Non-Steroidal Anti-inflammatory Drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used analgesic, anti-rheumatic and antipyretic drugs. The mechanism of action for NSAIDs may include an interference with oxidative phosphorylation, the displacement of endogenous anti-inflammatory peptide from plasma protein, interference with migration of leucocytes, inhibition of leucocytic-phagocytosis, stabilization of lysosomal membranes, inhibition of the generation of lipoperoxides, and hyperpolarization of neuronal membranes (Ferreira and Vane, 1974). The main effect is, however, the inhibition of the synthesis of prostaglandins.

On tissue injury algogens such as bradykinins, histamines, serotonin, dopamine, acetylcholine, acids and prostaglandins are released (Ferreira and Vane, 1974). Systemic administration of these algogens has been shown to produce pain-related behaviour in animals (Guzman and Lim, 1968). Prostaglandins (PGs) are synthesized from arachidonic acid following

a chain of reactions controlled by enzymes. The enzymes that catalyse the reactions are phospholipase A_2 and cyclooxygenase. NSAIDs prevent PG biosynthesis by inhibiting cyclooxygenase enzyme and thus block the conversion of arachidonic acid to cyclic endoperoxides. The mode of action serves to curtail the production of PGs which are potent inflammatory mediators (Vane and Botting, 1987). This effect can therefore explain the anti-inflammatory actions of NSAIDs (Ferreira, 1972).

The nociceptive response induced by PGs when administered alone is small, suggesting that they have minimal effects on pain receptors but facilitate the response to other stimuli affecting nociceptors (Ferreira, 1972; Handwerker, 1976; King *et al.*, 1976). It has been demonstrated that NSAIDs may also exert their analgesic effect centrally (Ferreira *et al.*, 1978; Ferreira, 1983; Hunskaar *et al.*, 1985a). Several PG biosynthesis inhibitors are capable of counteracting hyperalgesia induced by intrathecal administration of PGs, (Ferreira *et al.*, 1978; Ferreira, 1983). It has been shown that the behaviour caused by intrathecal administration of substance P is reduced by a pre-treatment with intraperitoneal aspirin or paracetamol (Hunskaar *et al.*, 1985a). Another study demonstrated that the serotonergic systems may play a role in the analgesic effect of paracetamol in mice (Hunskaar, 1987a). The differences in potency of NSAIDs also suggest that they have other modes of action in addition to their common effect on cyclooxgenase enzyme.

2:7.4 Cholinergic Drugs

The cholinergic or cholinomimetic drugs produce akinetic seizures, depression, lacrimation and salivation, similar to those associated to acetylcholine (Haley and McCormick, 1957). They have been categorized into two main groups vide; the direct acting receptor agonists

and indirect acting cholinesterase inhibitors. Acetylcholine is a neurotransmitter of the parasympathetic nervous system, the part of the peripheral nervous system responsible for the everyday work of the body (Dale, 1914). Acetylcholine causes physiological functions by interacting with either muscarinic or nicotinic receptors.

Systemic and intrathecal administration of muscarinic agonists produces potent antinociception in several species (Yaksh *et al.*, 1985; Gower, 1987; Gillberg *et al.*, 1989; Zhuo and Gebhart, 1991; Iwamoto and Marion, 1993; Abram and O'Connor, 1995; Lambert and Appadu, 1995). An involvement of nicotinic receptors in antinociception has been known for several decades. In 1932, antinociception of nicotine was reported by Davis *et al.* (1932), an effect that has been verified by other studies (Sahley and Berntson, 1979; Iwamoto, 1991). Other nicotinic agonists that produce antinociception after supraspinal or systemic administration are epibatidine (Qian *et al.*, 1993; Curzon *et al.*, 1998; Lawand *et al.*, 1999), A-85380 (Curzon *et al.*, 1998) and ABT-594 (Bannon *et al.*, 1998; Bitner *et al.*, 1998). The antinociceptive effects of nicotinic agonists administered into the spinal cord are somewhat controversial, since both nociceptive and antinociceptive effects have been observed by Khan *et al.*, (1998). However, neuronal nicotinic receptors are considered a promising target in pain treatment (Flores and Hargreaves, 1998).

It has been shown in rodents, that an increased release of spinal acetylcholine after intravenous administration of the muscarinic receptor agonist oxotremorine is associated with an increased pain threshold (Abelson and Hõglund, 2002b). Other studies in rats showed that the acetylcholine release was increased after intraspinal treatment with several substances associated with antinociception, such as lidocaine (Abelson and Hõglund, 2002a), α2-adrenoceptors agonists

(Abelson and Höglund, 2004), epibatidine (Kommalage and Höglund, 2004), serotonergic and GABAergic receptor agonists (Kommalage and Höglund, 2005a; 2005b). The involvement of the acetylcholinergic system in antinociception has been evaluated in other species, such as sheep (Bouaziz *et al.*, 1995; 1996), cats (Yaksh *et al.*, 1985) and humans (Flodmark and Wramner, 1945; Lambert and Appadu, 1995).

Atropine, which is a muscarinic receptor antagonist, lowers the intraspinal levels of acetylcholine and cause hyperalgesia (Abelson and Höglund, 2002b). Another report by Ghelardini *et al.*, (1990) showed that atropine induces analgesia in rats in doses ranging from 1 to 100 µg/kg and hyperalgesia when 5mg/kg was administered. Epibatidine acts through the nicotinic (Bonhaus *et al.*, 1995) and partially through the muscarinic (Kommalage and Höglund, 2004) acetylcholine receptors. It has been reported that epibatidine is a more potent analgesic agent than morphine (Spande *et al.*, 1992; Sullivan *et al.*, 1994), serotonergic or GABAergic receptor agonists (Kommalage and Höglund, 2005a; 2005b). The use of nicotinic agonists for pain management has not been practical due to the adverse effects, such as salivation and tremors, which are well documented by Wang *et al.*, (2004).

The involvement of cholinergic systems in central antinociceptive actions of morphine (Chen and Pan, 2001), acetylsalicylic acid and paracetamol has been documented (Abelson *et al.*, 2004). The role of the cholinergic system in ketamine anesthesia has also been demonstrated (Abelson *et al.*, 2006). The indirect acting cholinergic agents such as neostigmine and physiostigmine have a greater duration of action than acetylcholine in humans because of their resistance to hydrolysis by plasma cholinesterase, anticholinesterase, or both (Flodmark and Wramner, 1945; Lambert and Appadu, 1995).

2:8 JUSTIFICATION OF STUDY

The study is expected to provide additional information on the physiology and the pharmacology of the pain systems of the naked mole-rat. The study will explore the antinociceptive effects of selected direct acting cholinergic drugs that may occur during spinally and supraspinally mediated reflexes. Considering that the naked-mole rat has unique features, the study might uncover novel aspects of the cholinergic system. The study on the naked mole-rat, a primitive animal model might provide information on the evolution of the pain systems in vertebrates. The data obtained will be compared with the similar published data in rats and mice (Abelson and Höglund, 2002a: 2002b; Abelson *et al.*, 2004). Data obtained will be utilized in the improvement of the animal's health standards with a view of enhancing its welfare in captivity or zoos. The findings will also form a basis for commencing electrophysiological and molecular investigations of pain mechanisms in the naked mole-rat. The study will boost the knowledge about the cholinergic involvement in antinociception, an essential field, to exploit the high potential of development of pain treatments for both animal and human use.

CHAPTER 3

3:0 MATERIALS AND METHODS

3:1 EXPERIMENTAL ANIMALS

Naked mole-rats (*Heterocephalus glaber)* were captured from Kathekani and Kambu areas in the Kibwezi district. Five experienced local people were identified and contracted to undertake the exercise of capturing the animals. A total of 362 male and female adult animals weighing 30-35 g were captured. The naked mole-rats were captured easily by opening the underground burrow system that was identified by the presence of fresh mole-hills ("volcanoes"). Any captured animal weighing less than 20g was returned immediately to the burrow system to give them a chance to join the parent colony. The animals were packed in 20 litre capacity, rectangular boxes, with tightly fitting lids that could be opened easily to allow inflow of fresh air. The boxes containing the naked mole-rats were transported by road to the University of Nairobi, Department of Veterinary Anatomy and Physiology, Chiromo campus. Thirty days acclimation period was allowed

The housing conditions were almost similar to those in the wild i.e. Temperatures of 28-31°C; relative humidity 45-50% and 24/0 dark/light cycle. The naked mole-rats were housed in colonies of 50-100 in well designed cages covered by non-transparent lids (**Plate 1**). The cages made of plastic glass and painted with black super gloss on the outside surface, measured 70 x 50 x 20 cm. Each cage comprised two compartments, with an interconnecting tunnel that measured 30 x 10 x 10 cm between the subdividing wall. The naked mole-rats used one compartment as a toilet and the other as a bedroom. Wood shavings mixed with sand were used as beddings and

were changed once a week to ensure the cages were well sanitized and fresh. The lids that covered the cages were not closely fitting, which allowed for free air flow to ensure animals were not suffocated. The beddings were fumigated every month using Dudukrin® shampoo (KAPI Ltd., Nakuru, Kenya) to check on flea infestation. To avoid dry skin in the animals, the humidity in the room was maintained at 45-50% using strategically placed humidifier system. The humidifiers comprised of a 20 liter pail and three two-litre water bottles fitted with 30 cm long cotton winks. Relative humidity was measured using a hygrometer courtesy of the Ministry of Livestock Development, Kabete, Central Veterinary Laboratory. The cages were put on metallic frames 15 cm above the cemented floor to guarantee sound and vibration proof conditions in the room. The animal house measured 2.25 x 3.25 x 2.25 m, and was warmed constantly using three infra-red (250W) lamp heating system (**Plate 1**). The animals were fed on fresh carrots and sweet potatoes *ad libitum*. The food ration estimated at 4 grams per animal per day was cut into 1 cm cube pieces and placed at the bedroom compartment. The naked mole-rats obtained water from the fresh succulent tubers.

Plate 1: - Animal cages warmed by the infrared lamps.

During the acclimation period the animals were handled and weighed daily using a Sartorius electric balance (Sartorius AG, Frankfurt, Germany) before commencement of the actual experiments. A room measuring 1.25 x 3.25 x 2.25 m next to the animal house but having similar conditions as the latter was used to carry out all the experiments during the study. The animals were handled humanely and with regard to alleviation of suffering in accordance with the Kenya Wildlife Services Animals Welfare and Ethics Committee guidelines. A total of 362 naked mole-rats were used in the study. Six adult male and female animals per dose were used. Animal was used only once. The animals were randomly selected. The experimenter was not aware of the drugs or vehicle injected until after data analysis. Each animal was gently restrained by picking the animal out of the cage by tip of the tail using the right hand (**Plate 2**). The animal was gently put on a table covered with manila paper to facilitate holding of the loose skin on the dorsal side of the neck. The thumb and pointing finger of the left hand were used (**Plate 3**). The animal was then turned to lie on the palm of the left hand with the tail held by the little finger to expose the ventral surface of the animal (**Plate 4**).

Plate 2: Naked mole-rat restrained by lifting the tail.

Plate 3: - Naked mole-rat restrained by holding the nape and the tail.

Plate 4:- Naked mole-rat restrained by holding on left hand palm.

3:2 DRUGS

The drugs used during the experiments were as follows: -

Oxotremorine sesquifumarate salt (Sigma-Aldrich, Sweden), (±)-Epibatidine dihydrochloride (Sigma-Aldrich, Sweden), Atropine sulfate salt (Sigma-Aldrich, Sweden), Naloxone hydrochloride dehydrate (Sigma-Aldrich, Sweden), Mecamylamine hydrochloride (Tocris

Bioscience, Copenhagen), All drugs were weighed and dissolved in 0.9% saline and stored as a stock-solution at a temperature of 2-4°C. All precautions regarding handling and stability of the drugs as recommended by the manufacturer were adhered to. Fresh preparations were reconstituted daily and injected intraperitoneally in a volume of 50 microlitres.

3:3 NOCICEPTIVE AND ANTINOCICEPTIVE BEHAVIOURAL STUDIES

3:3.1 Antinociceptive Behavioural Studies

During the development of baseline latency for the formalin test, two groups of 6 animals each were intraperitoneally injected with 0.9% saline and then thirty minutes later, 20µls of 10% formalin or saline was intradermally injected into the right dorsal paw. The pain behaviour response was monitored for one hour. The preliminary tests started at a dose level of oxotremorine (300 µg/kg body weight) and epibatidine (30 µg/kg body weight) that were serially diluted in multiplies of 3 using 6 animals per dose level in the formalin test to determine the effective doses of the selected direct acting cholinergic drugs. The dose levels for oxotremorine and epibatidine were (10, 20, 50, and 100 µg/kg body weight) (0.5, 1, 2, and 3 µg/kg body weight) respectively.

Based on the preliminary data, the four dose levels of oxotremorine (10, 20, 50, and 100 µg/kg body weight) and epibatidine (0.5, 1, 2, and 3 µg/kg body weight) were used in the formalin, hot-plate and tail-flick tests. Every test had one control (vehicle injected) group. Atropine sulfate (2.5 mg/kg body weight) and mecamylamine (50 µg/kg body weight) were used to block the effects of oxotremorine (20 µg/kg body weight) and epibatidine (2 µg/kg body weight) respectively. The effect of co-administration of either oxotremorine (20 µg/kg body

weight) or epibatidine (2 μg/kg body weight) with naloxone (2.5 mg/kg body weight) on pain behaviour of the naked mole-rat was also evaluated.

The antinociceptive effects of oxotremorine and epibatidine were evaluated 30 minutes after intraperitoneal administration.

3:3.2 The formalin test

Adaptation of the animal to the transparent observation chamber measuring 30 x 30 x 30 cm was undertaken one hour everyday during the acclimation period and also 30 minutes prior to the start of the experiments. The animal was gently restrained before the administration of 20 microlitres of 10% formalin. The latter was injected intradermally into the right dorsal hind paw using 100μl syringe and a 30 gauge needle. The animal was thereafter put back into the observation chamber for observation. A mirror placed directly opposite the observer and behind the observation chamber allowed an unobstructed view of the naked mole-rat. The time spent licking or biting the injected paw following the injection of formalin was recorded in blocks of 5 minute for 60 minutes. The volume and concentration of formalin was based on earlier studies (Kanui *et al.*, 1993, Karim *et al.*, 1993; Towett *et al.*, 2009). The experiments were performed between 8 a.m., and 2 p.m., at a room temperature of 26-28°C in a sound proof room.

3:3.3 The Hot-plate test

The Hot-plate test was performed using an IITC Inc. model 35D analgesiometer. Before the start of the experiments, the naked mole-rats were acclimated to the cold plate for thirty minutes per day for a period of 30 days. The temperature of the copper plate (27 x 29 cm) which was enclosed by a 30 x 30x 30 cm lidded perspex box, was set at 60°C.

The animal was examined to ascertain that they had normal sensorimotor function by observing the ability of the animal to explore and negotiate the corners of the perspex box prior to the test. Thirty minutes after drug or saline injection, a naked mole-rat was placed gently on the hot plate and the latency (in seconds) to escaping or jumping (hot plate response latency) was recorded. Animals that failed to respond by 60 seconds were removed from the plate and assigned a response latency of 60 seconds.

3:3.4 The Tail-flick test

The animal was restrained by placing them in a 2mm thick plastic tube measuring 10cm long by 3cm in diameter (**Plate 5**).

Plate 5: -Naked mole-rat being put in a restrainer for Tail-flick test.

The restrainer had a stopper fitted with a 0.5cm (diameter) tube that allowed free flow of air. while the rear plastic stopper had a slit that allowed the tail of the animal to emerge through it (**Plate 6**).

Plate 6: The naked mole-rat in a restrainer with tail resting flat on the surface of tail-flick apparatus.

The tail was placed on a level surface and a beam of radiant heat was directed onto the dorsal surface of the tail at a spot 1 cm from the distal tip of the tail. The test stimulus consisted of a linear temperature ramp that rose from a holding temperature of 35^0 C to 52^0 C in ten seconds (**Plate 7**).

Plate 7: - The Tail-flick analgesiometer

Tail-flick latency was measured using an IITC model 33 analgesia meter (IITC Inc., Woodland Hills, CA, USA) with a sensitivity setting of 10, beam at 8 and heat cut-off was set at 10 seconds or on the occurrence of a tail-flick. The baseline latency was computed as the average of 5 tail

flick trials. After the administration of the drug or saline the thermal stimulus was applied to the tail and the duration (latency) the animal took before flicking its tail from the heat exposure was recorded.

3:4. STATISTICAL ANALYSIS

All values were expressed as mean ± standard error of mean (S.E.M.). To determine significant differences among experimental groups, the data was analyzed with SPSS version 12.0.1. The two-tailed, unpaired Student's t-test was used when comparing two experimental groups. The one-way analysis of variance (ANOVA) and a Dunnett's post-hoc test was performed for multiple sample comparisons. P-values lower than 0.05 were considered significant.

CHAPTER 4

4:0 RESULTS

4:1 THE FORMALIN TEST

The administration of 20 microlitres of 10% formalin intradermally into the dorsal right hind paw elicited discernable pain behaviour. The naked mole-rats showed behavioural responses that included licking and biting of the injected paw (**Plate 8**). Two distinct periods of high activity were observed; the early phase lasting for the first 5 minutes and a late phase starting 30-35 minutes after injection of formalin (**Fig. 1**). The 20 µl of 0.9% saline only induced minimal pain response (**Fig. 1**).

In both the early and the late phases, the effect of formalin was significantly different from that of saline.

Plate 8: Naked mole-rat licking/biting the dorsal right hind-paw following injection of 20µl of 10% formalin.

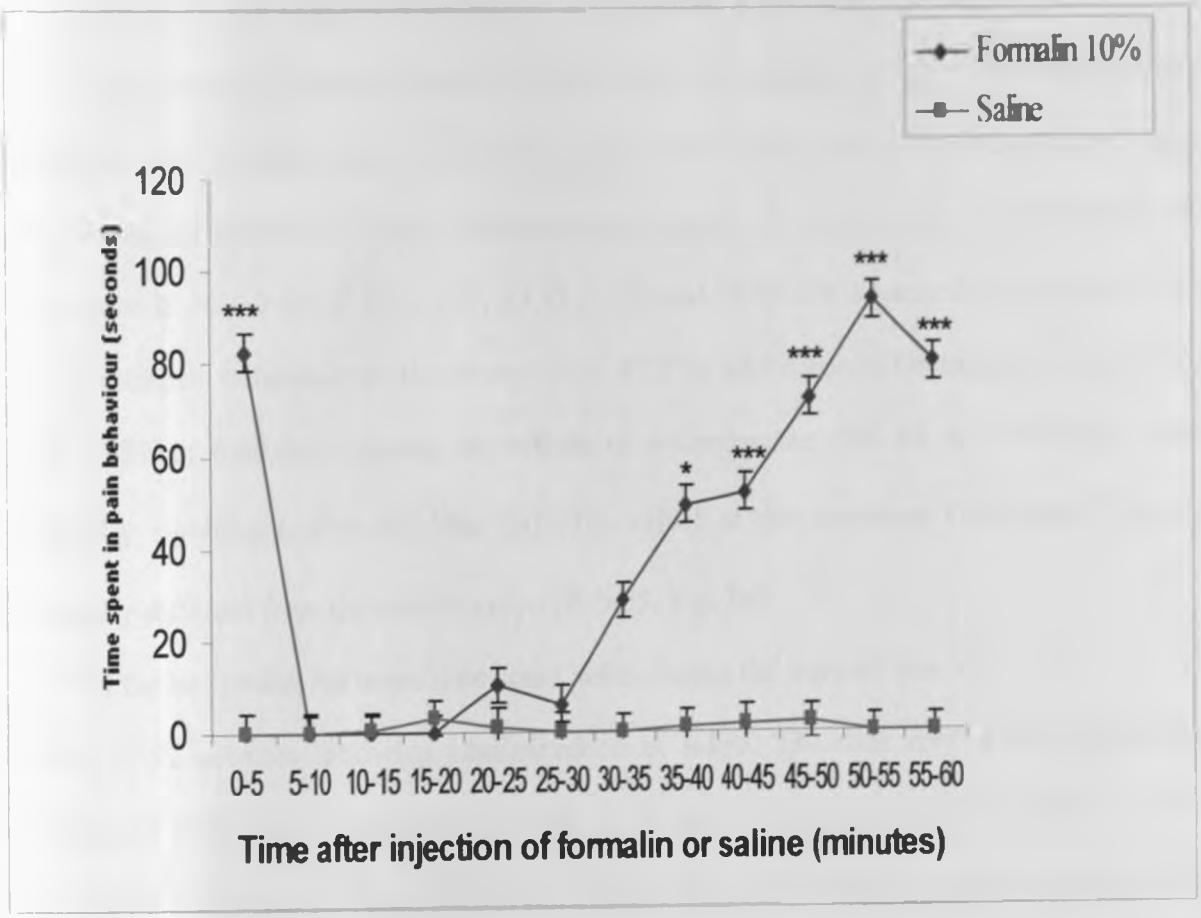

Fig. 1

Time-course of pain behaviour after intradermal injection of 20µl of 10% formalin or 20µl of physiological saline (0.9% NaCl) into the dorsal right hind paw (mean ± S.E.M.; n = 8; *and ***denotes $P<0.05$ and $P<0.001$ respectively, Student's t-test subsequent to ANOVA).

4:1.1 EFFECTS OF OXOTREMORINE, ATROPINE AND NALOXONE.

The effects of oxotremorine (10, 20, 50 or 100 μg/kg) on the mean licking/biting response in the formalin test were investigated. In the early phase, the mean times spent licking/biting the injected paw after intraperitoneal injection of oxotremorine (10, 20, 50 and 100 μg/kg) were 70.36 ± 7.57, 45.37 ± 6.41, 27.85 ± 4.58 and 24.56 ± 4.00 seconds, respectively. The time spent in pain behaviour for the controls, was 87.37 ± 9.80 seconds. On multiple comparisons of the different treatment means, the effects of oxotremorine (20, 50 or 100 μg/kg) were statistically significant ($P<0.05$; **Fig. 2a**). The effect of oxotremorine (10 μg/kg) was not statistically different from the control group ($P>0.05$; **Fig. 2a**).

In the late phase, the mean time spent licking/biting the injected paw was 60.43 ± 12.92 seconds following administration of saline. The time spent in pain behaviour following administration of oxotremorine (10, 20, 50 and 100 μg/kg) were 49.33 ± 4.01, 31.13 ± 8.15, 26.63 ± 13.85 and 22.76 ± 5.36 seconds, respectively. There was no statistically significant difference in time spent in pain behaviour between the control group and the group injected with 10 μg/kg of oxotremorine ($P>0.05$). However, oxotremorine (20, 50 or 100 μg/kg) caused a statistically significant reduction in pain behaviour ($P<0.05$; **Fig. 2a**). The times spent in pain behaviour following administration of oxotremorine (20, 50 or 100 μg/kg) were also statistically significant when compared with that of oxotremorine (10 μg/kg; $P<0.05$). In both the early and the late phases, treatment with oxotremorine (20, 50 and 100μg/kg) were significantly different ($P<0.05$) in comparison to the saline group.

The time spent in pain behaviour following intraperitoneal co-administration of oxotremorine (20 μg/kg) plus atropine (2.5mg/kg) was 70.41 ± 3.99 seconds in the early phase.

4:1.1 EFFECTS OF OXOTREMORINE, ATROPINE AND NALOXONE

The effects of oxotremorine (10, 20, 50 or 100 μg/kg) on the mean licking/biting response in the formalin test were investigated. In the early phase, the mean times spent licking/biting the injected paw after intraperitoneal injection of oxotremorine (10, 20, 50 and 100 μg/kg) were 70.36 ± 7.57, 45.37 ± 6.41, 27.85 ± 4.58 and 24.56 ± 4.00 seconds, respectively. The time spent in pain behaviour for the controls, was 87.37 ± 9.80 seconds. On multiple comparisons of the different treatment means, the effects of oxotremorine (20, 50 or 100 μg/kg) were statistically significant ($P<0.05$; **Fig. 2a**). The effect of oxotremorine (10 μg/kg) was not statistically different from the control group ($P>0.05$; **Fig. 2a**).

In the late phase, the mean time spent licking/biting the injected paw was 60.43 ± 12.92 seconds following administration of saline. The time spent in pain behaviour following administration of oxotremorine (10, 20, 50 and 100 μg/kg) were 49.33 ± 4.01, 31.13 ± 8.15, 26.63 ± 13.85 and 22.76 ± 5.36 seconds, respectively. There was no statistically significant difference in time spent in pain behaviour between the control group and the group injected with 10 μg/kg of oxotremorine ($P>0.05$). However, oxotremorine (20, 50 or 100 μg/kg) caused a statistically significant reduction in pain behaviour ($P<0.05$; **Fig. 2a**). The times spent in pain behaviour following administration of oxotremorine (20, 50 or 100 μg/kg) were also statistically significant when compared with that of oxotremorine (10 μg/kg; $P<0.05$). In both the early and the late phases, treatment with oxotremorine (20, 50 and 100μg/kg) were significantly different ($P<0.05$) in comparison to the saline group.

The time spent in pain behaviour following intraperitoneal co-administration of oxotremorine (20 μg/kg) plus atropine (2.5mg/kg) was 70.41 ± 3.99 seconds in the early phase.

When the mean time spent in pain behaviour for the combined treatment was compared with that of oxotremorine (20 μg/kg) alone, a statistically significant difference ($P<0.05$; **Fig. 2b**) was noted. The atropine-treated group, the group given the combined treatment (oxotremorine 20 μg/kg plus atropine 2.5mg/kg) and also the saline group were not significantly different ($P>0.05$).

In the late phase the time spent in pain behaviour following co-administration of oxotremorine (20 μg/kg) plus atropine (2.5mg/kg) was 50.08 ± 2.36 seconds. This was statistically significant when compared with that for oxotremorine (20 μg/kg) alone (31.13 ± 8.15 seconds) (($P<0.05$; **Fig. 2b**). The time spent in pain behaviour following administration of 0.9% saline, atropine (2.5mg/kg) alone and combined oxotremorine (20 μg/kg) plus atropine (2.5mg/kg) treated group was statistically different ($P<0.05$) in comparison to the group injected with oxotremorine (20 μg/kg) alone. In both early and late phases, the combined oxotremorine (20μg/kg) plus atropine (2.5 mg/kg) treated group showed statistically significant difference in comparison to the oxotremorine (20μg/kg) alone.

The times spent in pain behaviour following simultaneous intraperitoneal administration of oxotremorine (20 μg/kg) plus naloxone (2.5mg/kg), were 9.02 ± 1.90 seconds in the early phase, and 1.86 ± 1.63 seconds in the late phase. The pain behaviour following co-administration of oxotremorine (20 μg/kg) plus naloxone (2.5mg/kg) were statistically significant in both phases of the test ($P<0.05$, **Fig. 2c**) compared to oxotremorine (20 μg/kg) alone.

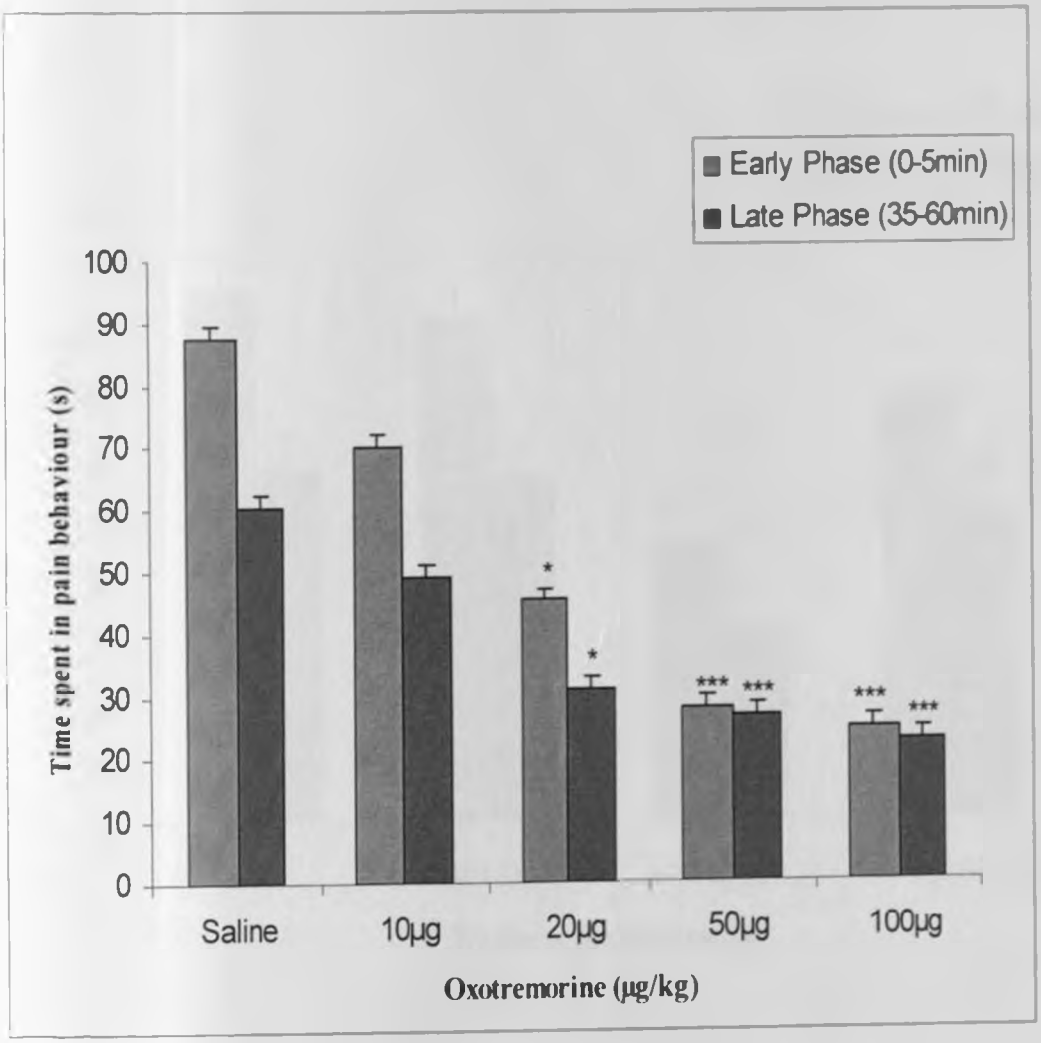

Fig. 2a

Effects of intraperitoneal administration of physiological saline (0.9% NaCl; controls) or oxotremorine (10, 20, 50 or 100 µg/kg) on the time spent in pain behaviour in the formalin test. Values presented are means ± S.E.M., and n=6 in each group. Treatment means were compared using Dunnett's post-hoc test subsequent to ANOVA. * and *** denotes $P<0.05$ and $P<0.001$ respectively (oxotremorine versus control groups).

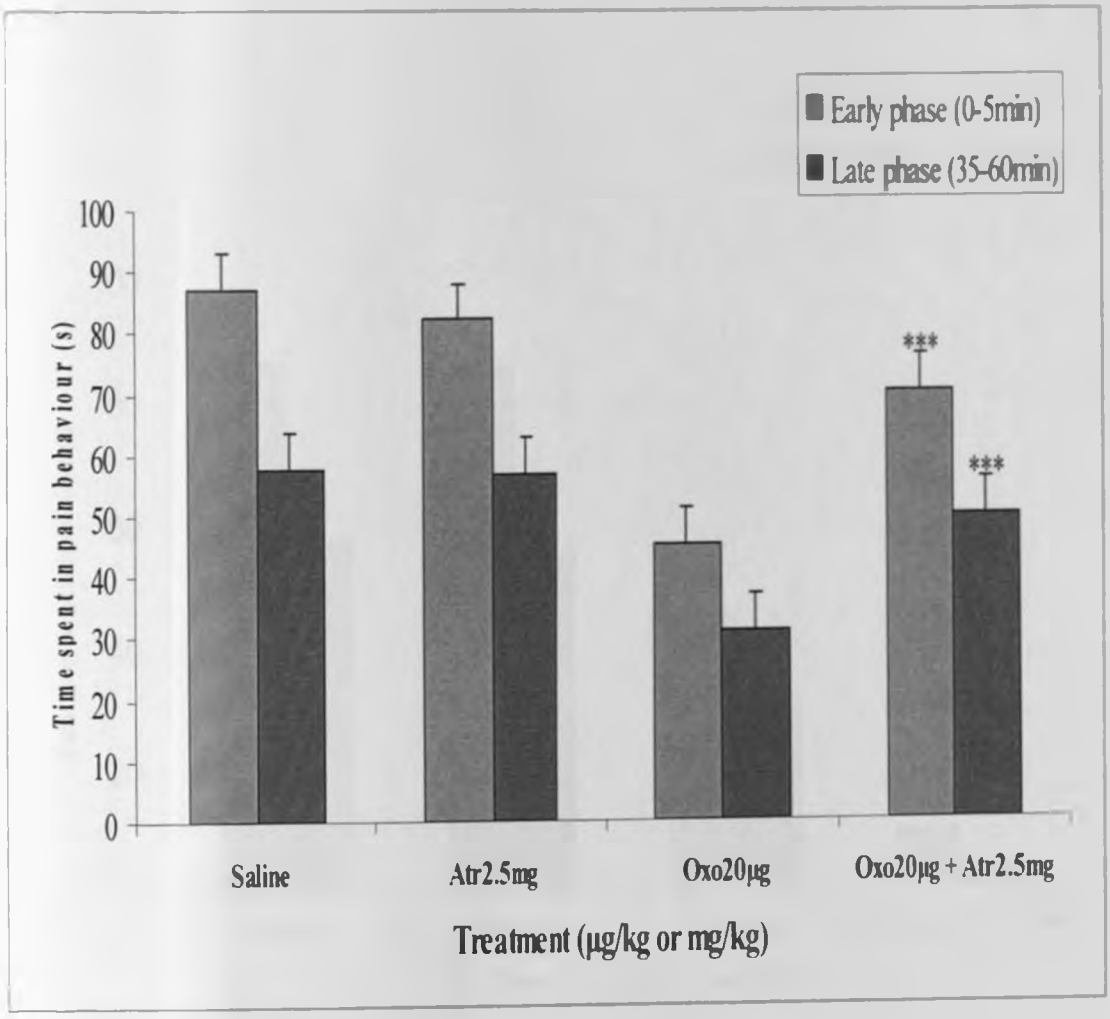

Fig. 2b

Effects of intraperitoneal administration of physiological saline (0.9% NaCl), atropine (Atr 2.5mg/kg), oxotremorine (Oxo 20µg/kg) or a combination of oxotremorine (Oxo 20µg/kg) plus atropine (Atr 2.5mg/kg) on the time spent in pain behaviour in the formalin test. Values presented are means ± S.E.M., and n=6 in each group. Treatment means were compared using Dunnett's post-hoc test, subsequent to ANOVA. ***denotes $P<0.001$ for oxotremorine (Oxo 20µg/kg) versus combined oxotremorine plus atropine (20µg/kg + 2.5mg/kg) groups).

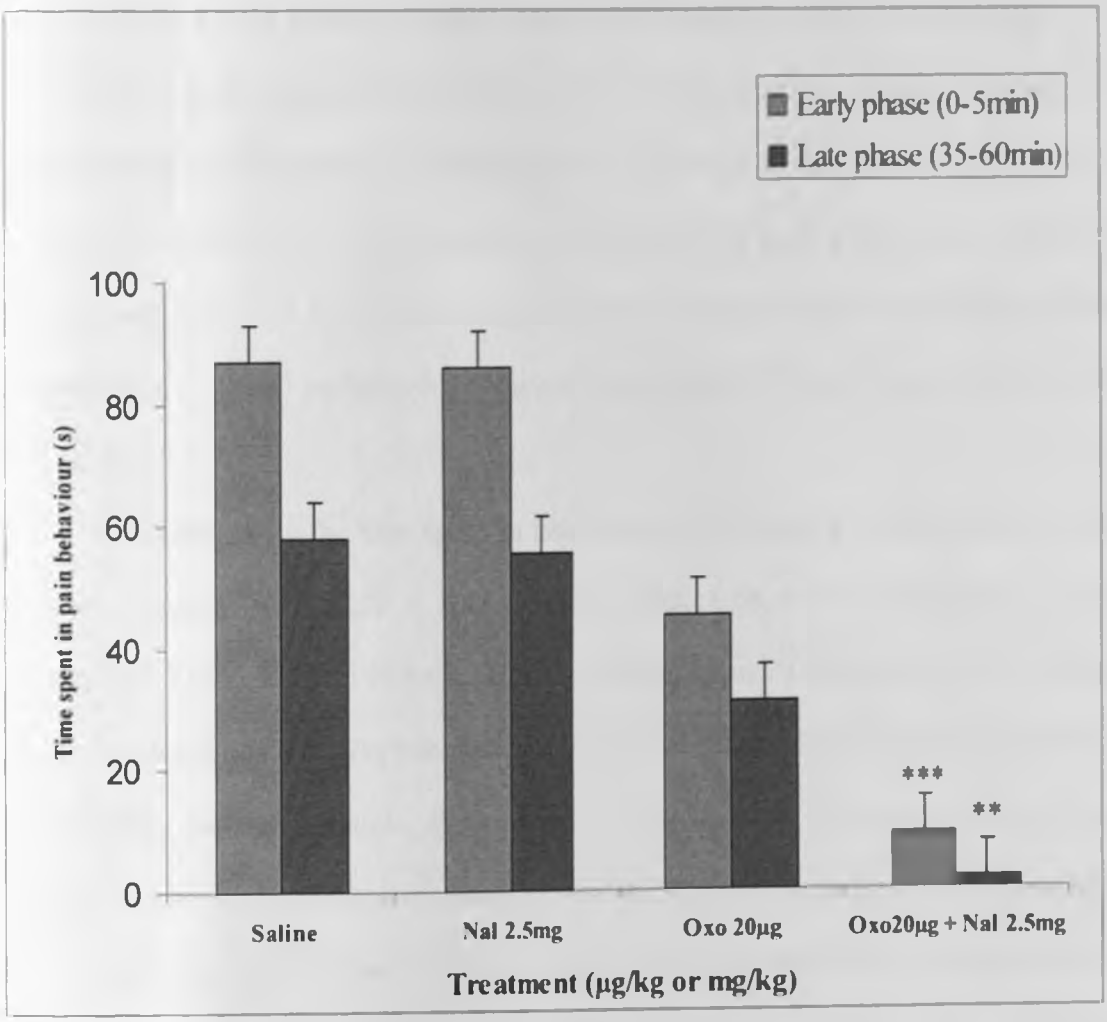

Fig. 2c

Effects of intraperitoneal administration of physiological saline (0.9% NaCl), naloxone (Nal 2.5mg/kg), oxotremorine (Oxo 20µg/kg) or a combination of oxotremorine (Oxo 20µg/kg) plus naloxone (Nal 2.5mg/kg) on the time spent in pain behaviour in the formalin test. Values presented are means ± S.E.M. and n=6 in each group. Treatment means were compared using Dunnett's post-hoc test, subsequent to ANOVA. ** and***denotes P<0.01and P<001 respectively for oxotremorine (20µg/kg) versus combined oxotremorine plus naloxone (20µg/kg + 2.5mg/kg) groups.

4:1.2 EFFECTS OF EPIBATIDINE, MECAMYLAMINE AND NALOXONE

In the early phase of the formalin test, the times spent in pain behaviour following intraperitoneal administration of epibatidine (0.5, 1, 2 or 3 μg/kg) were 76.21 ± 9.13, 57.68 ± 2.77, 44.30 ± 3.08, 31.67 ± 3.14 seconds, respectively. The time spent in pain behaviour for the controls, was 87.37 ± 9.80 seconds. The times spent in pain behaviour following administration of epibatidine (1, 2 or 3 μg/kg) were statistically significant ($P<0.05$, **Fig. 3a)** from those injected with saline.

In the late phase the time spent in pain behaviour following administration of epibatidine (1, 2 or 3 μg/kg) was 42.39 ± 8.75, 12.76 ± 4.92, 6.66 ± 3.71 and 0.41 ± 0.23 seconds respectively. The time spent in pain behaviour for the controls was 60.43 ± 12.92 seconds. The effect of epibatidine (0.5 μg/kg) on the time spent in pain behaviour was not statistically different ($P>0.05$, **Fig. 3a**) from controls. Epibatidine (1, 2 or 3 μg/kg) caused a statistically significant decrease in the time spent in pain behaviour ($P<0.05$, **Fig. 3a)** in comparison to the controls.

The times spent in pain behaviour following co-administration of epibatidine (2 μg/kg) plus mecamylamine (50 μg/kg) was 83.32 ± 2.11 seconds in the early phase and 51.36 ± 1.84 seconds in the late phase. The time spent in pain behaviour following the co-administration of epibatidine (2 μg/kg) plus mecamylamine (50 μg/kg) in the early phase, was significantly different ($P>0.05$; **Fig. 3b)** in comparison to the group given epibatidine (2 μg/kg) alone (44.30 ± 3.08 seconds). In the late phase, the mean response time of epibatidine (2 μg/kg) alone (6.66 ± 3.71 seconds) was statistically significant ($P<0.05$, **Fig. 3b**) in comparison to the mean of combined treatment (Epi 2 μg/kg + Mec 50 μg/kg). The times spent in pain behaviour following co-administration of mecamylamine (50 μg/kg) plus epibatidine (2 μg/kg) in both the early and

late phases were not statistically significant ($P>0.05$, **Fig. 3b)** in comparison to the saline and the mecamylamine treated groups.

The times spent in pain behaviour following intraperitoneal co-administration of epibatidine (2 μg/kg) and naloxone (2.5mg/kg) was 21.42 ± 2.29 seconds in the early phase and 0.93 ± 0.61 seconds in the late phase. The time spent in pain behaviour in both phases (44.30 ± 3.08 and 6.66 ± 3.71 seconds respectively) following administration of epibatidine (2 μg/kg) alone was statistically significantly different ($P<0.05$, **Fig. 3c**) in comparison to controls.

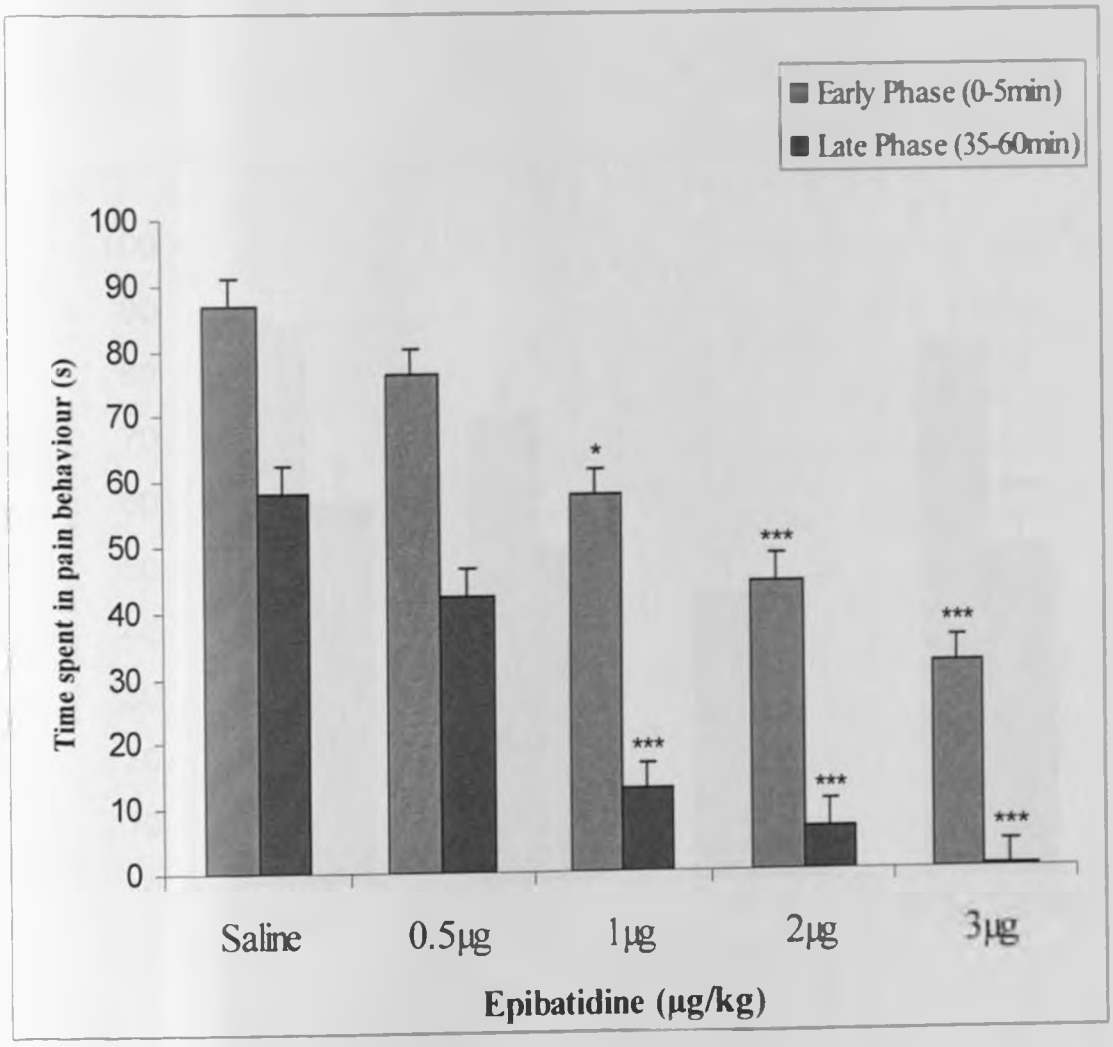

Fig. 3a

Effects of intraperitoneal administration of physiological saline (0.9% NaCl; controls) or epibatidine (0.5, 1, 2 or 3 µg/kg) on the formalin induced pain behaviour. Values presented are means ± S.E.M., and n=6 in each group. In both early and the late phases the treatment means were compared separately using Dunnett's post-hoc test, subsequent to ANOVA. * and *** denotes $P<0.05$ and $P<0.001$ respectively (epibatidine versus control groups).

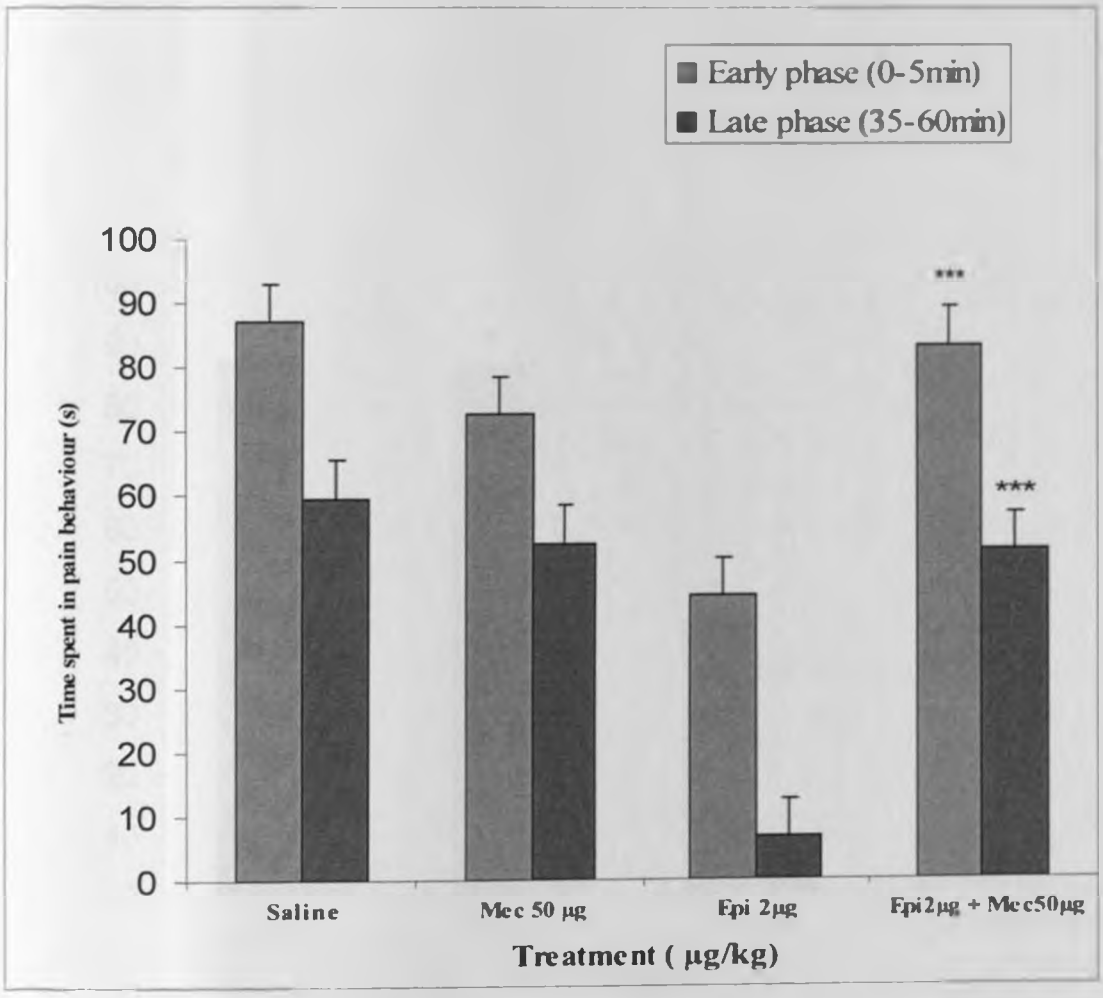

Fig. 3b

Effects of intraperitoneal administration of physiological saline (0.9% NaCl), mecamylamine (Mec 50µg/kg), epibatidine (Epi 2µg/kg) or a combination of epibatidine (Epi 2µg/kg) plus mecamylamine (Mec 50µg/kg) on the formalin induced pain behaviour. Values presented are means ± S.E.M., and n=6 in each group. Treatment means in the early phase and those in the late phase were compared separately using Dunnett's post-hoc test, subsequent to ANOVA. ***denotes $P<0.001$ for epibatidine (Epi 2µg/kg) versus combined (epibatidine plus mecamylamine (Epi 2µg/kg + Mec 50µg/kg) groups).

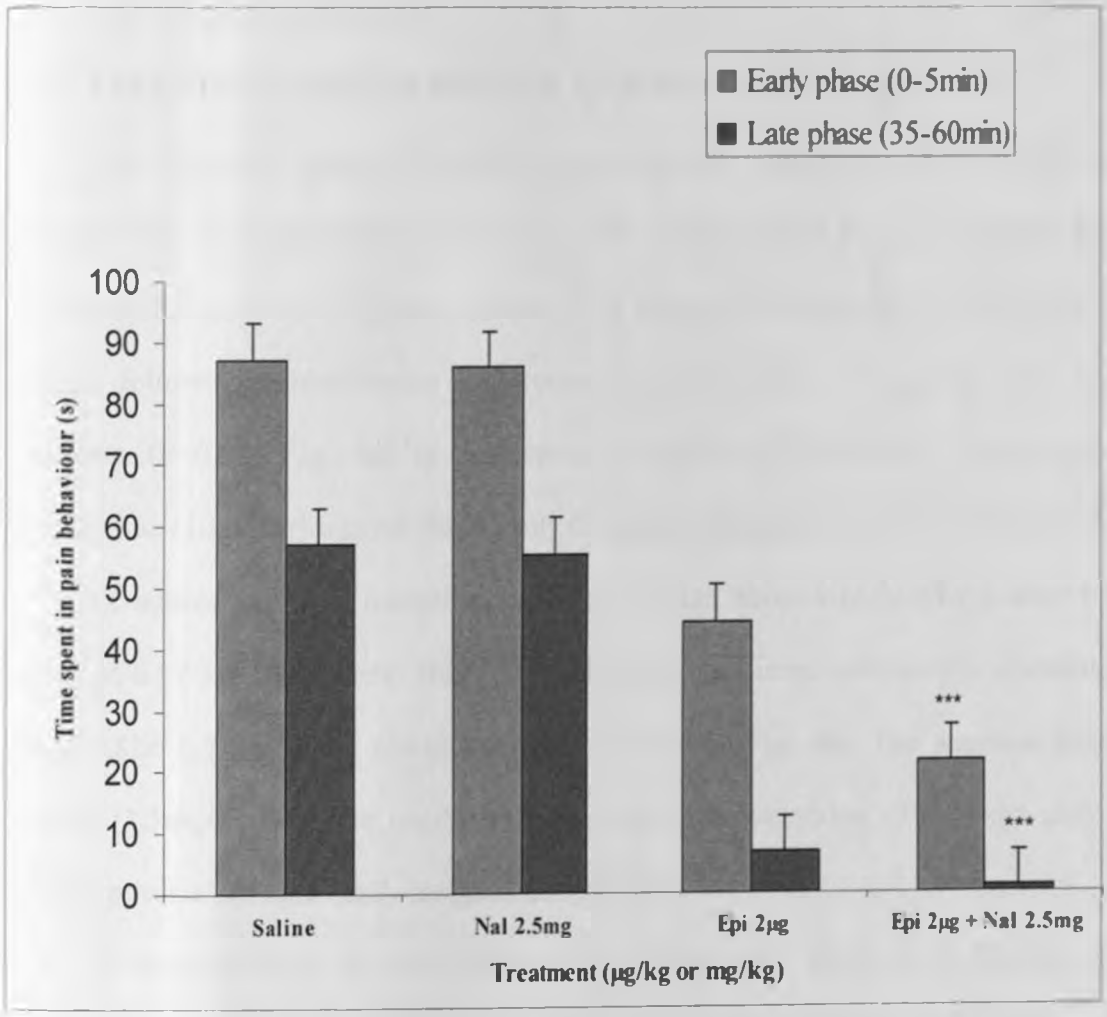

Fig. 3c

Effects of intraperitoneal administration of physiological saline (0.9% NaCl), naloxone (Nal 2.5mg/kg), epibatidine (Epi 2 µg/kg) or a combination of epibatidine (Epi 2µg/kg) plus naloxone (Nal 2.5mg/kg) on the time spent in pain behaviour in the formalin test. Values presented are means ± S.E.M., and n=6 in each group. Treatment means in the early phase and the late phase were compared separately using Dunnett's post-hoc test, subsequent to ANOVA. ***denotes $P<0.001$ for epibatidine (Epi 2µg/kg) versus epibatidine plus naloxone (Epi 2µg/kg + Nal 2.5mg/kg) groups.

4:2 THE HOT-PLATE TEST

4:2.1 EFFECTS OF OXOTREMORINE, ATROPINE AND NALOXONE

The response latency following intraperitoneal administration of 0.9% saline or administration of oxotremorine (10, 20, 50 or 100 μg/kg) were 5.29 ± 0.10 seconds and 5.70 ± 0.17, 9.81± 0.72, 14.38 ± 1.05 and 18.88 ± 0.78 seconds, respectively. The hot plate response latencies following administration of oxotremorine (20, 50 or 100 μg/kg) were statistically significant ($P<0.05$, **Fig. 4a**) in comparison to that for the control group. Injection of oxotremorine (10 μg/kg) was not statistically different when compared to that for controls.

Co-administration of oxotremorine (20 μg/kg) and atropine (2.5mg/kg) caused a response latency of 6.02 ± 0.20 seconds. This was statistically significant compared to oxotremorine (20 μg/kg: 9.81± 0.71 seconds) administered alone ($P<0.05$, **Fig. 4b**). The response latencies for atropine (2.5mg/kg) and the combined treatment of oxotremorine (20 μg/kg) plus atropine (2.5mg/kg) were not statistically insignificant ($P>0.05$).

Co-administration of oxotremorine (20 μg/kg) plus naloxone (2.5mg/kg) caused a response latency of 11.99 ± 0.75 seconds. This was statistically different when compared to that for oxotremorine (20 μg/kg: 9.81± 0.71 seconds) treatment alone ($P<0.05$, **Fig. 4c**). The means for naloxone (2.5mg/kg) and oxotremorine (20 μg/kg) were statistically significant ($P<0.05$). The mean response latencies for the combined treatment (oxotremorine, 20 μg/kg plus naloxone 2.5mg/kg), naloxone (2.5mg/kg) and saline-treated groups were statistically significant ($P<0.05$).

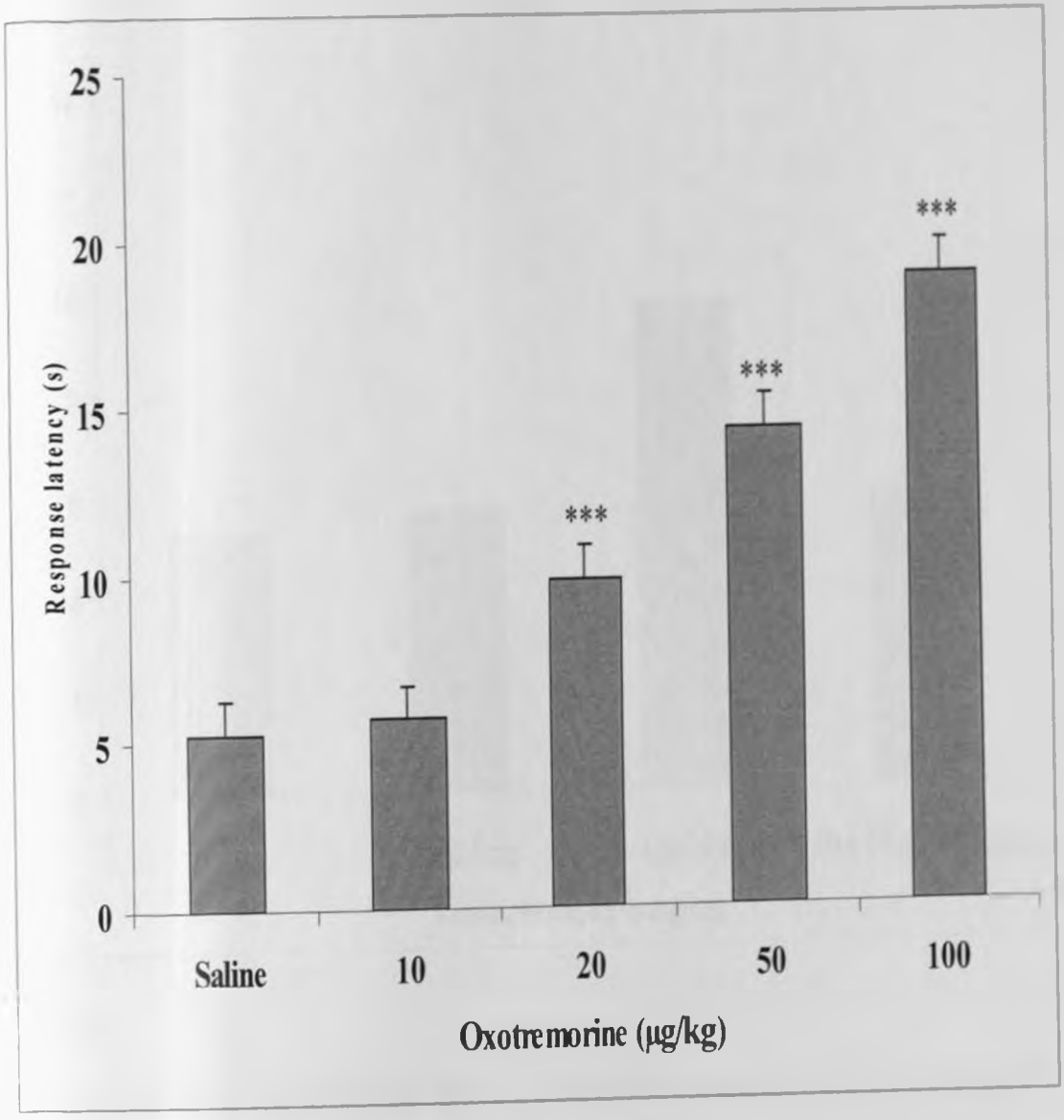

Fig. 4a

Effects of intraperitoneal administration of physiological saline (0.9% NaCl; controls) or oxotremorine (10, 20, 50 or 100 µg/kg) in the mean response latency in the hot plate test. Values are presented as means ± S.E.M., and n=6 in each group. Treatment means were compared using Dunnett's pos-hoc test, subsequent to ANOVA. *** denotes $P<0.001$ (oxotremorine versus control groups).

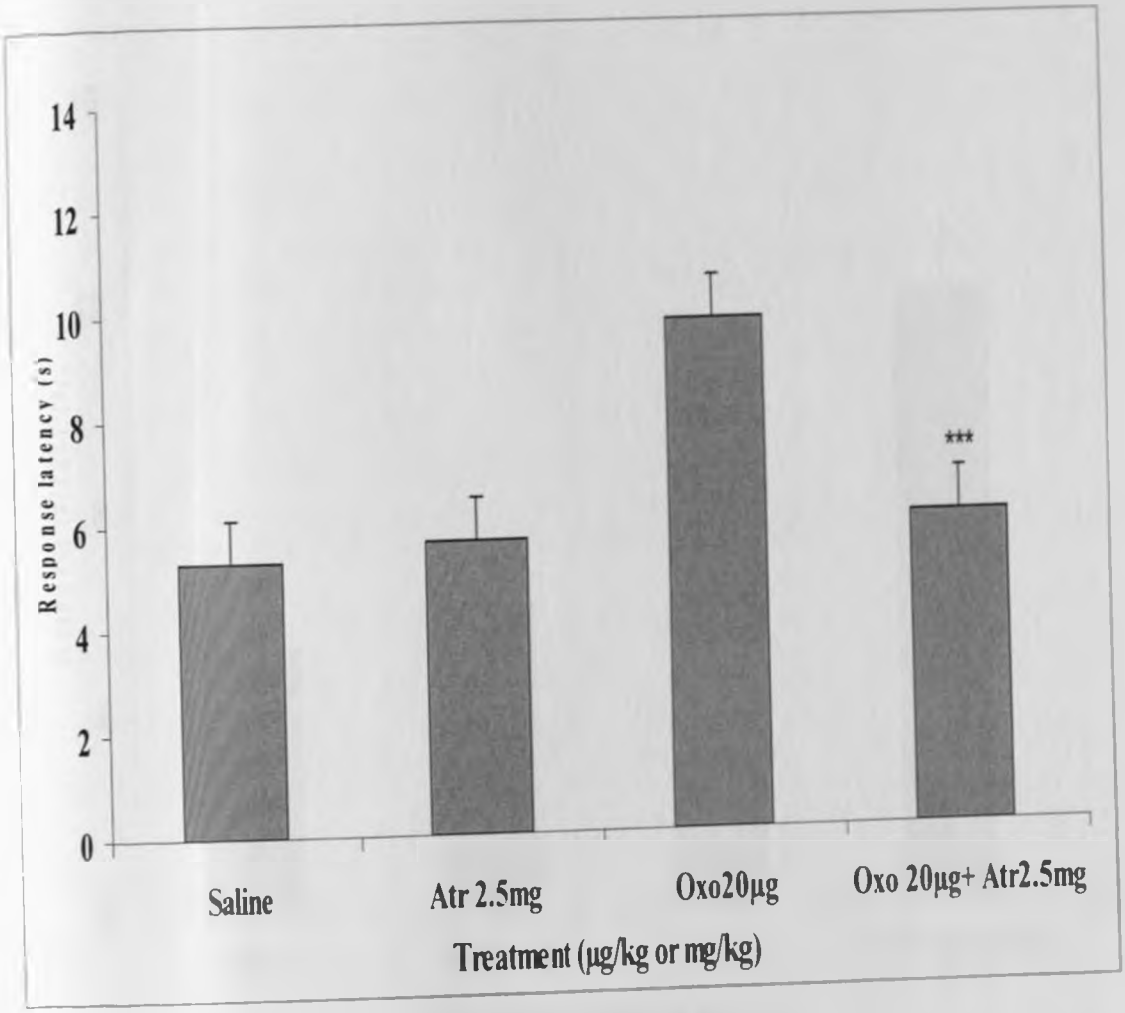

Fig. 4b

Effects of intraperitoneal administration of physiological saline (0.9% NaCl), atropine (Atr 2.5mg/kg), oxotremorine (Oxo 20µg/kg) or combination of oxotremorine (Oxo 20µg/kg) plus atropine (Atr 2.5mg/kg) in the mean response latency in the hot plate test. Values are presented as means ± S.E.M., and n=6 in each group. Treatment means were compared using Dunnett's post-hoc test, subsequent to ANOVA. *** denotes $P<0.001$ for oxotremorine (Oxo 20µg/kg) alone versus oxotremorine plus atropine (Oxo 20µg/kg + Atr 2.5mg/kg) groups.

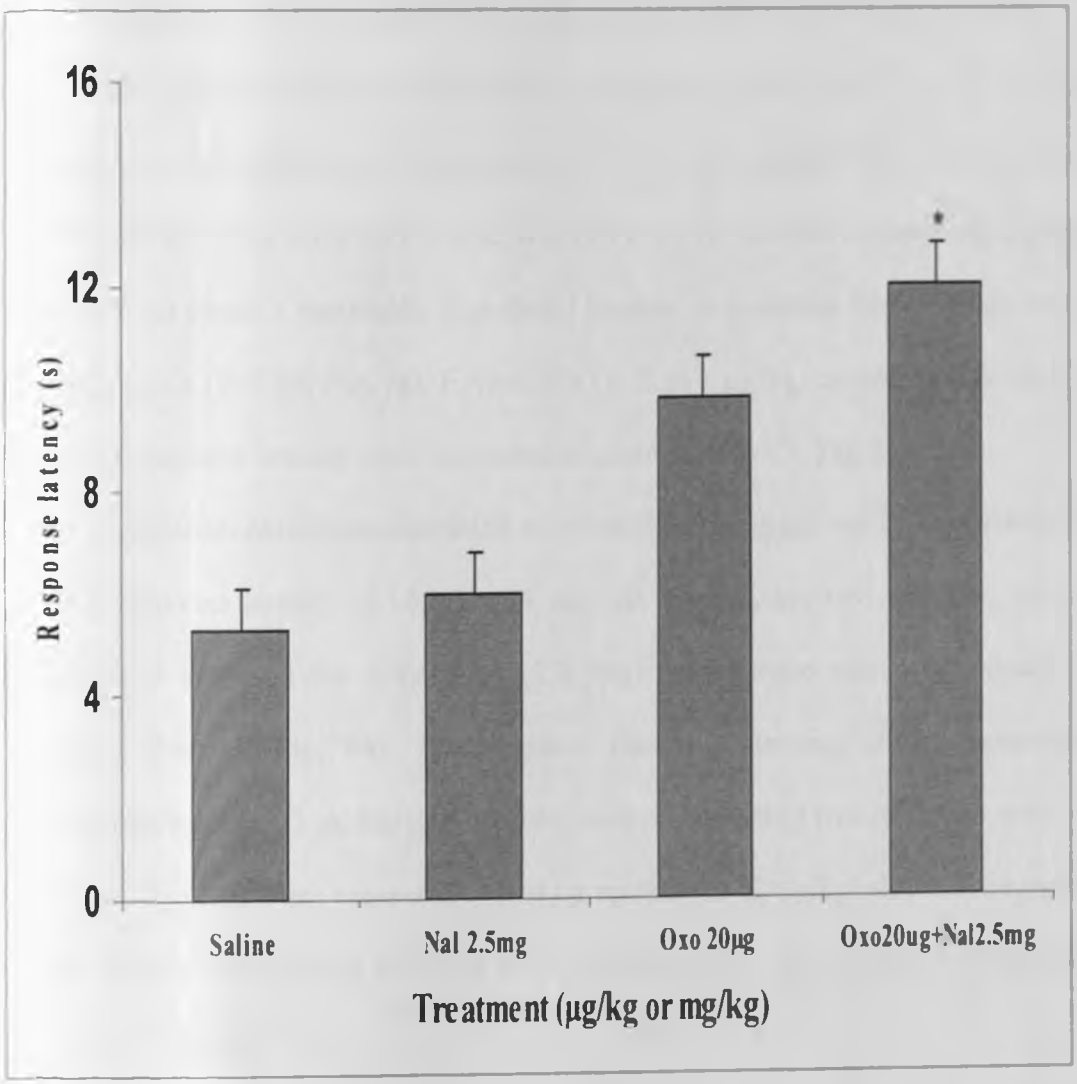

Fig. 4c

Effects of intraperitoneal administration of physiological saline (0.9% NaCl), naloxone (Nal 2.5mg/kg), oxotremorine (Oxo 20µg/kg), or a combination of oxotremorine (Oxo 20µg/kg) plus naloxone (Nal 2.5mg/kg) in the mean response latency in the hot plate test. Values are presented as means ± S.E.M., and n=6 in each group. Treatment means were compared using Dunnett's post-hoc test, subsequent to ANOVA. *denotes $P<0.05$ for oxotremorine (Oxo 20µg/kg) alone versus oxotremorine plus naloxone (Oxo 20 µg/kg + Nal 2.5mg/kg) groups.

4:2.2 EFFECTS OF EPIBATIDINE, MECAMYLAMINE AND NALOXONE

The response latency for control experiments was 5.43 ± 0.19 seconds, while intraperitoneal administration of epibatidine (0.5, 1, 2 or 3 μg/kg) resulted in response latencies of 5.96 ± 0.09, 7.73 ± 0.23, 9.12 ± 0.75 and 14.99 ± 0.72 seconds respectively. Epibatidine (0.5 μg/kg) did not cause a statistically significant increase in response latency when compared with that for controls ($P>0.05$, **Fig. 5a**). Epibatidine (1, 2, or 3 μg/kg) caused a statistically significant increase in response latency when compared to controls ($P<0.05$; **Fig. 5a**).

Intraperitoneal co-administration of epibatidine (2 μg/kg) and mecamylamine (50 μg/kg) caused a response latency of 5.65 ± 0.14 seconds. When compared with the response latency (9.12 ± 0.75 seconds) for epibatidine (2 μg/kg) alone, there was a statistically significant difference ($P<0.05$; **Fig. 5b**). The response latency following saline, mecamylamine and combined epibatidine (2 μg/kg) plus mecamylamine (50 μg/kg) treated groups were statistically significant ($P<0.05$) when compared to that for epibatidine (2 μg/kg) alone. The response latency for the saline-treated group and that for the group given the combined treatment were not statistically different ($P>0.05$).

Co-administration of epibatidine (2 μg/kg) and naloxone (2.5mg/kg) caused a response latency of 11.34 ± 0.67 seconds. The response latency of the combined treatment (11.34 ± 0.67 seconds) was statistically significant when compared with that for epibatidine (2 μg/kg: 9.12 ± 0.75 seconds) alone ($P<0.05$, **Fig. 5c**).

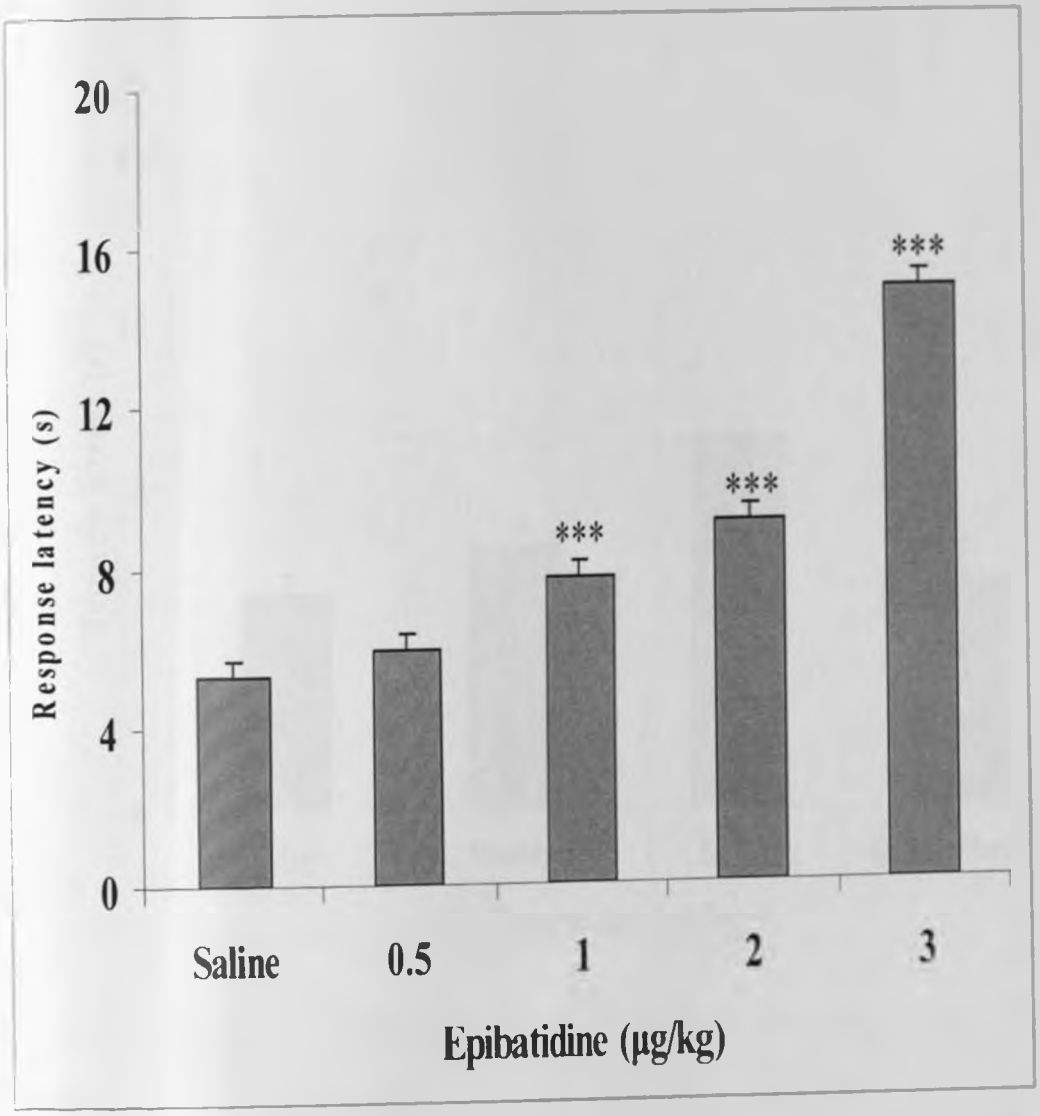

Fig. 5a

Effects of intraperitoneal administration of physiological saline (0.9% NaCl; controls) or epibatidine (0.5, 1, 2 or 3 µg/kg) in the mean response latency in the hot plate test. Values are presented as means ± S.E.M., and n=6. Treatment means were compared using Dunnett's post-hoc test, subsequent to ANOVA. *** denotes $P<0.001$ (epibatidine versus the control groups).

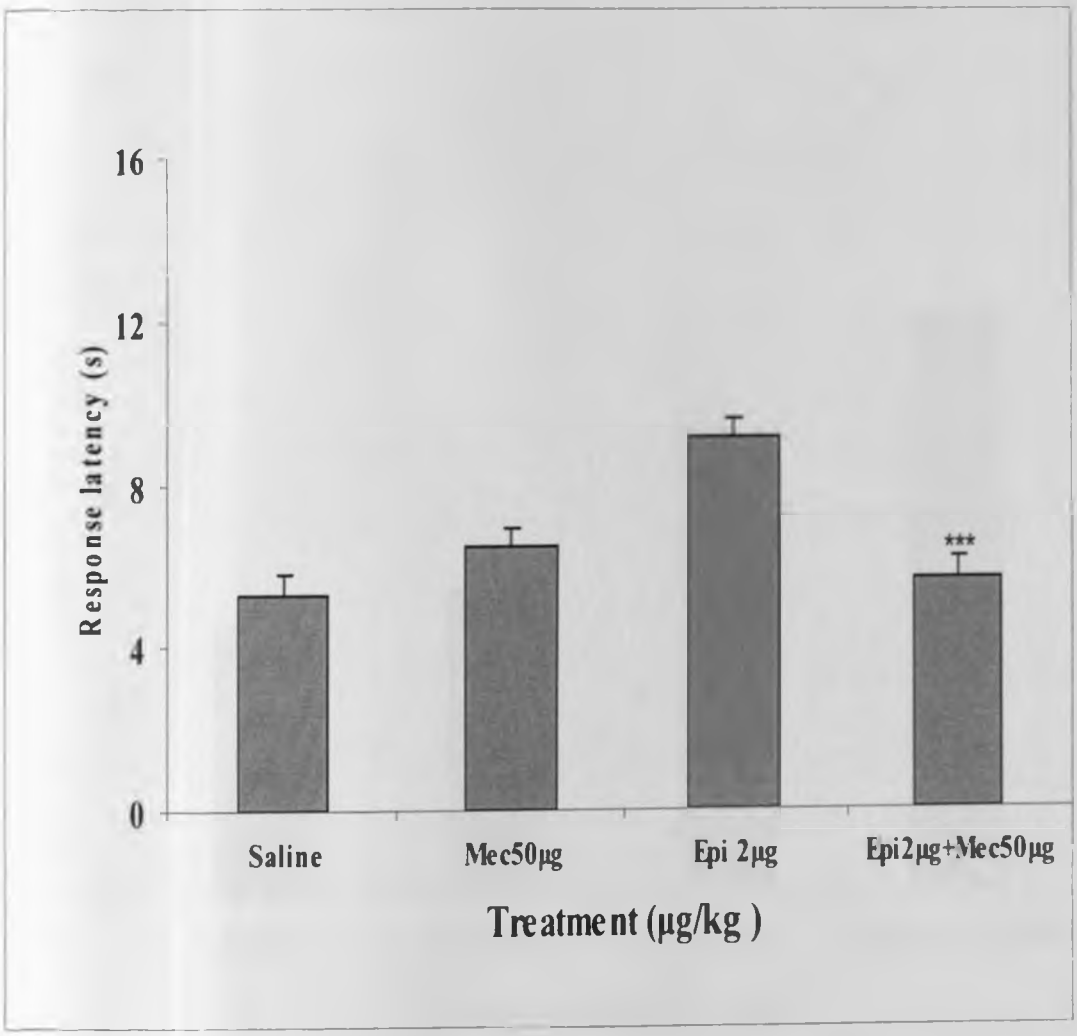

Fig. 5b

Effects of intraperitoneal administration of physiological saline (0.9% NaCl), mecamylamine (Mec 50µg/kg), epibatidine (Epi 2µg/kg) or a combination of epibatidine (Epi 2µg/kg) plus mecamylamine (Mec 50µg/kg) in the mean response latency in the hot plate test. Values are presented as means ± S.E.M., and n=6. Treatment means were compared using Dunnett's post-hoc test, subsequent to ANOVA. ***denotes $P<0.001$ for epibatidine (Epi 2µg/kg) versus epibatidine plus mecamylamine (Epi 2µg/kg + Mec 50µg/kg) groups.

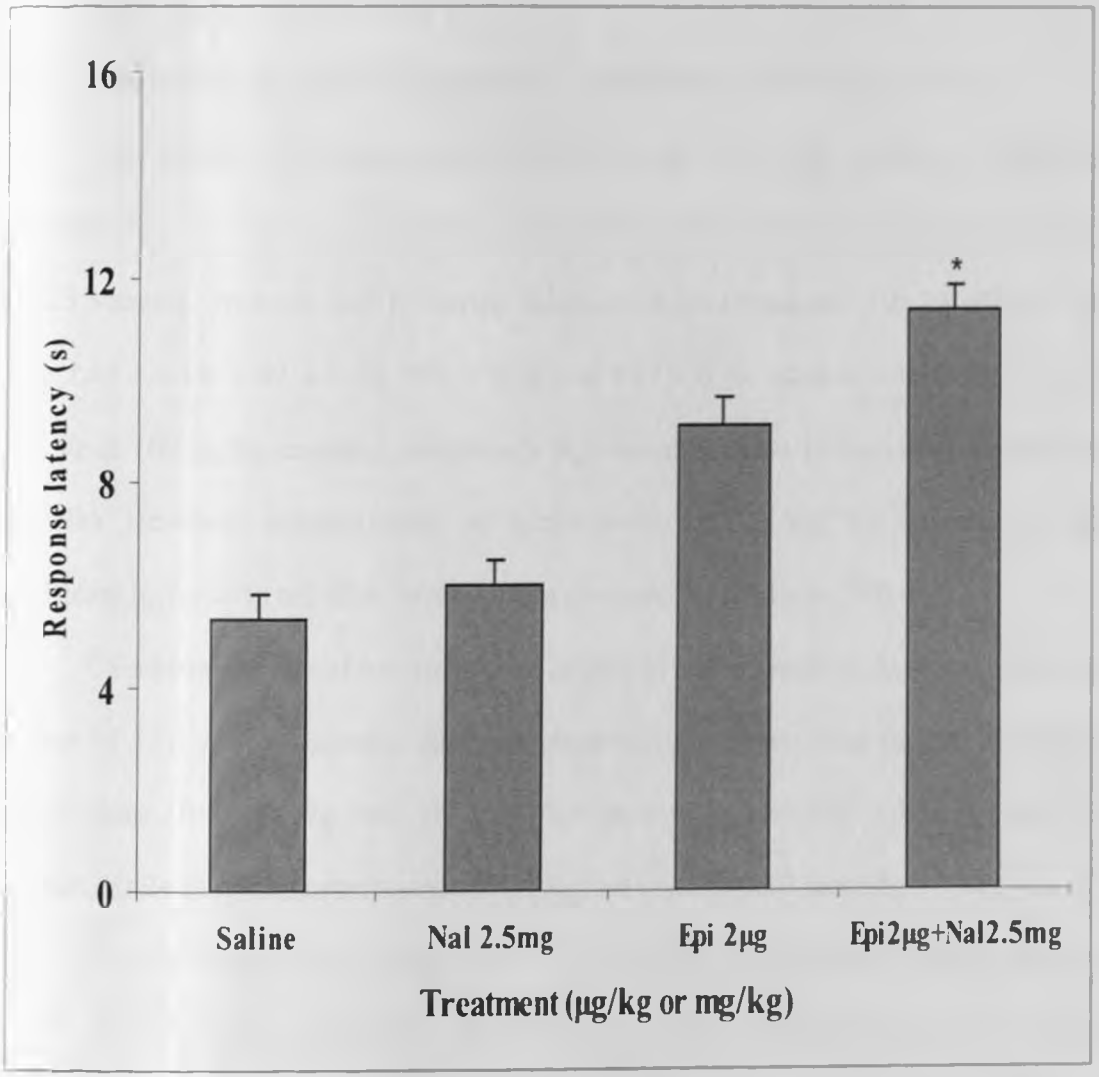

Fig. 5c

Effects of intraperitoneal administration of physiological saline (0.9% NaCl), naloxone (Nal 2.5mg/kg), epibatidine (Epi 2µg/kg) or a combination of epibatidine (Epi 2µg/kg) plus naloxone (Nal 2.5mg/kg) in the mean response latency in the hot plate test. Values are presented as means ± S.E.M., and n=6. Treatment means were compared using Dunnett's post-hoc test, subsequent to ANOVA. *denotes $P<0.05$ for epibatidine (Epi 2µg/kg) versus epibatidine plus naloxone (Epi 2µg/kg + Nal 2.5 mg/kg) groups.

4:3 THE TAIL-FLICK TEST

4:3.1 EFFECTS OF OXOTREMORINE, ATROPINE AND NALOXONE

The effects of intraperitoneal administration of 0.9% saline or administration of oxotremorine (10, 20, 50 or 100 μg/kg) were studied. The tail-flick latency for controls was 5.48 ± 0.23 seconds, whereas that following injection of oxotremorine (10, 20, 50 and 100 μg/kg) were 5.54 ± 0.15, 6.62 ± 0.30, 8.03 ± 0.12 and 8.82 ± 0.15 seconds respectively. Oxotremorine (20, 50 or 100 μg/kg caused a statistically significant increase in the tail-flick latency ($P<0.05$, **Fig. 6a**). However, administration of oxotremorine (10 μg/kg) did not cause a statistically significant increase in tail-flick latency when compared to controls ($P>0.05$).

Co-administration of oxotremorine (20 μg/kg) and atropine (2.5mg/kg) caused a tail-flick latency of 5.66 ± 0.31 seconds. This was statistically different from that for oxotremorine (20 μg/kg) alone ($P<0.05$, **Fig. 6b**). The tail-flick latency for atropine (2.5mg/kg) was 6.17± 0.11 seconds, while that for oxotremorine (20 μg/kg) was 6.62 ± 0.30 seconds.

Co-administration of oxotremorine (20 μg/kg) and naloxone (2.5mg/kg) caused tail-flick latency of 8.89 ± 0.14 seconds. The tail-flick latency for the combined treatment was statistically significant ($P<0.05$, **Fig. 6c**) compared to that for the oxotremorine (20 μg/kg) alone. The tail-flick latency following injection of naloxone (2.5mg/kg) and oxotremorine (20 μg/kg) were statistically significant ($P<0.05$) compared to that for the combined treatment.

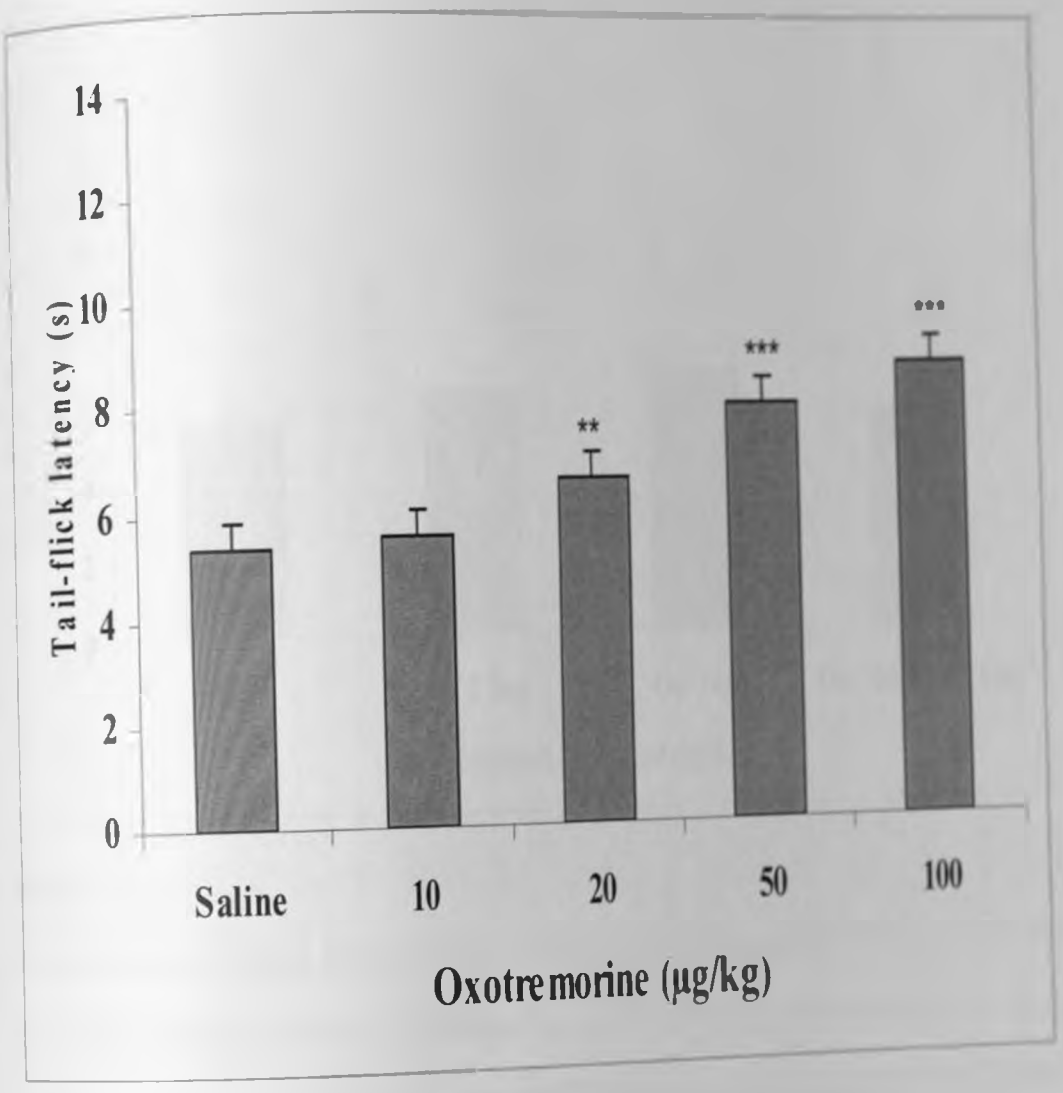

Fig. 6a

Effects of intraperitoneal administration of physiological saline (0.9% NaCl; controls) or oxotremorine (10, 20, 50 or 100 µg/kg) in the mean tail-flick latency. Values are presented as means ± S.E.M., and n=6. Treatment means were compared using Dunnett's pos-hoc test, subsequent to ANOVA. ** and *** denotes, $P<0.01$ and $P<0.001$ respectively (oxotremorine versus the control groups).

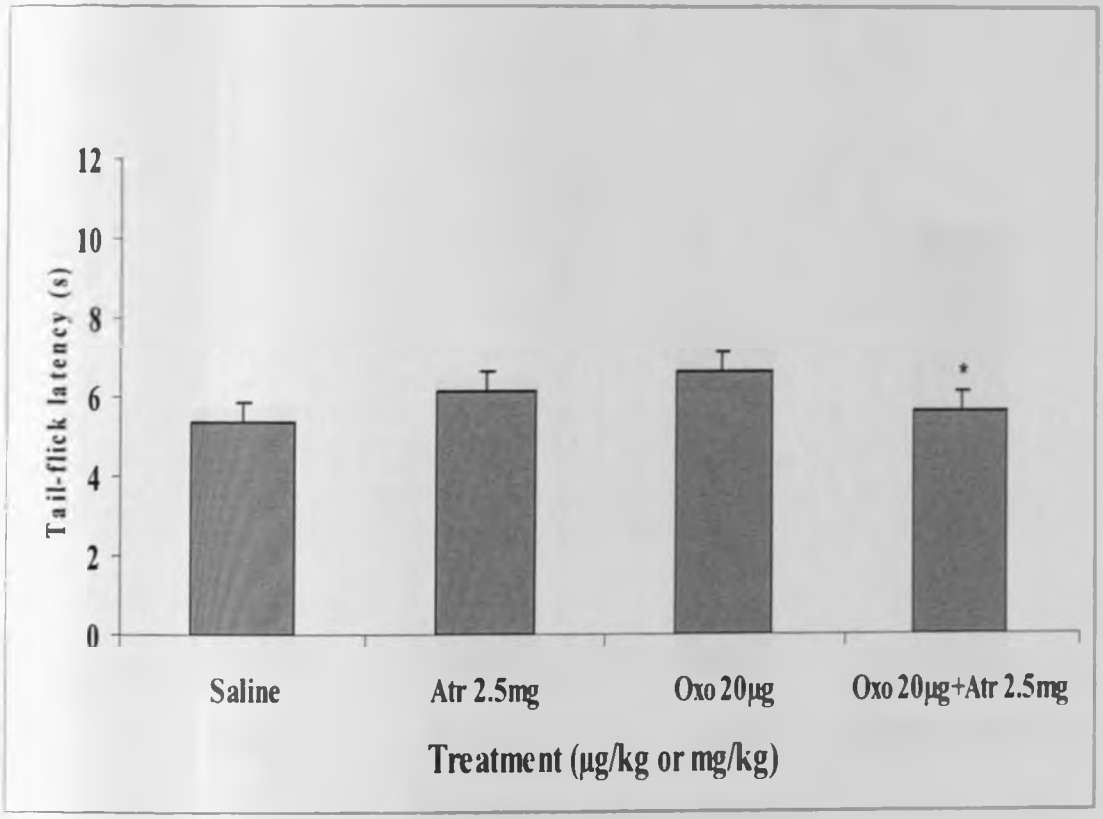

Fig. 6b

Effects of intraperitoneal administration of physiological saline (0.9% NaCl), atropine (Atr 2.5mg/kg), oxotremorine (Oxo 20µg/kg) or co-administration of oxotremorine (Oxo 20µg/kg) plus atropine (Atr 2.5mg/kg) in the mean tail-flick latency. Values are presented as means ± S.E.M., and n=6. Treatment means were compared using Dunnett's post-hoc test, subsequent to ANOVA. *denotes $P<0.05$ for oxotremorine (Oxo 20µg/kg) versus oxotremorine plus atropine, (Oxo 20µg/kg + Atr 2.5mg/kg) groups.

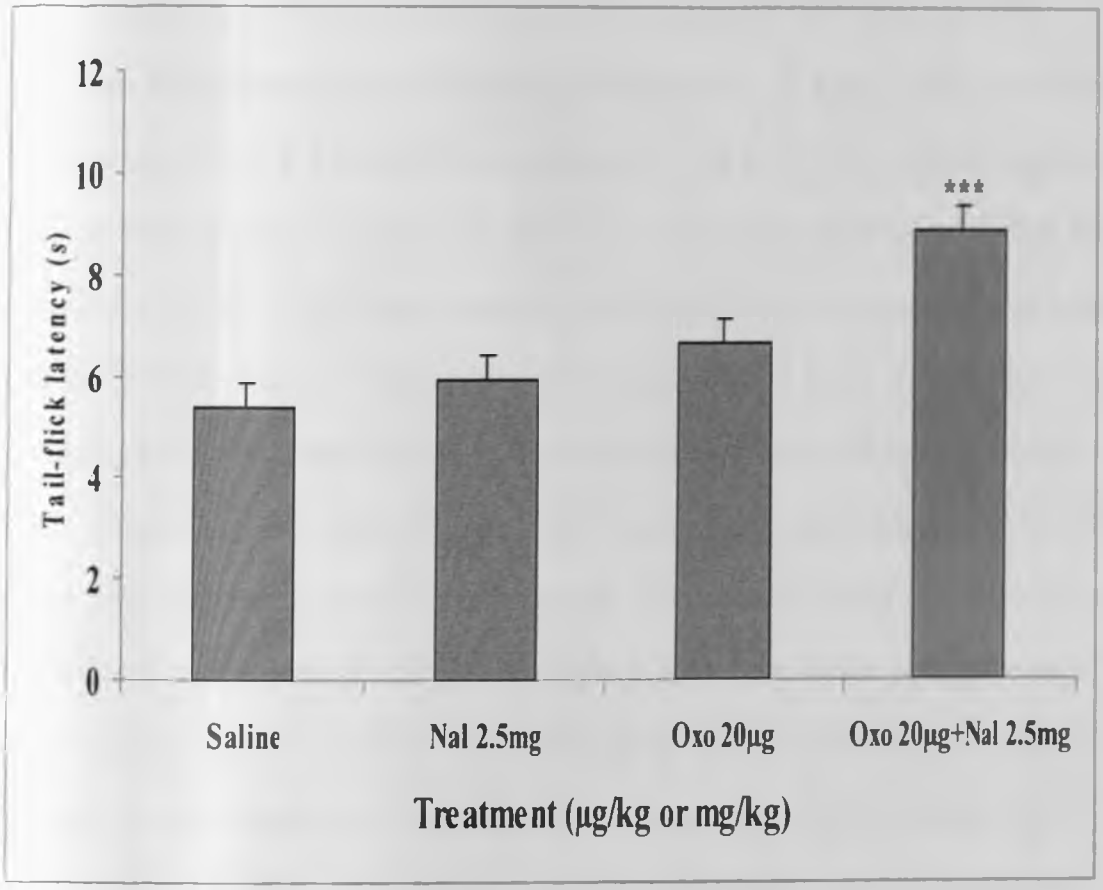

Fig. 6c

Effects of intraperitoneal administration of physiological saline (0.9% NaCl), naloxone (Nal 2.5mg/kg), oxotremorine (Oxo 20µg/kg) or co-administration of oxotremorine (Oxo 20µg/kg) plus naloxone (Nal 2.5mg/kg) in the mean tail-flick latency. Values are presented as means ± S.E.M., and n=6. Treatment means were compared using Dunnett's post-hoc test, subsequent to ANOVA. ***denotes $P<0.001$ for oxotremorine (Oxo 20µg/kg) versus oxotremorine plus naloxone (Oxo 20µg/kg + Nal 2.5mg/kg) groups.

4:3.2 EFFECTS OF EPIBATIDINE, MECAMYLAMINE AND NALOXONE

The intraperitoneal administration of epibatidine (0.5, 1, 2 or 3 μg/kg) caused mean tail-flick latencies of 5.57 ± 0.41, 6.68 ± 0.49, 7.58 ± 0.35 and 8.43 ± 0.11 seconds respectively. The mean tail-flick latency for the controls was 5.23± 0.37 seconds. The mean tail-flick latency for epibatidine (1, 2, or 3 μg/kg) was statistically significant when each was compared with that for controls ($P<0.05$; **Fig. 7a**). Epibatidine (0.5 μg/kg) did not cause a statistically significant increase in the tail-flick latency ($P>0.05$, **Fig. 7a**) when compared with that for controls.

Co-administration of epibatidine (Epi 2 μg/kg) and mecamylamine (Mec 50 μg/kg) caused a tail-flick latency of 5.77 ± 0.34 seconds. This was statistically significant from that for epibatidine (2 μg/kg) alone ($P<0.05$; **Fig. 7b**). The tail-flick latency for mecamylamine (Mec 50 μg/kg) was 6.39 ± 0.34 seconds, while that for epibatidine (Epi 2 μg/kg) was 7.58 ± 0.35 seconds. Co-administration of epibatidine (Epi 2 μg/kg) plus mecamylamine (Mec 50 μg/kg) caused a tail-flick latency of 5.77 ± 0.34 seconds) that was not statistically significant ($P>0.05$) when compared to that for the controls.

Co-administration of epibatidine (Epi 2 μg/kg) and naloxone (Nal 2.5mg/kg) caused a tail-flick latency of 8.43 ± 0.11 seconds. When the mean tail-flick latency for the combined treatment (8.43 ± 0.11seconds) was compared with that for epibatidine (Epi 2 μg/kg: 7.58 ± 0.35 seconds) alone, a statistically significant difference was recorded ($P<0.05$, **Fig. 7c**).

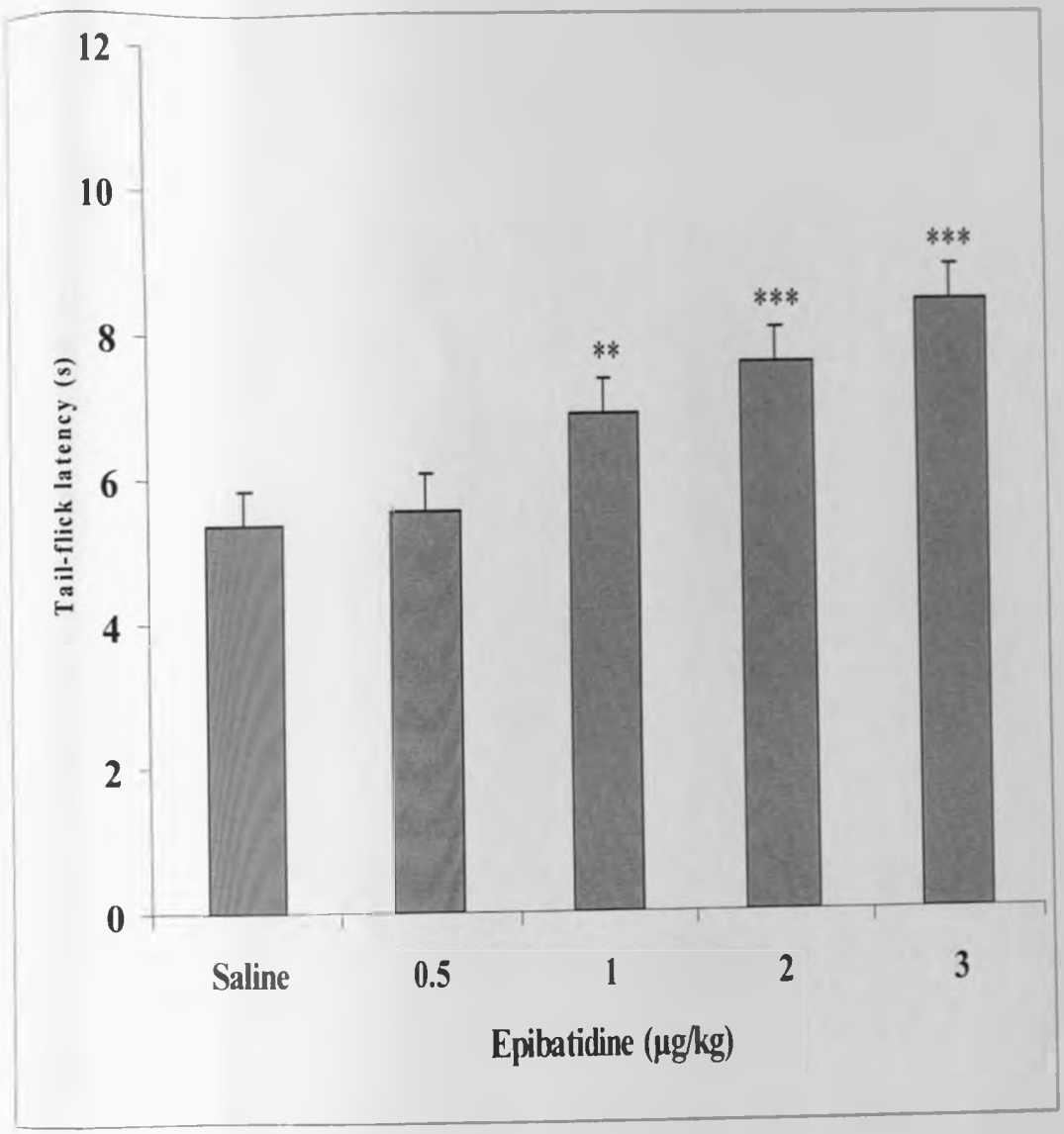

Fig. 7a

Effects of intraperitoneal administration of physiological saline (0.9% NaCl; controls) or epibatidine (0.5, 1, 2 or 3 µg/kg) in the tail-flick test. Values are presented as means ± S.E.M., and n=6. Treatment means were compared separately using Dunnett's pos-hoc test, subsequent to ANOVA. ** and *** denotes $P<0.01$ and $P<0.001$ respectively (epibatidine versus the control groups).

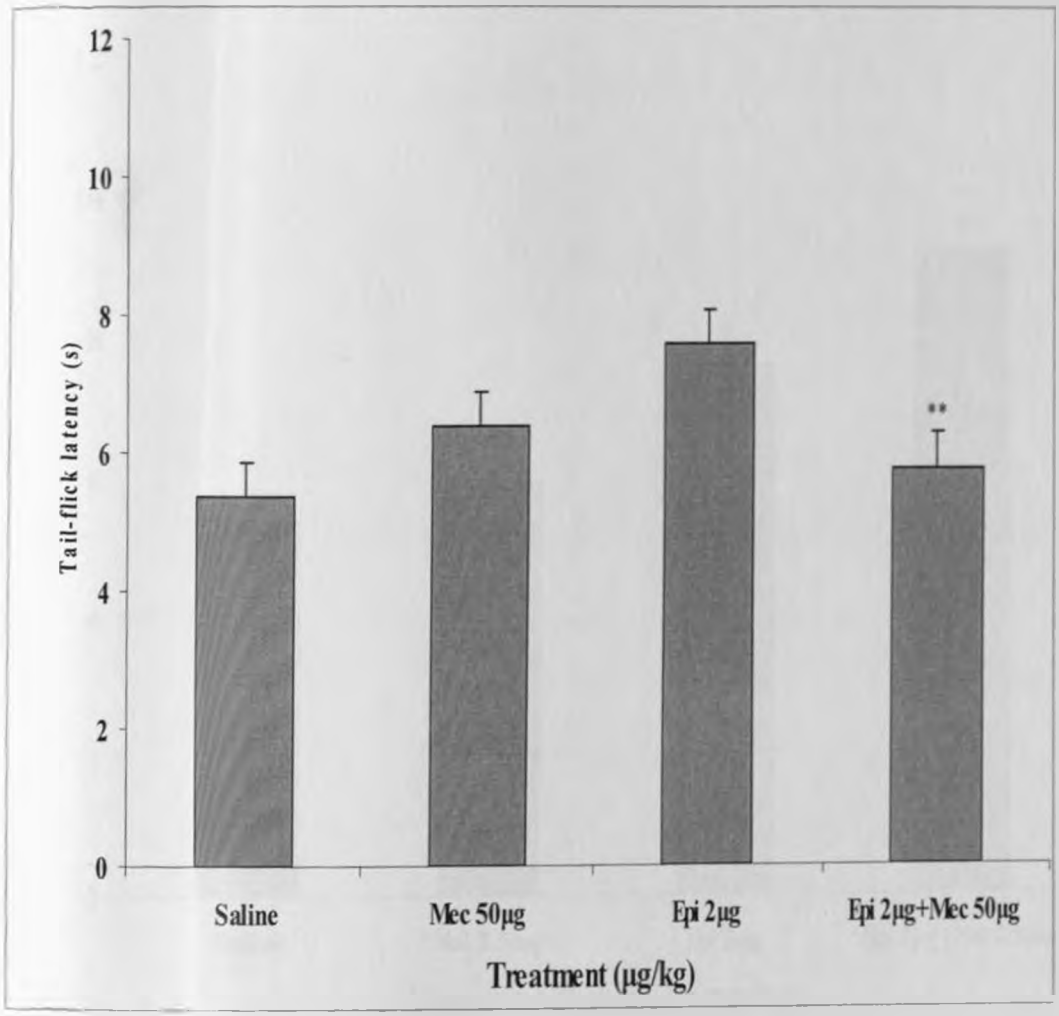

Fig. 7b

Effects of intraperitoneal administration of physiological saline (0.9% NaCl), mecamylamine (Mec 50µg/kg), epibatidine (Epi 2µg/kg), or co-administration of epibatidine (Epi 2µg/kg) plus mecamylamine (Mec 50µg/kg) in the mean tail-flick latency. Values are presented as means ± S.E.M., and n=6. Treatment means were compared using Dunnett's post-hoc test, subsequent to ANOVA. **denotes $P<0.01$ for epibatidine (Epi 2µg/kg) versus epibatidine plus mecamylamine (Epi2µg/kg + Mec 50µg/kg) groups.

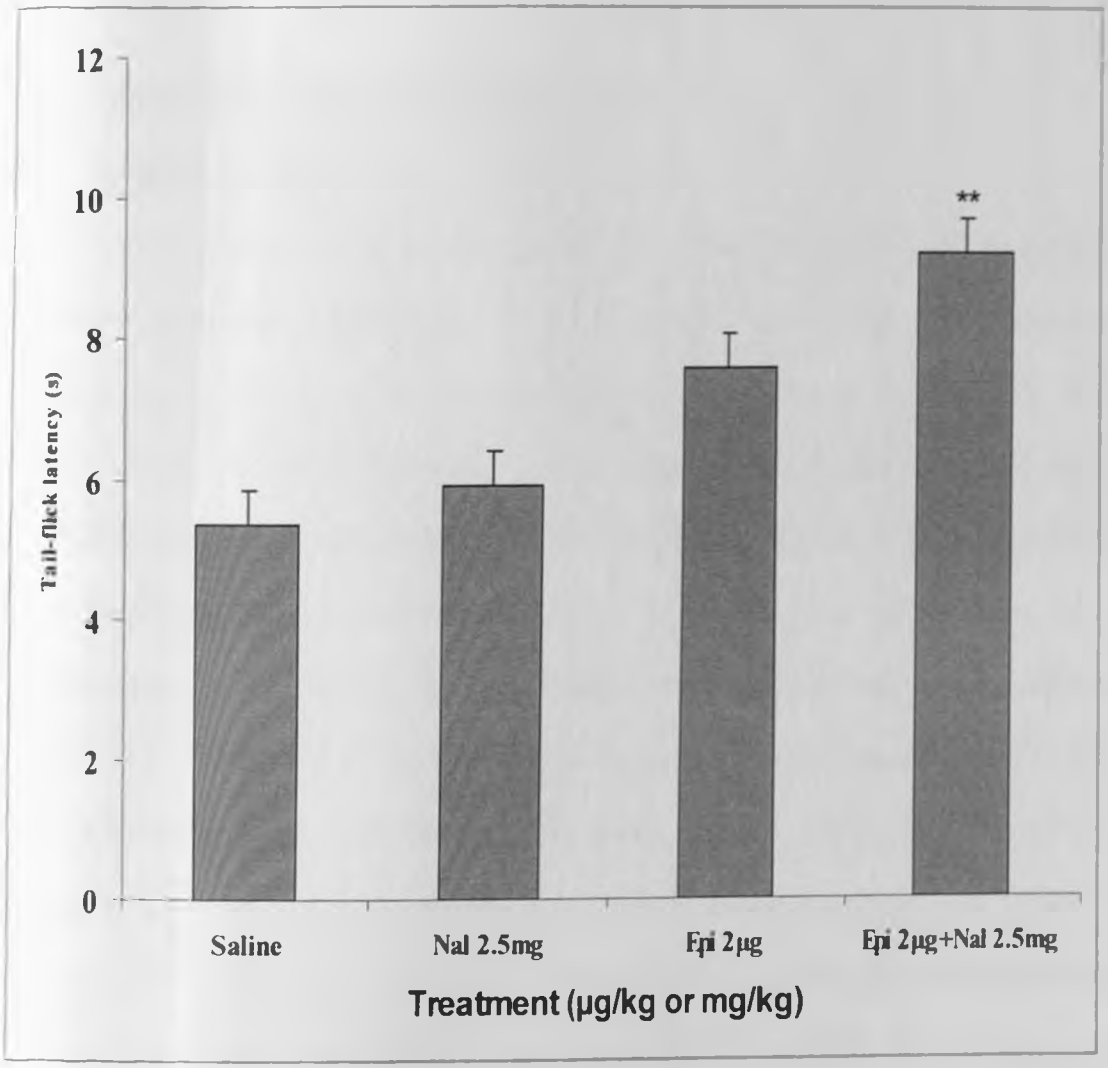

Fig. 7c

Effects of intraperitoneal administration of physiological saline (0.9% NaCl), naloxone (Nal 2.5mg/kg), epibatidine (Epi 2µg/kg) or co-administration of epibatidine (Epi 2µg/kg) plus naloxone (Nal 2.5mg/kg) on the mean tail-flick latency. Values are presented as means ± S.E.M., and n=6. Treatment means were compared using Dunnett's post-hoc test, subsequent to ANOVA. **denotes $P<0.01$ for epibatidine (Epi 2µg/kg) versus epibatidine plus naloxone (Epi2µg/kg + Nal 2.5 mg/kg) groups.

CHAPTER 5

5:0 DISCUSSION AND CONCLUSIONS

5:1 FORMALIN TEST

The study showed that the formalin test is a suitable nociceptive test for evaluating animal behavioural responses to persistent pain, and is useful for examining antinociceptive effects for various analgesic drugs such as the cholinergic ones. Contrary to the phasic and short-lasting noxious stimuli, formalin induced pain is prolonged and therefore resembles many painful conditions encountered clinically, such as post operative pain (Porro and Cavazzuti, 1993).

The pain-like behaviour monitored in the formalin test was licking/biting of the injected right hind paw. The formalin induced two distinct periods of pain behaviour, an early phase (0-5 minutes) and a late phase (35-60 minutes) in the naked mole-rat. This is similar to the findings reported in other animal species, such as rats (Dubuisson and Dennis, 1977; Curzon *et al.*, 1998; Capone *et al.*, 1999), primates (Alreja *et al.*, 1984) and mice (Hunskaar *et al.*, 1985; Hunskaar and Hole, 1987). The formalin-induced behaviour in the naked mole-rat is also similar to published reports on the same rodent (Kanui *et al.*, 1993; Karim *et al.*, 1993; Towett *et al.*, 2009).

The early phase response is due to the stimulation of peripheral nociceptors while the late phase may be due to varied inflammatory processes (Dubuisson and Dennis, 1977; Hunskaar *et al.*, 1985a; Hunskaar and Hole, 1987; Shibata *et al.*, 1989). The intradermal injection of 10% formalin at the dorsal right hind-paw inflicts a moderate continuous pain generated by mildly injured tissue (Dubuisson and Dennis, 1977). The injury to the tissue stimulates C-fibres that induce a series of neuronal modifications that increase the excitability of motorneurons and lead to an increase of the flexor reflex. The main chemical mediators implicated in the irritation of

free nerve endings during the early phase response are bradykinins (Dray, 1995). Peripheral injection of formalin also activates descending inhibitory pathways that can significantly reduce this facilitation (Gozariu *et al.*, 1997). The nociceptive input following the formalin induced pain is transduced by beta-endorphin as the main neurotransmitter. This is present at the arcuate projection to brain areas involved in pain modulation (Capone *et al.*, 1999). It has been demonstrated previously that the septo-hippocampal cholinergic activity, inhibited by beta-endorphin is depressed by formalin in other species, such as rats (Aloisi *et al.*, 1995).

The late phase in the naked mole-rat started 35 minutes following intradermal administration of formalin whereas in the other species it occurs at 15-20 minutes (Capone *et al.*, 1999), suggesting a slow inflammatory reaction. This may be explained by the lack of sensory fibres immunoreactive to substance P and calcitonin gene related peptide (CGRP) in the naked mole rat (Park *et al.*, 2003). These two chemical modulators play a major role in sensitization of chemoreceptors. Neuromodulators such as histamines, prostaglandins, bradykinins, serotonin, protons, nitric oxide, leukotrienes and cytokines are also reported to have a role in the late phase of the formalin test (Shibata *et al.*, 1989; Dray, 1995). The second messengers that signal the biochemical communication within cells after the action of neurotransmitters at the pain receptors and trigger the licking/biting response include substance P, potassium ions and adenosine triphosphate (ATP). The role of various peptides, acetylcholine and the type of receptors involved in the formalin test in the naked mole-rat has not been investigated.

In the present study, oxotremorine administered intraperitoneally, reduced the time spent licking/biting the injected paw in both phases of the test. The antinociceptive effect of oxotremorine was dose dependent. This is similar to what has been reported in Sprague-Dawley

rats (Yaksh *et al.*, 1985; Capone *et al.*, 1999; Machelska *et al.*, 1999; Abelson and Höglund, 2000b; Abelson *et al.*, 2004) and mice (Wang *et al.*, 2004). In these reports the effects of the muscarinic agonist, oxotremorine, were blocked by atropine (Ishii and Kurachi, 2006; Langmead *et al.*, 2008).

The intraperitoneal administration of atropine (2.5 mg/kg) alone did not have any significant effect on the time spent in pain behaviour in naked mole-rats. The findings were contrary to Ghelardini *et al.*, (1990) who observed that atropine in low doses produced analgesia in rats. Conversely, it was opposite to the findings in a later study where atropine produced increased pain sensitivity in rats in both high and low doses (Abelson and Höglund, 2002b). This might suggest that different strains, species and animals belonging to different classes respond differently to the atropine treatment.

Intraperitoneal administration of epibatidine caused a decrease in pain behaviour in both phases of the formalin test in the naked mole-rat. The antinociceptive effect of epibatidine was dose-dependent and mecamylamine-reversible. This is similar to previous reports in mice (Qian *et al.*, 1993) and rats (Curzon *et al.*, 1998; Boyce *et al.*, 2000; Kommalage and Höglund, 2004). However, this was the first study to be conducted in the naked mole-rat, a primitive fossorial rodent. The effect of mecamylamine on epibatidine induced antinociception suggests that the antinociceptive effect of the agonist was mediated by nicotinic acetylcholine receptors. Similar observation has been noted in rats (Badio and Daly, 1994). Lower doses of epibatidine compared to those of oxotremorine were effective in reducing the licking/biting behaviour in the naked mole-rat. This may be associated to the pharmacological binding properties of epibatidine which have to be investigated in the naked mole-rat.

In the current study, the intraperitoneal administration of mecamylamine and naloxone alone did not have any significant effect on the time spent in pain behaviour. Naloxone had been reported to have no effect on formalin induced pain in earlier studies in the naked mole- rat (Towett *et al.*, 2009).

Co-administration of oxotremorine or epibatidine with naloxone exhibited synergistic increase of the antinociceptive effects of the selected direct acting cholinergic agonists. The interaction between the opioid and the cholinergic systems has been well-documented (Dewey and Pedigo, 1981; Lewis *et al.*, 1983; Sperber *et al.*, 1986). There is evidence indicating that opiate agonists inhibit cholinergic activity in several brain regions (Lamour and Epelbaum, 1988) by reducing the release of acetylcholine (Cheney *et al.*, 1975; Wood and Stotland, 1980). Naloxone is a general blocker of opioid receptors in the central nervous system (Cheney *et al.*, 1975; Beani *et al.*, 1982; Walker *et al.*, 1991). Perhaps naloxone caused synergism with exogenously administered cholinergic agonists by blocking opioid receptors the site of action of endogenous opioids associated to nociceptive stimulation, a feature that needs to be investigated in the naked mole-rat. The unclear mechanisms in the naked mole-rat resulted in an enhanced antinociceptive effect of oxotremorine and epibatidine in the three nociceptive tests used in this study.

Furthermore, very low concentrations of cholinergic drugs have been shown to enhance neurogenic inflammation by their action at nociceptors (Mayhan and Sharpe, 1998; Miao et al., 1992; 1997). This enhancement is usually not observable due to tonic suppression by endogenous opioids (Miao *et al.*, 2001) suggesting the importance of opioid system on cholinergic activity.

5:2 HOT-PLATE TEST

The data presented suggest that the hot plate test is good for studying reflex response of the animal. The test involves a phasic stimulus of high intensity and short duration and has been used in mice (Ankier, 1974; Hunskaar *et al.*, 1986a) and rats (Hunskaar *et al.*, 1986a). The test has been successfully used before to evaluate the antinociceptive effects of various drugs. It has been reported earlier that the naked mole-rats exhibited a reliable and specific pain-related behaviour when tested on the hot plate set at 60^0 C (Kanui and Hole, 1990; Towett and Kanui, 1993; Towett *et al.*, 2006).

In the present study, oxotremorine or epibatidine caused an increase in the response latency when they were injected intraperitoneally, suggesting analgesia. This effect is different from the effects of opioids observed by (Towett and Kanui, 1993; Towett *et al.*, 2006), who found the opioid agonists caused increased pain insensitivity in the animal. The effects of oxotremorine (20µg/kg) or epibatidine (2µg/kg) were reversed by atropine (2.5 mg/kg) or mecamylamine (50µg/kg, respectively. This suggests that oxotremorine and epibatidine acted on muscarinic and nicotinic receptors, respectively, to cause analgesia in the naked mole-rat. As expected, atropine on its own had no significant effect on the response latency in the naked mole rat. It was observed that naloxone (2.5 mg/kg) alone had no effect on response latency but caused an increase in the response latency when combined with either oxotremorine or epibatidine. The combined treatment of naloxone with either oxotremorine or epibatidine exhibited synergism of the antinociceptive effect of oxotremorine or epibatidine. The possible mechanism for the synergistic effect of naloxone is as explained for the formalin test.

5:3 TAIL-FLICK TEST

The study showed that the tail-flick test is easy to replicate and generates reliable data to quickly evaluate antinociceptive for direct acting cholinergic drugs in the naked mole-rat. Tail-flick pain is primarily a spinally integrated reflex. Naked mole-rats showed clear quantifiable tail withdrawal response when tested on a radiant beam set at 56°C, thus affirming that the tail-flick test can be adapted for use in the naked mole-rat. In the current study, oxotremorine at the selected doses (20, 50 or 100 μg/kg significantly increased the mean tail-flick latency. This means that the naked mole-rats became less sensitive to noxious stimulation after the administration of oxotremorine. The findings are similar to earlier reports on other rodents, such as mice (Yael *et al.*, 2000), rats (Hai-chun *et al.*, 2001; Illes *et al.*, 2006). The analgesia caused by oxotremorine, a muscarinic agonist, suggests that the cholinergic system play a role in pain regulation in the naked mole-rat. This was the first time antinociception was assessed in the naked mole-rat using the tail-flick test.

Administration of atropine (2.5 mg/kg) reversed the effect of oxotremorine in the tail-flick test. The finding is similar to previous studies in rats (Vilaro *et al.*, 1992; Eisenach, 1999) and suggests that antinociceptive actions of oxotremorine are mediated by muscarinic acetylcholine receptors in the naked mole-rat. Contrary to the possible involvement of non- M_1 in the naked mole-rat it has been demonstrated that M_1 receptor subtype is involved in the muscarinic antinociception, in rats (Bartolini *et al.*, 1992). Naloxone, in combination with oxotremorine increased the tail-flick latency, suggesting a synergistic effect. The possible mechanism for the synergistic effect of naloxone is as explained for the formalin test.

In the current study, epibatidine at the selected doses (1, 2 or 3 μg/kg) significantly increased the mean tail-flick latency, suggesting antinociceptive effect. The antinociceptive effect of epibatidine was dose-dependent. Co-administration of mecamylamine (50 μg/kg) together with epibatidine (2 μg/kg) reversed the effect of epibatidine, on the tail-flick latency. In earlier studies, epibatidine antinociception was demonstrated in rats using the tail-flick (Rao *et al.*, 1996; Caban *et al.*, 2004). Treatment with mecamylamine or naloxone alone had no significant effect on the mean response latency. It was observed that naloxone, in combination with epibatidine increased the tail-flick latency, suggesting a synergistic effect. The possible mechanism for the synergistic effect of naloxone is as explained for the formalin test.

5:4 CONCLUSIONS

This study has demonstrated that oxotremorine and epibatidine have a potent antinociceptive effect in the naked mole-rat and that the effect is reversible by atropine and mecamylamine respectively. It is imperative from the findings that the selected direct acting cholinergic drugs may be used for therapeutic purposes at dose levels oxotremorine (20 μg/kg body weight) and epibatidine (2 μg/kg body weight).

The interaction between the cholinergic and opioidergic system in pain control is exemplified by the observed synergistic antinociceptive effect when oxotremorine or epibatidine were combined with naloxone in the naked mole-rat.

REFERENCES:

Abbott, F.V. (1988): Peripheral and central antinociceptive actions of ethylketocylazocine in the formalin test. European Journal of Pharmacology, **152**:93-100.

Abbott, F.V., Melzack, R., and **Samwel, C. (1982)**. Morphine analgesia and tolerance in the tail-flick and formalin tests is mediated by different neural systems. Experimental Neurology, **75**:644-651.

Abelson, K.S.P. (2005): Acetylcholine in Spinal pain modulation: An in vivo study in the Rat. Acta Universitatis Upsaliensis, Uppsala, ISBN: 91-554-6178-6: Ph.D. thesis, Uppsala Universitet, Sweden.

Abelson, K.S.P. and **Höglund, A.U. (2002a)**: Intravenously administered oxotremorine and atropine in doses known to affect pain threshold, affect the intraspinal release of acetylcholine in rats. Pharmacology and Toxicology, **90**:187-192.

Abelson, K.S.P. and **Höglund, A.U. (2002b)**: Intravenously administered lidocaine in therapeutic doses increases the intraspinal release of acetylcholine in rats. Neuroscience Letters, **317**:93-96.

Abelson, K.S.P. and **Höglund, A.U. (2004)**: The effects of α2-adrenergic agonists clonidine and rilmenidine and antagonists yohimbine and efaroxan, on the cholinergic receptor system in the rat. Basic Clinical Pharmacology and Toxicology, **94**:153-160.

Abelson, K.S.P., Kommalage, M. and **Höglund, A.U. (2004)**: Spinal cholinergic involvement after treatment with aspirin and paracetamol in rats. Neuroscience Letters, **368**:116-120.

Abelson, K.S.P., Röstlinger Goldkuhl, R., Nylund, A. and **Höglund, A.U. (2006):** The effect of ketamine on intraspinal acetylcholine release: Involvement of spinal nicotinic receptors. European Journal of Pharmacology, **534**:122-128.

Abram, S.E. and **O'Connor, T.C. (1995)**: Characteristics of the analgesic effects and drug interactions of intrathecal carbachol in rats. Anesthesiology, **83**:844-849.

Akaike, A., Shibata, T., Satoh, M. and **Takagi, H. (1978)**: Analgesia induced by microinjection of morphine into, and electrical stimulation of, the nucleus reticularis paragigantocellularis of rat medulla oblongata. Neuropharmacology, **17**:775-778.

Algiers, B.E. and **Nicoll, R.A. (1982)**: Pharmacological evidence for the kinds of GABA receptors on rat hippocampal pyramidal cells studied in vitro. Journal of Physiology, **328**:125-141.

Almeida, T.F.S., Roizenblatt and **Tufik, S. (2004)**: Afferent pain pathways: a neuroanatomical review. Brain Research, **1000**:40-56.

Aloisi, A.M., Sacerdote, P, Albonetti, M.E. and **Carli, G. (1995)**: Sex-related effects on behaviour and β-endorphin of different intensities of formalin pain in rats. Brain Research, **699**:242-249.

Alreja, M., Mutalik, P., Nayar. U. and **Manchanda, S.K. (1984)**: The formalin test: A tonic pain model in the primate. Pain, **20**:97-105.

Ankier, S.I. (1974): New hot-plate tests to quantify antinociceptive and narcotic Antagonists activities. European Journal of Pharmacology. **27**:1-4.

Armstrong, D., Dry, R.M.L., Keele, C.A. and **Markham, J.W. (1953)**: Observations on Chemical excitants of cutaneous pain in man. Journal of Physiology, **120**:236-251.

Azami, J., Wright, D.M. and **Roberts, M.H.T. (1981)**: Effects of morphine and naloxone on the responses to noxious stimulation of neurons in the nucleus reticularis paragigantocellularis. Neuropharmacology, **20**:869-876.

Baba, H., Kohno, T., Okamoto, M., Goldstein, P.A., Shimoji, K. and **Yoshimura, M. (1998):** Muscarinic facilitation of GABA release in substantia gelatinosa of the rat spinal dorsal horn. Journal of Physiology (London). **508**:83-93.

Badio, B. and **Daly, J.W. (1994):** Epibatidine, a potent analgesic nicotinic agonist. Molecular Pharmacology, **45**:563-569.

Baker, R.A. (1988): The basal ganglia and pain. International Journal of Neuroscience, **41**:29-34.

Bannon, A.W., Decker, M.W., Holladay, M.W., Curzon, P., Donnely-Roberts, D., Puttfarcken, S., Bitner, R.S., Diaz, A., Dickenson, A.H., Porsolt, R.D., Williams, M. and **Arneric, S.P. (1998):** Broad-spectrum, non-opioid analgesic activity by selective modulation of neuronal nicotinic acetylcholine receptors. Science, **279**:77-81.

Bartolini, A., Gheraldini, C., Fantelli, L., Malcangio, M., Malmberg-Aiello, P. and **Giotti, A. (1992):** Role of muscarinic receptor subtypes in central antinociception. British Journal of Pharmacology, **105**:77-82.

Basbaum, A.I. (1999): Regional anesthesia. Pain Medicine, **24**:59-67.

Beani, L., Bianchi, C. and **Siniscalchi, A. (1982)**: The effect of naloxone on opioid-induced inhibition and facilitation of acetylcholine release in brain slices. British Journal of Pharmacology, **76(3)**:393-401.

Beck, P.W., Handwerker, H.O. and **Zimmerman, M. (1974):** Nervous outflow from the cat's foot during noxious radiant heat stimulation. Brain Research, **67**:373-386

Beecher, H.K. (1957): The measurement of pain. Pharmacology Review, **9**:9-209.

Beital, R.E. and **Dubner, R. (1976):** Response of unmyelinated (C) polymodal nociceptors to thermal stimuli applied to monkey's face. Journal of Neurophysiology, **39**:1160-1175.

Belcher, G., Ryall, R.W. and **Schaffer, R. (1978)**: The differential effects of 5-hydroxytryptamine, noradrenaline and raphe stimulation on nociceptive and non-nociceptive dorsal horn interneurons in cat. Brain Research, **151**: 307-321.

Bennet, G.J., Abdelmoumene, M., Hayashi, H. and **Dubner, R. (1980)**: Physiology and morphology of substantia gelatinosa neurons intracellularly stained with horseradish peroxidase, **194**:809-827.

Bentley, G.A., Newton, S.H. and **Starr, J. (1981)**: Evidence for an action of morphine and the enkephalins on sensory nerve endings in the mouse peritoneum. British Journal of Pharmacology, **73**:325-332.

Berge, O.G. (1986): Regulation of pain sensitivity, influence of prostaglandins. Cephalagia Supplementarius, **4**: 21-31.

Berge, O.G., Fasmer, O.B., Flatmark, T. and **Hole, K. (1983)**: Time course of changes in nociception after 5, 6-dihydroxytryptamine lesions of descending 5-hydroxytryptamine pathways. Pharmacology, Biochemistry and Behavior, **18**:637-643.

Berge, O.G, Fasmer, O.B, Jørgensen, H.A. and **Hole, K. (1985)**: Test-dependent antinociceptive effect of spinal serotonin release induced by intrathecal p-chloroamphetamine in mice. Acta Physiologica Scandanavica, **123**: 35-41.

Besson, J.M. and **Chauoch, A. (1987)**: Peripheral and spinal mechanisms of nociception. Physiology Review, **67**:68-186.

Bessou, P. and **Perl, E.R. (1969)**: Responses of cutaneous sensory units with unmyelinated fibres to noxious stimuli. Journal of Neurophysiology, **32**:1025-1043.

Bevan, S. (1996): Intracellular messengers and signal transduction in nociceptors: In neurobiology of nociceptors (Belmonte C. and Cervero F. **eds**) pp 298-324, Oxford University Press, Oxford.

Beydoun, A. and **Misha-Miroslav, B. (2003)**: Mechanistic stratification of antineuralgic agents.Journal of Pain and Symptom Management. **25**, No. 5.

Biggio, D., Dell Bella, D., Frigeni, V. and **Guidotti, A. (1977)**: Potentiation of morphine analgesia by mucimol. Neuropharmacology, **16**:149-150.

Bitner, R.S., Nikkel, A.L., Curzon, S.P., Arneric, S.P., Bannon, A.W. and **Decker, M.W. (1998)**: Role of nucleus raphe magnus in antinociception produced by ABT-594: immediate early gene responses possibly linked to neuronal nicotinic acetylcholine receptors on serotonergic neurons. Journal of Neuroscience. **18**:5426-5432.

Bodman, R.J., Romero, M.T. and **Kramer, E. (1988)**: Organismic variables and pain inhibition: Roles of gender and aging. Brain Research Bulletin **21**:947-953.

Bonhaus, D.W., Bley, K.R., Broka, C.A., Fontana, D.J., Leung, E., Lewis, R., Shieh, A. and **Wong, E.H. (1995)**: Characterization of the electrophysiological, biochemical and behavioural actions of d-epibatidine. Journal of Pharmacology and Experimental Therapeutics, **272**:1199-1203.

Bouaziz, H., Tong, C. and **Eisenach, J.C.** (1995): Postoperative analgesia from intrathecal neostigmine in sheep. Anesthesia and Analgesia, **80**:1140-1144.

Bouaziz, H., Tong, C., Yoon, Y., Hood, D.D. and **Eisenach, J.C.** (1996): Intravenous opioids stimulate norepinephrine and acetylcholine release in spinal cord dorsal horn. Anesthesiology, **84**:143-154.

Bowery, N.G., Price, G.W., Hudson, A.L., Hill, D.R., Wilkin, G.P. and **Turnbull, M.J. (1984)**: GABA receptor multiplicity visualization of different receptor types in the mammalian CNS. Neuropharmacology, **23**:219-231.

Boyce, S., Webb, J.R., Shepheard, S.L., Russell, M.G.N., Hill, R.G. and **Rupmai, N.M.J. (2000)**: Analgesic and toxic effects of ABT-394 resemble epibatidine and nicotine in rats. Pain. **85**:443-450.

Buffenstein, R. (2005): The naked mole-rat: a new long-living model for human aging research. Journal of Gerontology, Aging and Biological Medical Science. **60**:1369-77.

Buffenstein, R. and **Jarvis, J.U. (2002)**: The naked mole-rat: a new record for the oldest living rodent. Science of Aging and Knowledge Environment. **21**:7.

Burgess, P.R. and **Perl, E.R. (1967)**: Myelinated afferent fibres responding specifically to noxious stimulation of the skin. Journal of Physiology, **190**:541-562.

Burgess, P.R. and **Perl, E.R. (1973)**: Cutaneous mechanoreceptors and nociceptors. In Handbook of Sensory Physiology; edited by Iggo, A., Heidelberg, Springer, ***Vol. 2***:29-43.

Burgess, P.R., Petit, D. and **Warren, R.M. (1968)**: Receptor types in cat hairy skin supplied by myelinated fibres. Journal of Neurophysiology, **31**:833-848.

Burstein, R., Cliffer, K.D. and **Giesler, G.L. (1987)**: Direct somatosensory projections from the spinal cord to the hypothalamus and telencephalon. Journal of Neuroscience, 7:4159-4164.

Burstein, R., Cliffer, K.D. and **Giesler, Jr. G.L. (1990)**: Cells of origin of the spinohypothalamic tract in the rat. Journal of Comparative Neurology, **291**:329-344.

Caban, A.J., Hama, A.T., Lee, J.W. and **Sagen, J. (2004)**: Enhanced antinociception by nicotinic receptor agonist epibatidine and adrenal medullary transplants in the spinal subarachnoid space. Neuropharmacology, **47**:106-116.

Cajal, R.Y. (1909): Histologie du system nerveux de l'homme et des vertebres. **Vol. I**. Institute Cajal, Madrid. Reprinted in 1952.

Campell, J.N. and **LaMotte, R.H. (1983):** Latency to detection of first pain. Brain Research, **266**:203-208.

Capone, F., Aloist, A.M., Giancarlo C., Sacerdore, P. and **Pavone, F. (1999):** Oxotremorine induced modifications of the behavioural and neuroendocrine responses to formalin pain in male rats. Brain Research, **830**:292-300.

Carr, D.B. and **Cousins, M.J. (1998):** Spinal route of analgesia: Opioids and future options In: Cousins MJ, Bridenbaugh PO (Eds). Neural blockade in clinical anesthesia and management of pain, **3rd ed**. Philadelphia: Lippincot-Raven, pp. 915-984.

Carli, G., Farabolini, F. and **Fontani, G. (1981):** Effects of pain, morphine and naloxone on the duration of animal hypnosis. Behavior Brain Research, **2**, 373-385.

Casey, K.L. (1999): Forebrain mechanisms of nociception and pain: Analysis through imaging. Proceedings of the National Academy of Sciences, **96**:7668-7674.

Caulfield, M.P. and **Birdsall, N.J. (1998):** International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. Pharmacology Review, **50** (2):279-290.

Cervero, F. (2009): Spinal cord hyperexcitability and its role in pain and hyperalgesia. Experimental Brain Research, **196**:129-137.

Cervero, F, Iggo, A and **Ogawa, H. (1976):** Nociceptor-driven dorsal horn neurons in the lumbar spinal cord of cat. Pain, **2**:5-24.

Cervero, F., Molony, V. and **Iggo, A. (1979)**: Ascending projections of nociceptor driven lamina I neurones in the cat. Experimental Brain Research, **35**:135-149.

Chakour, M.C., Gibson, S.J. and **Bradbeer, M. (1996):** The effect of age on A delta and C fibre thermal pain perception. Pain, **64**:143-152.

Chen, S-R., and **Pan, H-L. (2001):** Spinal endogenous acetylcholine contributes to the analgesic effect of systemic morphine in rats. Anesthesiology, **9**:525-530.

Chen, S.R. and **Pan, H.L. (2003)**: Up-regulation of spinal muscarinic receptors and increased antinociceptive effect of intrathecal muscarine in diabetic rats. Journal of Pharmacology and Experimental Therapeutics, **307**:676-681

Chen, N., Mali, A., Blacque, O.E., Chu, J., Phgora, K., Bakhoum, M.W., Newbury, H.R.C., Khattia, J., Chan, S., Go, A., Efimenko, E., Johnsen, R., Phirke, P., Swoboda, P., Marra, M., Moerman, D.G., Leroux, M.R., Braillie, D.L. and **Stein, L.D. (2006):** Identification of ciliary and ciliopathy genes in *Caenorhabditis elegans* through comparative genomics. Genome Biology 7:R126 doi: 10.1186/gb-2006-7-12-r126.

Cheney, D.L., Trabucchi, M., and **Racagni, G. (1975)**: Steady-state Concentrations of Choline and Acetylcholine in Rat Brain Parts during a Constant Rate Infusion of Deuterated Choline. Naunyn-Schiedeberg's Archives of Pharmacology, **290**:99-105.

Christensen, B.N. and **Perl, E.R. (1970):** Spinal neurones specifically excited by noxious or thermal stimuli: marginal zone of the dorsal horn. Journal of Neurophysiology, **33**:293-307.

Chu, Z. and **Moenter, S.M**. **(2005)**: Endogenous Activation of metabotropic Glutamate Receptors modulates GABAergic Transmission to Gonadotropin –Releasing Hormone Neurons and alters their Firing rate: A possible feedback current. Journal of Neuroscience, **25(24)**:5740-5749.

Chudler, E.H. and **Dong, W.K. (1995):** The role of the basal ganglia in nociception and pain. Pain, **60**:3-38.

Clarke, R.W. and **Harris, J. (2004):** The organization of motor responses to noxious stimuli. Brain Research Reviews, **46(2):**163-172.

Coderre, T.J., Katz, J., Vaccarino, A.L. and **Melzack, R. (1993):** Contribution of central neuroplasticity to pathological pain: Review of clinical and experimental evidence. Pain, **52**:259-285.

Coggeshall, R.E. and **Carlton, S.M. (1997):** Receptor localization in the mammalian dorsal horn and primary afferent neurons. Brain Research Review, **24**:28-66.

Collier, H.O.J., Dinneen, L.C., Johnson, C.A. and **Schneider, C. (1968):** The abdominal constriction response and its suppression by analgesic drugs in the mouse. British Journal of Pharmacology and Chemotherapeutics, **32**:295-310.

Contreras, E., Tamayo, L. and **Quiijada, L. (1979):** Effects of the irreversible inhibition of GABA transaminase upon some morphine effects. Neuropharmacology, **18**:309-313.

Corringer, P.J., Le Novere, N. and **Changeux, J.P. (2000):** Nicotinic receptors at the amino acid level. Annual Review of Pharmacology and Toxicology, **40**:431-458.

Cox, B, Lee, T.F. and **Martin, D. (1981):** Different hypothalamic receptors mediate 5-hydroxytryptamine and tryptamine-induced temperature changes in the rat. British Journal of Pharmacology, **72**:477-482.

Cross, S.A. (1994): Pathophysiology of pain. Mayo Clinical Proceedings, **69**:375-383.

Curzon, P., Nikkel, A.L., Bannon, A.W., Arneric, S.P. and **Decker, M.W. (1998):** Differences between the antinociceptive effects of the cholinergic channel activators A-85380 and (+/-) epibatidine in rats. Journal of Pharmacology and Experimental Therapeutics, **287**:847-853.

D'Amour, F.E. and **Smith, D.L. (1941):** A method for determining loss of pain sensation. Journal of Pharmacology and Experimental Therapeutics, **72:**74-79.

Dale, H.H. (1914): The action of certain esters and ethers of choline and their relation to muscarine. Journal of Pharmacology and Experimental Therapeutics, **6**:147-196.

Davis, L., Pollock, L.J. and **Stone, T.T. (1932):** Visceral pain. Surgical Gynecology and Obstetrics, **55**:418.

Davis, K.D., Taylor, S.J., Crawley, A.P., Wood, M.L. and **Mikulis, D.J. (1997):** Functional MRI of pain- and attention-related activations in the human cingulate cortex. Journal of Neurophysiology, **77**:3370-3380.

Dash, M.S. and **Deshpande, S.S. (1975):** Pain and itch sensations: Specific chemosensory mechanisms in the human skin. In the Somatosensory system. H.H. Kornhuber (**ed.**), pp.78. Georg. Thieme Publishers Stuttgart.

Dennis, S.G. and **Melzack, R. (1980):** Pain modulation by 5-hydroxytryptaminergic agents and morphine as measured by three pain tests. Experimental Neurology, **69**:260-270.

Dennis, S.G. and **Melzack, R. (1983):** Effects of cholinergic and dopaminergic agents on pain and morphine analgesia as measured by three pain tests. Experimental Neurology, **81**:167-176.

Dennis, S.G., Melzack, R., Gutman, S. and **Boucher, F. (1980)**: Pain modulation by adrenergic agents and morphine as measured by three pain tests. Life Sciences, **26**:1247-1259.

Detweiler, D.J., Eisenach, J.C., Tong, C. and **Jackson, C. (1993):** A cholinergic interaction in *alpha*$_2$ adrenoceptor-mediated antinociception in sheep. Journal of Pharmacology and Experimental Therapeutics, **265**:536-542.

Dewey, W.L. and **Pedigo, N.W. (1981)**: Comparison of the antinociceptive activity of intraventricularly administered acetylcholine to narcotic antinociception. Neuroscience Letters, **26(1)**:85-90.

Dickenson, A.H., Fardin, V., Le Bars, D. and **Besson, J.M. (1979):** Antinociceptive action following microinjection of methionine-enkephaline in the nucleus raphe magnus of the rat. Neuroscience Letters, **15**:265-270.

Dolan, S. and **Nolan, A.M. (1999):** N-Methyl D-aspartate induced mechanical allodynia is blocked by nitric oxide synthetase and cyclooxygenase-2 inhibitors. Neuroscience Report, **10**:449-452.

Dolan, S., Field, L.C. and **Nolan, A.M. (2000):** The role of nitric oxide and prostaglandin signaling pathways in spinal nociceptive processing in chronic inflammation. Pain, **86**:311-320.

Dougherty, P.M. and **Willis, W.D. (1992)**: Enhanced responses of spinothalamic tract neurons to excitatory amino acids accompany capsaicin-induced sensitization in the monkey. Journal of Neuroscience, **12**:883-894.

Dray, A. (1995): Inflammatory mediators of pain. British Journal of Anaesthesia, **751**:125-131.

Dubuisson, D. and **Dennis, S.G. (1977):** The formalin test: a quantitative study of the analgesic effects of morphine, meperidine and brain stem stimulation in rats and cats. Pain, **4**:161-174.

Duggan, A.W., Hall, J.G. and **Headly, P.M. (1977):** Suppression of transmission of nociceptive impulses by morphine selective effects of morphine administered in the region of the substantia gelatinosa. British Journal of Pharmacology, **61**:65-76.

Eddy, N.B. and **Leimbuch, D. (1953):** Synthetic analgesics. II. Dithienybutenyl- and dithienylbutylamines. Journal Pharmacology and Experimental Therapeutics, **107**: 385-392.

Eddy, N.B., Touchberry, C.F. and **Lieberman, J.E. (1950):** Synthetic analgesics. I. methadone isomers and derivatives. Journal Pharmacology and Experimental Therapeutics, **98**.121-137.

Einspahr, F.J. and **Piecey, M.F. (1980):** Morphine depresses dorsal horn responses to controlled noxious and non-noxious cutaneous stimulation. Journal Pharmacology and Experimental Therapeutics, **213**:456-461.

Eisenach, J.C. (1999): Muscarinic-mediated analgesia. Life Sciences, **64**:549-554.

Emele, J.F. and **Shanaman, J. (1963):** Bradykinin writhing, a method for measuring analgesia. Proceedings of Society of Biology and Medicine, **114**:680-682.

Emilio, F.E. and **Eladio, G. (1998)**: Antagonism of peripheral 5-HT_4 receptors reduces visceral and cutaneous pain in mice and induces visceral analgesia after simultaneous inactivation of 5-HT_3 receptors. Brain Research, **788**:20-24.

Engberg, I. and **Ryall, R.W. (1966):** The inhibitory actions of noradrenaline and other monoamines on spinal neurons. Journal of Physiology, **185**:298-322.

Ephrem, F. and **Dennis, T.C. (1994)**: Demand characteristics underlying differential ratings of sensory versus affective components of pain. Journal of Behavioural Medicine, **17**:375-390.

Faulkes, C.G., Verheyen, E., Verheyen, W., Jarvis, J.U. and **Bennett, C. (2004):** Phylogeographical patterns of genetic divergence and speciation in African mole-rats (Family: Bathyergidae). Molecular Ecology, **13**:613-629.

Fasmer, O.B., Berge, O.G., Walther, B. and **Hole, K. (1983):** Changes in nociception after intrathecal administration of 5, 6-dihydroxytryptamine in mice. Neuropharmacology, **22:**1197-1201.

Fasmer, O.B., Berge, O.G. and **Hole, K. (1985):** Changes in nociception after intrathecal administration of 5, 6-dihydroxytryptamine in mice. Different effects in formalin and tail-flick tests. Neuropharmacology, **24**:729-734.

Ferreira, S.H. (1972): Prostaglandins, aspirin-like drugs and analgesia. Nature New Biologist, **240**:200-203.

Ferreira, S.H. (1983): Prostaglandins: peripheral and central analgesia. Advances in Pain Research and Therapeutics, **5**:627-634.

Ferreira, S.H., Moncada, S. and **Vane, J.R. (1973):** Prostaglandins and the mechanism of analgesia produced by aspirin-like drugs. British Journal of Pharmacology, **49**:86-97.

Ferreira, S.H. and **Vane, J.R. (1974):** New aspects of mode of action of non-steroidal anti-inflammatory drugs. Annual Review of Pharmacology, **14**:57-73.

Ferreira, S.H., Lorenzetti, B.B. and **Correa, F.M.A. (1978):** Central and peripheral anti-analgesic action of aspirin-like drugs. European Journal of Pharmacology, **53**:29-48.

Fields, H.L., Basbaum, A.I., Clanton, C.H. and **Anderson, S.D. (1977):** Nucleus raphe magnus inhibition of spinal dorsal horn neurons. Brain Research, **126**: 441-453.

Fields, H.L., Emson, P.C., Leigh, B.K., Gilbert, R.F.T. and **Iversen, L.L. (1980):** Multiple opiate receptor sites on primary afferent fibres. Multiple opiate receptor sites on primary afferent fibres. Nature, **284**:351-353.

Fitzgerald, M.J.T. and **Lynn, B. (1977):** The sensitization of high threshold mechanoreceptors with myelinated axons by repeated heating. Journal of Physiology, **265**:549-563.

Fjalibrant, N. and **Iggo, A. (1961):** The effect of histamine, 5-hydroxytryptamine and acetylcholine on cutaneous afferent fibres. Journal of Physiology, **156**:578-590.

Flodmark, S. and **Wramner, T. (1945):** The analgesic action of morphine, eserine and prostigmine studied by a modified Handy-Wolff Goodell method. Acta Physiologica Scandinavica, **9**:88-96.

Flores, C.M. and **Hargreaves, K.M. (1998):** Neuronal nicotinic receptors: New targets in the treatment of pain. In: Neuronal nicotinic receptors: Pharmacology and therapeutic opportunities. *(***eds***)* S.P. Arneric and J.D. Brioni. Wiley-Liss, Inc pp.359-378.

Foreman, R.D., Beall, J.E., Applebaum, A.E., Coulter, J.D. and **Willis, W.D. (1976):** Effects of dorsal column stimulation on primate spinothalamic tract neurons. Journal of Neurophysiology **39**:534-546.

Fürst, S. (1999): Transmitters involved in antinociception in the spinal cord. Brain Research Bulletin, **48**. 129-141.

Gamble, G.D. and **Milne, R.J. (1989)**: Habituation to Sham testing procedures modifies tail-flick latencies: effects on nociception rather than vasomotor tone. Pain, **39(1)**:103-107.

Gamble, G.D. and **Milne, R.J. (1990):** Hypercapnia depresses nociception: endogenous opioids implicated. Brain Research, **514**:198-205.

Gamse, R., Holzer, P. and **Lembeck, F. (1979):** Indirect evidence for presynaptic location of opiate receptors on chemosensitive primary sensory neurons. Naunyn-Schmiedeberg's Archives of Pharmacology, **308**:281-285.

Gentle, M.J. and **Hill, F.L. (1987):** Oral lesions in chicken: Behavioural responses following nociceptive stimulation. Physiology and Behavior, **40**:781-783.

Georgopoulos, A.P. (1976): Functional properties of primary afferent units probably related to pain mechanisms in primate glabrous skin. Journal of Neurophysiology, **39**:71-83.

Gheraldini, C., Malmberg-Aiello, P., Giotti, A., Malcangio, M. and **Bartolini, A. (1990):** Investigation into atropine-induced antinociception. British Journal of Pharmacology, **101**:49-54.

Gillberg, P.G. and **Aquilonius, S.M. (1985):** Cholinergic, opioid and glycine receptor binding sites localized in human spinal cord by *in vitro* autoradiography. Acta. Neurologica Scandinivica, **72**:299-306.

Gillberg, P.G., Gordh, P., Hartvig, P., Jansson, I., Pettersson, J. and **Post, C. (1989):** Characterization of the antinociception induced by intrathecally administered carbachol. Pharmacology and Toxicology, **64**:340-343.

Gobel, S. (1975): Golgi studies of the substantia gelatinosa neurones in the spinal trigeminal nucleus. Journal of Comparative Neurology, **162**:397-416.

Gobel, S. (1978): Golgi studies of the neurons in layer I of the dorsal horn of the medulla (Trigeminal Nucleus Caudalis). Journal of Comparative Neurology **180**:395-414.

Gower, A.J. (1987): Effects of acetylcholine agonists and antagonists on yawning and analgesia in the rat. European Journal of Pharmacology **139**:79-89.

Gozariu, M., Bragard, D., Willer, J.C. and **Le Bars, D. (1997)**: Temporal summation of C-fiber afferent inputs: competition between facilitatory and inhibitory effects on C-fiber reflex in the rat. Journal of Neurophysiology, **78**:3165-3179.

Gregor, M. and **Zimmerman, M. (1972):** Characteristics of spinal neurons responding to cutaneous myelinated and unmyelinated fibres. Journal of Physiology, **221**:555-576.

Guilbaud, G., Oliveras, J.L., Giesier, G. and **Besson, J.M. (1977):** Effects induced by stimulation of the centralis inferior nucleus of the raphe on dorsal horn interneurons in cats spinal cord. Brain Research, **126**:355-360.

Guzman, F. and **Lim, R.K.S. (1968):** The mechanisms of action of non-narcotic analgesics. Medical Clinician of North America, **52**:3-14.

Hai-Chun, M.A., Shuji, D., Yan-Fen, W., Yumiko, I. and **Fumi, Y. (2001)**: The antinociceptive and sedative effects of carbachol and oxycodone administered into brainstem pontine reticular formation and spinal subarachnoid space in rats. Anesthesia and Analgesia, **92**:1307-1315.

Haley, J.E., Sullivan, A.F. and **Dickenson, A.H. (1990):** Evidence for spinal N-methyl-D-aspartate receptor involvement in prolonged chemical nociception in the rat. Brain Research, **518**:218-226.

Haley, T.J. and **McCormick, W.G. (1957)**: Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. British Journal of Pharmacology, **12**:12-15.

Hamm, R.J. and **Kinsley, J.S. (1988):** Developmental aspects of nociception. Brain Research Bulletin, **21**, 933-946.

Hammond, D.L. and **Yaksh, T.L. (1984):** Antagonism of stimulation-produced antinociception by intrathecal administration of methysergide or phentolamine. Brain Research, **298**:329-337.

Handwerker, H.O. (1976): Pharmacological modulation of the discharge of the nociceptive C-fibres. In Sensory Function of the Skin in Primates. Y. Zotterman (**ed**.), pp.427-437. Pergamon Press, Oxford, U.K.

Handwerker, H.O., Kilo, S. and **Reeh, P.W. (1991)**: Unresponsive afferent nerve fibres in the sural nerve of the rat. Journal of Physiology (London), **435**:229-242.

Handwerker, H.O., Kessler, W., Kirchoff, C. and **Reeh, P.W. (1992)**: Excitation of cutaneous afferent nerve endings in vitro by a combination of inflammatory mediators and conditioning effect of substance P. Experimental Brain Research, **91(3)**:467-476.

Hannon, J. and **Hoyer, D. (2008):** Molecular Biology of 5-HT receptors. Behavioural Brain Research, **195**:198-213.

Hao-Jun, Y., Jun, C., Carsten, D.M. and **Lars A-N. (2002):** Differential effect of peripheral glutamate (NMDA, non-NMDA) receptor antagonists on bee venom – induced spontaneous nociception and sensitization. Brain Research Bulletin, **58**:561-567.

Hao-Jun, Y., Jun, C., Carsten, D.M. and **Lars A-N. (2003):** Role of Central NMDA verses non-NMDA receptor in spinal anesthetized rats under normal and hyperexcitable conditions. Brain Research, **981**:12-22.

Hardy, J.D., Wolff, G. and **Goodell, H. (1950):** Experimental evidence on the nature of cutaneous hyperalgesia. Journal of Clinical Investigation, **29**:115-140.

Hargreaves, K.M., Dubner, R. and **Joris, J. (1988):** Peripheral actions of opiates in the blockade of carrageenin-induced inflammation. In Pain Research and Clinical Management, (1988): **Vol.3** R. Dubner, G.F. Gebhart and M.R. Bond (**eds**.), pp. 55-60: Proceedings of the Fifth (Vth) World congress on Pain, Elsevier Science Publishers BV (Biomedical division).

Harris, L.S., Dewey, L.W., Howes, J.F., Kennedy, J.S. and **Pars, H. (1969):** Narcotic antagonist analgesics: interactions with cholinergic systems. Journal of Pharmacology and Experimental Therapeutics, **169**:17-22.

Hayashi, G. and **Takemori, A.E. (1971):** The type of analgesic-receptor interaction involved in certain analgesic assays. European Journal of Pharmacology, **16**:63-66.

Hayes, R.L., Price, D.D., Ruda, M. and **Dubner, R. (1979):** Suppression of nociceptive responses in the primate by electrical stimulation of the brain or morphine administration: Behaviour and electrophysiological comparisons. Brain Research, **167**: 417-421.

Hensel, H. and **Boman, K.K.A. (1960):** Afferent impulses in cutaneous sensory nerves in human subjects. Journal of Neurophysiology, **23**:564-578.

Hilman, P. and **Wall, P.D. (1969):** Inhibitory and excitatory factors influencing the receptive fields of Lamina V spinal cord cells. Experimental Brain Research, **9**: 284-306.

Hodge, C.J., Apkarian, A.V., Stevens, R.T., Vogesisang, G.D., Brown, O. and **Frank, J.I. (1983):** Dorsolateral pontine inhibition of dorsal horn cell responses to cutaneous stimulation: lack of dependence of catecholaminergic systems in the cat. Journal of Neurophysiology, **240**:415-420.

Hodge, C.J. and **Apkarian, A.V. (1990):** The spinothalamic tract. Critical Review of Neurobiology, **5**:363-397.

Hole, K. and **Berge, O.G. (1981):** Regulation of pain sensitivity in the central nervous system. Cephalagia, **1**:51-59.

Hole, K. and **Tjølsen, A. (1993):** The tail flick and formalin tests in rodents: changes in skin temperature as a confounding factor. Pain, **53**:247-254.

Honda, K., Koga, K., Moriyama, T., Koguchi, M., Takano, Y. and **Kamiya, H. (2002):** Intrathecal α_2 adrenoceptor agonist clonidine inhibits mechanical transmission in mouse spinal cord via activation of muscarinic M_1 receptors. Neuroscience Letters, **322**:161-164.

Honda, K., Murao, N., Ibuki, T,. Kamiya, H. and **Takano, Y. (2003):** The role of spinal muscarinic acetylcholine receptors in clonidine-induced anti-nociceptive effects in rats. Biology and Pharmacology Bulletin, **26**:1178-1180.

Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxane, P.R. and **Humphrey, P.P. (1994):** International Union of Pharmacology Classification of receptors for 5 – hydroxytryptamine (serotonin). Pharmacology Review, **46**:157.

Höglund, A.U. and **Baghdoyan, H.A. (1997):** M2, M3, and M4, but not M1, muscarinic receptor subtypes are present in rat spinal cord. Journal of Pharmacology and Experimental Therapeutics, **281**:470-477.

Hughes, R.A.. and **Sufka, K.J. (1991):** Morphine hyperalgesic effects on the formalin test in domestic fowl (*Gallus gallus*). Pharmacology, Biochemistry and Behavior, **38**:247-251.

Hunskaar, S. (1987a): Non-narcotic antinociceptive drugs. Studies with special emphasis on appropriate behavioural tests in mice. Ph.D. thesis, University of Bergen, Norway.

Hunskaar, S. (1987b): Similar effects of acetylsalicylic acid and morphine on immediate responses to acute noxious stimulation. Pharmacology and Toxicology, **60**:167-170

Hunskaar, S., Berge, O.G. and **Hole, K. (1986a):** A modified hot-plate test sensitive to mild analgesics. Behaviour Brain Research, **21**:101-108.

Hunskaar, S., Berge, O.G. and **Hole, K. (1986b):** Dissociation between antinociceptive and anti-inflammatory effects of acetylsalicylic acid and indomethacin in the formalin test. Pain, **25**:125-132.

Hunskaar, S., Fasmer, O.B. and **Hole, K. (1985a):** Acetylsalicylic acid, paracetamol and morphine inhibit behavioural responses to intrathecal administration of substance P or capsaicin. Life Science, **37**:1835-1841.

Hunskaar, S., Fasmer, O.B. and **Hole, K. (1985b):** Formalin test in mice, a useful technique for evaluating mild analgesics. Journal of Neuroscience Methods **14**:69-76.

Hunskaar, S. and **Hole, K. (1987):** The formalin test in mice dissociation between inflammatory and non-inflammatory pain. Pain, **30**:103-114.

IASP. (1979): Pain Terms: A list with definitions and notes on usage. Pain, **6**:249-252.

Iggo, A. (1959): Cutaneous heat and cold receptors with slowly conducting C-afferent fibres. Quarterly Journal of Experimental Physiology, **44**:362-370.

Iggo, A. (1960): Cutaneous mechanoreceptors with afferent C-fibres. Journal of Physiology, **15**:337-353.

Iggo, A. and **Ogawa H. (1971):** Primate thermal nociceptors. Journal of Physiology, **216**: 77P-78P

Illes, P., Wirkner, K., Güntner, A., Weber, M., Guzman, S.J., Krause, T., Fuchs, J., Köles, L. and **Nörenberg, W. (2006):** Modulation of NMDA receptor current in layer V pyramidal neurons of the rat prefrontal cortex by P2Y receptor activation. Cerebral Cortex doi: 10.1093/cecor/bhk012.

Innes, D.G.L. and **Kavaliers, M. (1987):** Opiates and deer mouse behaviour: differences between island and mainland populations. Canadian Journal of Zoology, **65**:2504-2512.

Ishii, M. and **Kurachi, Y. (2006)**: Muscarinic acetylcholine receptors. Current Pharmacological Designs, **12(28)**:3573-3581.

Iwamoto, E.T. (1991): Characterization of the antinociception induced by nicotine pedunculopontine tegmental nucleus and the nucleus raphe magnus. Journal of Pharmacology and Experimental Therapeutics, **257**:120-133.

Iwamoto, E.T. and **Marion, L. (1993):** Characterization of the antinociception induced by intrathecally administered muscarinic agonists in rats. Journal of Pharmacology and Experimental Therapeutics, **266**:329-338.

Iwamoto, E.T. and **Marion, L. (1994a):** Pharmacologic evidence that spinal muscarinic analgesia is mediated by L-arginine/nitric oxide/cyclic GMP cascade in rats. Journal of Pharmacology and Experimental Therapeutics, **271**:601-608.

Iwamoto, E.T. and **Marion, L. (1994b):** Pharmacological evidence that nitric oxide mediates the antinociception produced by muscarinic agonists in the rostral ventral medulla of rats. Journal of Pharmacology and Experimental Therapeutics, **269**:699-708.

Jarvis, J.U.M. (1978): Energetics of survival in *Heterocephalus glaber* (Rüppell) the naked mole-rat (Rodentia: Bathyergidae). Bulletin of the Carnegie Museum of Natural History, **6**:81-87.

Jarvis, J.U.M. (1981): Eusociality in a mammal; cooperative breeding in naked mole-rat colonies. Science, **212**:571-573.

Ji, R.R., Befort, K., Brenner, G.J. and **Woolf, C.J. (2002)**: ERK MAP kinase activation in superficial spinal cord neurons induces prodynorphin and NK-1 upregulation and contributes to persistent inflammatory pain hypersensitivity. Journal of Neuroscience, **22**:478-485.

Ji, R.R., Kohno T., Moore, K.A. and **Woolf, C.J. (2003):** Central sensitization and LTP: do pain and memory share similar mechanisms? TINS, **26**:696-705.

Jones, S.L. and **Gebhart, G.F. (1986):** Characterization of coeruleospinal inhibition of the nociceptive tail-flick reflex in the rat: mediation by spinal α_2-adrenoreceptors. Brain Research, **364**:315-330.

Kakidani, H., Furutani, Y.and **Takahashi, H. (1982):** Cloning and sequence analysis of cDNA for porcine beta-neo-endorphin/dynorphin precursor. Nature, **298:**245-249

Kajander, K.C., Wakisaka, S. and **Bennett, G.J. (1992):** Spontaneous discharges originates in the dorsal root ganglion at the onset of a painful peripheral neuropathy in the rat. Neuroscience Letters, **138**:225-228.

Kanui, T.I. and **Hole, K. (1990):** Morphine induces aggression but not analgesia in the naked mole-rat (*Heterocephalus glaber*). Comparative Biochemistry and Physiology, **96C, No.1**:131-133.

Kanui, T.I., Hole, K. and **Miaron, J.O. (1990)**: Nociception in crocodiles-capsaicin instillation, formalin and hot plate tests. Zoological Sciences, 7:537-540.

Kanui, T.I., Karim, F. and **Towett, P.K. (1993):** The formalin test in the naked mole-rat (*Heterocephalus glaber*) analgesic effects of morphine, nefopam and paracetamol. Brain Research, **600**:123-126.

Karim, F., Kanui, T.I. and **Mbugua, S. (1993):** Effects of codeine, naproxen and dexamethasone on formalin induced pain in the naked mole-rat. Neuroreport, **4**:25-28.

Kavaliers, M. (1984): Opioid peptides, pineal gland and rhythmic behaviour in fishes. Trends American Fish Society, **113**:432-438.

Kavaliers, M. (1988): Evolutionary and Comparative aspects of nociception. Brain Research Bulletin, **21**:923-931.

Kavaliers, M. and **Hirst, M. (1983):**Tolerance to morphine-induced thermal response in the terrestrial snail *Cepaea nemoralis.* Neuropharmacology, **22:**1321-1326.

Khan, I.M., Buerkle, H., Taylor, P. and **Yaksh, T.L. (1998):** Nociceptive and antinociceptive responses to intrathecally administered nicotinic agonists. Neuropharmacology, **37**:1515-1525.

Kenshalo, D.R., Leonard, R.B., Chung, J.M. and **Willis, W.D. (1979)**: Responses of primate spinothalamic neurons to graded and to repeated noxious heat stimuli. Journal of Neurophysiology, **42**:1370-1389.

Kevetter, G.A., Haber, L.H., Yezierski, R.P., Chung, J.M., Martin, R.F. and **Willis, W.D. (1982)**: Cells of origin of the spinoreticular tract in the monkey. Journal of Comparative Neurology, **207**:61-74.

King, J.S., Gallant, P., Meyerson, V. and **Perl, E.R. (1976):** The effects of anti-inflammatory agents on the responses and sensitization of unmyelinated (C) fibre polymodal nociceptors. In sensory Function of the Skin in Primates. Y. Zotterman (**ed.**) pp.441-44. Pergamon Press, Oxford, U.K.

Klemm, W.R. (1981): Opiate mechanisms: Evaluation of research involving neuronal action potentials. Progressive Neuro-Psychopharmacology, **5**:1-33.

Klimscha, W., Tong, C. and **Eisenach, J.C. (1997):** Intrathecal α_2-adrenergic agonists stimulate acetylcholine and norepinephrine release from the spinal cord dorsal horn in sheep. An in vivo microdialysis study. Anesthesiology, **87**:110-116.

Kommalage, M. and **Höglund, A.U. (2004):** (+/-) Epibatidine increases acetylcholine release partly through an action on muscarinic receptors. Basic Clinical Pharmacology and Toxicology, **94**:238-244.

Kommalage, M. and **Höglund, A.U. (2005a):** Involvement of spinal GABA receptors in the regulation of intraspinal acetylcholine release. European Journal of Pharmacology, **2**:9-73.

Kommalage, M. and **Höglund, A.U. (2005b):** Involvement of spinal serotonin receptors in the regulation of intraspinal acetylcholine release. European Journal of Pharmacology, **509**:127-34.

Krogsgaard-Larsen, P. (1984): THIP, a specific and clinically active GABA agonist. Neuropharmacology, **23**:837-839.

Kumazawa, T. and **Perl, E.R. (1978):** Excitation of marginal and substantia gelatinosa neurons in the primate spinal cord. Indications of their place in dorsal horn functional organization. Journal of Comparative Neurology, **177**:417-434.

Kuraishi, Y., Harada, Y., Aratani, S., Satoh, M. and **Takagi, H. (1983):** Separate involvement of spinal noradrenergic and serotonergic systems in morphine analgesia: the differences in mechanical and thermal tests. Brain Research, **273**:245-252.

Kuraishi, Y., Hirota, N., Satoh, M. and **Takagi, H. (1985):** Antinociceptive effects of intrathecal opioids, noradrenaline and serotonin in rats. Brain Research, **326:**168-171.

Lambert, D.G. and **Appadu, B.L. (1995):** Muscarinic receptor subtypes. Do they have a role in clinical anaesthesia. British Journal of Anaesthesia, **74**:497-499.

LaMotte, C., Pert, C.B., and **Synder, S.H. (1976):** Opiate receptor binding in primate spinal cord: distribution and changes after dorsal root section. Brain Research, **112**:407-412.

LaMotte, R.H., Simone, D.A., Baumann, T.K., Shain, C.N. and **Alreja, M. (1988):** Hypothesis for novel classes of chemoreceptors mediating chemogenic pain and itch. In Pain Research and Clinical Management, **Vol. 3.** R. Dubner, G.F. Gebhart and M.R. Bond (**eds**.), pp. 529-535. Proceedings of the Vth World congress on Pain, 1988, Elsevier Science Publishers BV (Biomedical division).

LaMotte, R.H., Shain, C.N., Simone, D.A. and **Tsai, E.F. (1991):** Neurogenic hyperalgesia: psychophysical studies of underlying mechanisms. Journal of Neurophysiology, **66**:190-211.

LaMotte, R.H., Thalhammer, J.G., Torebjörk, H.E. and **Robinson, C.J. (1982):** Peripheral neural mechanisms of cutaneous hyperalgesia following mild injury by heat. Journal of Neuroscience, **6**:765-781.

Langmead, C.J., Watson, J. and **Reavill, C. (2008)**: Muscarinic acetylcholine receptors as CNS drug targets. Pharmacology and Therapeutics, **117(2)**:232-243.

Lamour, Y. and **Epelbaum, J. (1988)**: Interactions between cholinergic and peptidergic system in the Cerebral Cortex and hippocampus. Progress in Neurobiology, **31**:109-148.

Lawand, N.B., Lu, Y. and **Westlund, K.N. (1999):** Nicotinic cholinergic receptors: potential targets for inflammatory pain relief. Pain, **80**:291-299.

Le Bars, D., Dickenson, A.H. and **Besson, J.M. (1980):** Microinjection of morphine within nucleus raphe magnus and dorsal horn neuron activities related to nociception in the rat. Brain Research, **189**:467-481.

Le Bars, D., Menetrey, D., Conseillie, R. C. and **Besson, J.M. (1975):** Depressive effects of morphine upon lamina V cells activities in the dorsal horn of the spinal cat. Brain Research, **98**:261-277.

Le Novere, N. and **Changeux, J.P. (2001):** LGICdb: the ligand-gated ion channel database. Nucleic Acids Research, **29**:294-295.

Lembeck, F. and **Holzer, P. (1979):** Substance P as a neurogenic mediator of antidromic vasodilation and neurogenic plasma extravasation. Archives of Pharmacology, **310**:175-183.

Levine, J.D. and **Reichling, D.B. (1999)**: Peripheral mechanisms of inflammatory pain. In: Wall PD, Melzack R, eds. Textbook of Pain. Edinburgh: Churchill Livingstone, **4th ed.**:59-84.

Lewis, M.E., Pert, A., Pert, C.B. and **Herkenham, M. (1983)**: Opiate receptor localization in rat cerebral cortex. Journal of Comparative Neurology, **216**:339-358.

Lewis, V.A. and **Gebhart, G.F. (1977):** Evaluation of the periaqueductal central gray (PAG) as a morphine–induced locus of action and examination of morphine- induced and stimulation produced analgesia at coincident PAG loci. Brain Research, **124**:283-303.

Liebeskind, J.C., Guilbaud, G., Besson, J.M. and **Oliveras, J.L. (1973):** Analgesia from electrical stimulation of periaqueductal gray matter in the cat: Behavioural observations and inhibitory effects on spinal cord interneurons. Brain Research, **50**:441-446.

Light, A.R. and **Perl, E.G. (1979a):** Re-examination of dorsal horn including observations on the differential termination of coarse and fine fibers. Journal of Comparative Neurology, **186**:117-132.

Light, A.R. and **Perl, E.G. (1979b):** Spinal termination of functionality identified primary afferent neurons with slowly conducting myelinated fibers. Journal of Comparative Neurology, **186**:151-172.

Light, A.R., Trevino, D.A. and **Perl, E.R. (1979):** Morphological features of functionally defined neurons in the marginal zone and substantia gelatinosa of the spinal dorsal horn. Journal of Comparative Neurology, **186**:151-172.

Lim, R.K.S. (1970): Pain. Annual Review of Physiology. **32**:269-288.

Lin, M.T., Wu, J.J. and **Ysay, B.L. (1983):** Serotonergic mechanisms in the hypothalamus mediates thermoregulatory responses in rats. Naunyn-Schmiedeberg's Archives of Pharmacology, **322**:271-278.

Lovick, T.A.. (1985): Ventrolateral medullary lesions block the antinociceptive and cardiovascular responses elicited by stimulating the dorsal periaqueductal gray matter in rats. Pain, **21**, 241-252.

Lynn, B. (1977): Cutaneous hyperalgesia. British Medical Bulletin, **33**:103-108.

Ma, W., Ribeiro-Da-Silva, A., De Koninck, Y., Radhakrishnan, V., Cuello, A.C. and **Henry, J.L. (1997)**: Substance P and enkephalin immunoreactivities in axonal boutons presynaptic to physiologically identified dorsal horn neurons. An ultrastructural multiple-labelling study in the cat. Neuroscience, 77:793-811.

Machelska, H., Pavone, F., Capone, F., and **Przewlocka, B. (1999):** Antinociception after both peripheral and intrathecal injection of oxotremorine is modulated by spinal nitric oxide. European Neuropsychopharmacology, **9**:213-217.

Malmberg, A.B. and **Yaksh, T.L. (1992):** Hyperalgesia mediated by spinal glutamate or substance P receptor blocked by spinal cyclooxygenase inhibitor. Science, **257**:1267-1279

Mao, J. (1999): NMDA and opioid receptors: Their interactions in antinociception, tolerance and neuroplasticity. Brain Research Review, **30**:289-304.

Mauk, M.D., Olson, R., LaHoste, G.J. and **Olson, G.A. (1981):** Tonic immobility produces hyperalgesia and antagonizes morphine analgesia. Science, **21**:353-354.

Mayer, D.J., Wolfle, T.L., Akil, H., Carder, B. and **Liebeskind, J.C. (1971):** Analgesia from electrical stimulation in the brain stem of the rat. Science, **174**:1351-1354.

Mayer, M.L. and **Hill, R.G. (1978):**The effects of intravenous fentanyl, morphine and naloxone on nociceptive responses in the rat caudal medulla. Neuropharmacology, **17**:533-539.

Mayhan, W.G. and Sharpe, G.M. (1998): Nicotine impairs histamine-induced increases in macromolecular efflux: role of oxygen radicals. Journal of Applied Physiology, **84**:1589-1595.

McCreery, D.B., Bloedel, J.R. and **Hames, E.G. (1979):** Effects of stimulating in raphe nuclei and reticular formation on response of spinothalamic neurons to mechanical stimuli. Journal of Neurophysiology, **42:**166-182.

McNab, B.K. (1966): The metabolism of fossorial rodents: a study of convergence. Ecology, **47:**712-733.

McNab, B.K. **(1968):** The influence of fat deposits on the basal rates of metabolism in desert homeotherms. Comparative Biochemistry and Physiology, **26**:337-343.

Meller, S.T., Cummings, C.P., Traub, R.J. and **Gebhart, G.F. (1994):** The role of nitric oxide in the development and maintenance of the hyperalgesia produced by intraplantar injection of carrageenan in the rat. Neuroscience, **60**, 367-374.

Melzack, R. and **Casey, K.L. (1968):** Sensory, motivational and central control of determinants of pain. In The Skin Senses. edited by Kenshalo, D.R., Springfield, I.L., Charles, C. Thomas, pp.423-439.

Melzack, R. and **Wall, P.D. (1965):** Pain mechanisms: A new theory. Science, **150**: 971-979.

Menetrey, D., Giesler, G.J. and **Besson, J.M. (1977):** An analysis of spinal cord dorsal horn neurons to non-noxious and noxious stimuli in the spinal rat. Experimental Brain Research, **27**:15-33.

Merskey, H., Mumford, J.M., Nathan, P.W., Noordenbos, W. and **Sunderland, S. (1986):** Pain terms, Part II. Pain Supplement, **3**: S216.

Meyer, R.A.. and **Campbell, J.N. (1981):** Myelinated nociceptive afferents account for hyperalgesia that follows a burn to the hand. Science, **213**:1527-1529.

Miao, F.J.-P., Benowitz, N.L., Basbaum, A.I. and **Levine, J.D. (1992)**: Sympathoadrenal contribution to nicotine and muscarinic modulation of bradykinin-induced plasma extravasation in the knee joint of the rat. Journal of Pharmacology and Experimental Therapeutics, **262**:889-895.

Miao, F.J.-P., Benowitz, N.L., Heller, P.H. and **Levine, J.D. (1997)**: Contribution of adrenal hormones to nicotine-induced inhibition of synovial plasma extravasation. British Journal of Pharmacology, **120**:298-304.

Miao, F.J.-P., Benowitz, N.L. and **Levine, J.D. (2001)**: Endogenous opioids suppress activation of nociceptors by sub-nanomolar nicotine. British Journal of Pharmacology, **133**:23-28.

Millan, M.J. (1999): The induction of pain: An Integrative review. Progressive Neurobiology, **57**:1-164.

Milne, P.J., Foreman, R.D., Giesler, Jr G.J. and **Willis, W.D. (1981):** Convergence of cutaneous, pelvic visceral nociceptive inputs onto primate spinothalamic neurons. Pain **11**:163-183.

Mizumura, K. and **Kumazawa, T. (1996):** Modulations of nociceptor responses by inflammatory mediators and second messengers implicated in their action – a study in canine testicular polymodal receptors. In: The Polymodal Receptor-A Gateway to Pathological pain. Progress in Brain Research **Vol. 113 Ed**. By Kumazawa T., Kruger L. and Mizumura K. Elsevier: Amsterdam, pp. 115-141.

Mogil, J.S., Grisel, J.E., Zhang's, G. Belknap, J.K. and **Grandy, D.K. (1996):** Functional antagonism of mu-, delta- and kappa-opioid antinociception by orphanin FQ. Neuroscience Letters, **214**:131-134.

Mogil, J.S., Richards, S.P., O'Toole, L.A., Helms, M.L., Mitchell, S.R. and **Belknap, J.K. (1997)**: Genetic sensitivity to hotplate nociception in DBA/2J and C57BL/6J inbred mouse strains: possible sex-specific mediation by δ_2-opioid receptor. Pain, **70**:267-277.

Moncada, S.S., Ferreira, S.H. and **Vane, J.R. (1975):** Inhibition of prostaglandin biosynthesis as the mechanism of analgesia of aspirin-like drugs in the dog knee joint. European Journal of Pharmacology, **31**:250-260.

Moroni, F., Cheney, D.L. and **Costa, E. (1977)**: Beta endorphins inhibits Acetylcholine turnover in nuclei of rat brain. Nature, **267**:267

Morris, B.J. and **Herz, A. (1987):** Distinct distribution of opioid receptor types in rat lumbar spinal cord Naunyn-Schmiedeberg's Archives of Pharmacology, **336**:240-243.

Nakamura-Craig, M. and **Smith, T.W. (1989):** Substance P and peripheral inflammatory hyperalgesia. Pain, **38**:91-98.

Nicoll, R.A., Malenka, R.C. and **Kauer, J.A. (1990):** Functional comparison of neurotransmitter receptor subtypes in mammalian central nervous system. Physiological Review, **70**:513-565.

Noda, M., Furutani, Y. and **Takahashi, H. (1982):** Cloning and sequence analysis of cDNA for bovine adrenal preproenkephalin. Nature, **295**:202-206.

Nygren, L.G. and **Oslon, L.A. (1977):** A new major protection from locus coeruleus: the main source of noradrenergic nerve terminals in the ventral and dorsal columns of the spinal cord. Brain Research, **132**:85-93.

O'Callaghan, J.P. and **Holtzman, S.G. (1975):** Quantification of the analgesic activity of narcotic antagonists by a modified hot-plate procedure. Journal of Pharmacology and Experimental Therapeutics, **192**:497-505.

Ogren, S.B. and **Berge, O.G. (1984):** Test-dependent variations in the antinociceptive effect of p-chloroamphetamine. Neuropharmacology, **23:**915-924.

Olson, G.A., Kastin, A.J., Mitchell, G.F, Olson, R.D., Coy, D.H. and **Montalbano, D.M. (1978):** Effects of endorphin and enkephalin analogs on fear habituation in goldfish. Pharmacology, Biochemistry and Behavior, **9**:111-114.

Otsuka, M. and **Konishi, S. (1976):** Release of substance P like immunoreactivity from isolated spinal cord of newborn rats. Nature, **264**:83-84.

Ossipov, M.H., Lai, J., King, T., Vanderah, T.W., Malan, T.P.J., Hruby, V.J. and **Porreca, F. (2004):** Antinociceptive and nociceptive actions of opioids. Journal of Neurobiology, **61**, 126-148.

Palecek, J., Paleckova, V. and **Willis, W.D. (2002)**: The roles of pathways in spinal cord lateral and dorsal funiculi in signaling nociceptive somatic and visceral stimuli in rats. Pain, **96**:297-307.

Pan, H.L., Chen, S.R. and **Eisenach, J.C. (1999):** Intrathecal clonidine alleviates allodynia in neuropathic rats: interaction with spinal muscarinic and nicotinic receptors. Anesthesiology, **90**:509-514.

Park, T.J., Comer, C., Carol, A., Lu, Y., Hong, H.S. and **Rice, F.L. (2003):** Somatosensory organization and behaviour in naked mole-rats:II. Peripheral structure innervation and selective lack of neuropeptides associated with thermoregulation and pain. Journal of Comparative Neurology, **465**:104-120.

Park, T.J., Lu, Y., Jüttner, R., Smith EStJ., Hu, J., Brand, A., Wetzel, C., Milenkovic, N., Erdmann, B., Heppenstall, P.A., Laurito, C.E., Wilson, S.P. and **Lewin, G.R. (2008):** Selective Inflammatory Pain Insensitivity in the African Naked Mole-Rat (*Heterocephalus glaber*). *PloS Biology I* http:// www.plosbiology.org. **6**; Issue 1, *e13:0156*-0170.

Perl, E.R. (1968): Myelinated afferents innervating the primate skin and their response to noxious stimuli. Journal of Physiology, **197**, 593-615.

Pert, A. (1975): The cholinergic system and nociception in the primate: interactions with morphine. Psychopharmacologia, **44**:131-137.

Pert, A. (1987): Cholinergic and catecholaminergic modulation of nociceptive reactions. Interaction with opiates. In Neurotransmitters and Pain Control. Akil, H. and Lewis, J.W (**eds.**), pp. 1-63. Karger, Basel, U.S.A.

Pezalla, P.D. (1983): Morphine-induced analgesia and explosive motor behaviour in amphibian. Brain Research, **273**:297-305.

Pezalla, P.D. and **Stevens C.W. (1984):** Behavioural effects of morphine, levorphanol, dextrophan and Naloxone in the frog *Rana papiens.* Pharmacology, Biochemistry and Behavior, **21**:213-217.

Pircio, A.W., Fedele, C.T. and **Bierwagen, M.E. (1975):** A new method for the evaluation of analgesic activity using adjuvant –induced arthritis in the rat, European Journal of Pharmacology, **31**, 207-215.

Pomeranz, B., Wall, P.D. and **Webber, W.V. (1968):** Cord cells responding to the fine myelinated afferents from viscera, muscle and skin. Journal of Physiology, **199:**447-451.

Porro, C.A. and **Cavazzuti, M. (1993):** Spatial and temporal aspects of spinal cord and brainstem activation in the formalin pain model. Progressive Neurobiology, **41**:565-607.

Prado, W.A. and **Gonçalves, A.S. (1997)**: Antinociceptive effect of Intrathecal neostigmine evaluated in rats by two different pain models. Brazilian Journal of Medical and Biological Research, **30**:1225-1231.

Price, D.D. (1999): Psychological mechanisms of pain and analgesia. In Progress in Pain Research and Management, Vol. 15, IASP Press, Seattle, USA.

Price, D.D., Bennett, G.J. and **Raffi, A. (1989):** Psychophysical observations on patients with neuropathic pain relieved by sympathetic block. Pain, **36**:273-288.

Price, D.D., Long, S. and **Huitt, C. (1992):** Sensory testing of pathological mechanisms of pain in patients with reflex sympathetic dystrophy. Pain, **49**:163-173.

Proudfit, H.K. and **Anderson, E.G. (1975):** Morphine analgesia: blockade by raphe magnus lesions. Brain Research, **98**:612-619.

Qian, C., Li T., Shen, T.Y., Liberune-Graham, L., Eckman, J. and **Biftu, T. (1993):** Epibatidine is a nicotinic analgesic. European Journal of Pharmacology, **250**:R13-14.

Rao, T.S., Correa, L.D., Reid, R.T. and **Lloyd, G.K. (1996)**: Evaluation of Anti-nociceptive effects of Neuronal Nicotinic Acetylcholine Receptor (NAChR) ligands in the Rat Tail-flick Assay. Neuropharmacology, **35(4)**:393-405.

Raja, S.N., Campbell, J.N. and **Meyer, R.A. (1984):** Evidence for different mechanisms of primay and secondary hyperalgesia following heat injury to the glabrous skin. Brain **107**:1179-1188.

Ralston, H.J. and **Ralston, D.D. (1979):** The distribution of dorsal root axons in laminae I, II and III of the macaque spinal cord: a quantitative electron microscope study. Journal of Comparative Neurology, **184**:643-684.

Randall, L.O. and **Selitto, J.J. (1957):** A method for measurement of analgesic activity on inflamed tissue. Archives of International Pharmacodynamics, **111**:409-419.

Reeh, P.W., Bayer, J., Kocher, L. and **Handwerker, H.O. (1987)**: Sensitization of nociceptive cutaneous nerve fibres from the rat's tail by noxious mechanical stimulation. Experimental Brain Research, **65**:505-512.

Reeh, P.W. and **Steen, K.H. (1996)**: Tissue acidosis in nociception and pain. Progressive Brain Research, **113**:143-151.

Reynolds, D.V. (1969): Surgery in the rat during electrical analgesia induced by local brain stimulation. Science, **164**:444-445.

Rexed, B. (1952): The cytoarchitectonic organization of the spinal cord in the cat. Journal of Comparative Neurology, **96**:415-495.

Rexed, B. (1954): The cytoarchitectonic atlas of the spinal cord in the cat. Journal of Comparative Neurology, **100**:297-380.

Riedel, W. and **Neeck, G. (2001):** Nociception, pain and antinociception: Current concepts. Zoological Rheumatology, **60**:404-415.

Roberts, M.H.T. (1984): 5-hydroxytryptamine and antinociception. Neuropharmacology, **23**:1529-1536.

Roberts, R.G., Stevenson, J.E., Westerman, R.A. and **Pennefather, J. (1995)**: Nicotinic acetylcholine receptors on capsaicin-sensitive nerves. Neuroreport, **31**:1578-1582.

Rosland, J.H., Tjølsen, A., Mæhle, B. and **Hole, K. (1990):** The formalin test in mice: effect of formalin concentration. Pain, **42**:235-242.

Rosland, J.H. (1991): The Formalin test in mice; the influence of ambient temperature. Pain, **42**:235-242.

Rossier, M.F., Python, C.P., Burnay, M.M., Schlegel, W., Valloton, M.B. and **Capponi, A.M. (1993):** Thapsigargin inhibits voltage-activated calcium channels in adrenal glomerulosa cells. Biochemical Journal, **295**:309-312.

Sabbe, M.B. and **Yaksh, T.L. (1990):** Pharmacology of spinal opioids Journal of Pain and Symptom Management, **5**:191-203.

Sagen, J. and **Proudfit, H.K. (1981):** Hypoalgesia induced by blockade of noradrenergic projections to the raphe magnus: reduced by blockade of noradrenergic projections to the spinal cord. Brain Research, **223**:391-396.

Sahley, T.L. and **Berntson, G.G. (1979):** Antinociceptive effects of central and systemic administration of nicotine in the rat. Psychopharmacology. **65**:279-283.

Sargent, P.B. (1993): The diversity of neuronal nicotinic acetylcholine receptors. Annual Review of Neuroscience, **16**:403-443.

Satoh, M., Akaike, A. and **Takagi, H. (1979):** Excitation by morphine and enkephalin of single neurons of nucleus reticularis paragiganto cellularis in the rat: a probable mechanism of analgesic action of opioids. Brain Research, **169**:406-410.

Scheibel, M.E. and **Scheibel, A.B. (1968):** Terminal axonal patterns in the cat spinal cord. II. The dorsal horn. Brain Research, **9**, 32-58.

Schmauss, C., Hammond, D.L., Ochi, J.W. and **Yaksh, T.L. (1983):** Pharmacological antagonism of the antinociceptive effect of serotonin in the rat spinal cord. European Journal of Pharmacology, **90**:349-357.

Schmauss, C. and **Yaksh, T.L. (1984):** In vivo studies on spinal opiate receptor systems mediating antinociception. II. Pharmacological profiles suggesting differential association of mu, delta and kappa receptors with visceral, chemical and cutaneous thermal stimuli in the rat. Journal of Pharmacology and Experimental Therapeutics, **228**:1-12.

Schmelz, M., Schmidt, R., Weidner, C., Marita, H., Torebjörk H.E. and **Handwerker, H.O. (2003):** Chemical response pattern of different classes of C-nociceptors to pruritogens and algogens. Journal of Neurophysiology, **89**:2441-2448.

Schmidt, R.F. (1973): Control of the access of afferent activity to somatosensory pathways. In Handbook of Sensory Physiology, Vol.II, Somatosensory system. Iggo, A. (ed.) pp. 151-206. Springer-Verlag, Heidelberg, Berlin.

Segal, M. and **Sandberg, D. (1977):** Analgesia produced by stimulation of catecholamine nuclei in rat brain. Brain Research, **123:**369-372.

Selzer, M. and **Spencer, W.A. (1969):** Convergence of cutaneous and visceral pathways. Brain Research, **14**:331-348.

Sheng, C. and **Gary, A-J. (1995)**: Anatomical evidence for inputs to ventro-lateral medullary catecholaminergic neurons from the midbrain periaqueductal gray of the rat. Neuroscience Letters, **195(2)**:140-144.

Sherman, P.W., Jarvis, J.U. and **Braude, S.H. (1992):** Naked mole-rats. Scientific American, **267**:42-48.

Sherman, P.W. and **Jarvis, J.U.M. (2002)**: Extraordinary life spans of naked mole-rats (*Heterocephalus glaber*). Journal of Zoology (London). **258**:307-311.

Shibata, M., Ohkubo, T., Takahashi, H. and **Inoki, R. (1989):** Modified formalin test: characteristic biphasic pain response. Pain, **38**:347-352.

Simone, D.A., Sorkin, L.S., Oh, U., Chung, J.M., Owens, C., LaMotte, R.H. and **Willis, W.D. (1991):** Neurogenic hyperalgesia: central neural correlates in responses of spinothalamic tract neurons. Journal of Neurophysiology, **66**:228-246.

Sindrup, S.H. and **Jensen, T.S. (1999):** Efficacy of pharmacological treatments of neuropathic pain: an update and effect related to mechanism of drug action. Pain, **83(3)**:389-400

Slater, P. and **Blundell, C. (1980):** Role of brain catecholamines and 5-hydroxytryptamine in morphine induced temperature changes in normal and tolerant rats and mice. Naunyn-Schmiedeberg's Archives of Pharmacology, **313**:125-130.

Slater, P. and **Patel, S. (1983):** Autoradiographic localization of opiate κ receptors in the rat spinal cord. European Journal of Pharmacology, **92**:159-160.

Sorkin, L.S. and **McAdoo, D.J. (1993):** Amino acids and serotonin are released into the lumbar spinal cord of the anaesthetized cat following intradermal capsaicin injections. Brain Research, **607**:89-98.

Spande, T.F., Garraffo, M.W. Edward, M.W., Yeh, H.J.C., Pannel, L. and **Daly, J.W. (1992):** Epibatidine: A novel (Chloropyridyl)azabicycloheptane with potent analgesic activity from Ecuadoran poison frog. Journal of American Chemical Society, **114**:3475-3478.

Sperber, E.S., Romero, M-T. and **Bodnar, R.J. (1986)**: Selective potentiations in opioid analgesia following scopolamine pretreatment. Psychopharmacology, **89**:175-176.

Spivak, V., Lin, A., Beebe P., Stoll L. and Gentille L. (2004): Identification of a Neurosteroid Binding site Contained within the $GluR_2$ – SIS2 Domain. Lipids, **39**: No.8

Steen, K.H., Reeh, P.W., Anton, F. and **Handwerker, H.O. (1992)**: Protons selectively induce lasting excitation and sensitization to mechanical stimulation of nociceptors in rat skin, in vitro. Journal of Neuroscience, **12**:86-95.

Steen, K.H. and **Reeh, P.W. (1993)**: Actions of cholinergic agonists and antagonists on sensory nerve endings in rat skin, in vitro. Journal of Neurophysiology, **70**:397-405.

Steinbusch, H.W.M. (1981): Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. Neuroscience, **6**:557-618.

Steiner, T.J. and **Turner, L.M. (1972):** Cytoarchitecture of the rat spinal cord. Journal of Physiology, **222:**123-124P.

Siegmund, E., Cadmus, R. and **Lu, G. (1957):** A method for evaluating both non-narcotic and narcotic analgesics. Proceedings of Society of Experimental Biology and Medicine, **95**:729-731.

Suguira, Y. (1975): Three dimensional analysis of neurones in the substantia gelatinosa Rolandi. Proceedings of Japanese Academy of Sciences, **51**, 336-341.

Surmeier, D.J., Honda, C.N. and **Willis, W.D. (1986)**: Responses of primate spinothalamic neurons to noxious thermal stimulation of glabrous and hairy skin. Journal of Neurophysiology, **56**:328-350.

Sullivan, J.P., Decker, M.W., Brioni, J.D., Donnelly, R. D., Anderson, D.J., Bannon, A.W., Kang, C.H., Adams, P., Piatoni, K. M., Buckley, M.J., Gopalakrishnan, M., Williams, M. and **Arneric, S.P. (1994):** (±)-Epibatidine elicits a diversity of in vitro and in vivo effects mediated by nicotinic acetylcholine receptors. Journal of Pharmacology and Experimental Therapeutics, **271**:624-631.

Szentagothai, J. (1964): Neuronal and synaptic arrangement in substantia gelatinosa rolandi. Journal of Comparative Neurology, **122**:219-240.

Taber, R.I. (1974) Predictive value of analgesic assays in mice and rats. In Advances in Biochemical Psychopharmacology, Narcotic Antagonists, **Vol 8.** Braude, M.C., Harris, L.S., May, E.L., Smith, J.P. and Villareal, J.E. (**eds**.), pp. 191-211. Raven Press, New York, U.S.A.

Taddese, A., Seung-Yeol, N. and **McCleskey, E.W. (1995):** Selective opioid inhibition of small nociceptive neurons. Science, **270**:1366-1369.

Takagi, H. (1980): The nucleus reticularis paragigantocellularis as a site of analgesic action of morphine and enkephalin. Trends Pharmacology Science, **1**:182-184.

Takahashi, T. and **Otsuka, M. (1975):** Regional distribution of substance P in the spinal cord and nerve roots of the cat and the effect of dorsal root section. Brain Research, **87**: 1-11.

Takahashi, T., Shibata, M., and **Naruse, S. (1984):** A modified formalin test for measuring analgesia in mice. Japanese Journal of Oral Biology, **26**:543-548.

Tania, B., Mahinda, M.S., Blaise, M., Lovell, M.S. and **Bradley K.T. (2004):** Morphine-induced analgesia, hypotension, and bradycardia are enhanced in hypertensive rats. Anesthesia and Analgesia, **98**:1698-1704.

Timmermann, L., Ploner, M., Haucke, K., Schmitz, F., Baltissen, R. and **Schnitzler, A. (2001):** Differential coding of pain intensity in the human primary and secondary somatosensory cortex. Journal of Neurophysiology, **86**:1499-1503.

Tjølsen, A., Berge, O.G., Eide, P.K., Broch, O.J. and **Hole, K. (1988):** Apparent hyperalgesia after lesions of the descending serotonergic pathways is due to increased tail skin temperature. Pain, **33**:225-231.

Torebjörk, H.E. and **Hallin, R.G. (1973):** Perceptual changes accompanying controlled preferential blocking of A- and C-fibre responses in intact human skin nerves. Experimental Brain Research, **16:**321-332.

Torebjörk, H.E. and **Hallin, R.G. (1974):** Identification of afferent C units in intact human skin nerves. Brain Research, **67**:387-403.

Torebjörk, H.E., Lundberg, L.E.R. and **LaMotte, R.H. (1992):** Central changes in processing of mechanoreceptive input in capsaicin-induced secondary hyperalgesia in humans. Journal of Physiology (London), **448**:765-780.

Torebjörk, H.E., Wahren, L.K., Walli, G., Hallin, R. and **Koltzenberg, M. (1995):** Noradrenaline-evoked pain in neuralgia. Pain, **63**:11-20.

Towett, P.K. and **Kanui, T.I. (1993):** Effects of pethidine, acetylsalicylic acid, and indomethacin on pain and behaviour in the mole-rat. Pharmacology, Biochemistry and Behavior, **4**:153-159.

Towett, P.K. and **Kanui, T.I. (1995):** Hyperalgesia following administration of morphine and pethidine in root rat (*Tachyoryctes splendens).* Journal of Veterinary Pharmacology and Therapeutics, **18**:68-71.

Towett, P.K., Kanui, T.I. and **Juma, F.D. (2006):** Stimulation of mu and delta opioid receptors induces hyperalgesia while stimulation of kappa receptors induces antinociception in the hot-plate test in the naked mole-rat. Brain Research Bulletin, **71**:60-68.

Towett, P.K., Kanui, T.I., Maloiy, G.M.O., Juma, F. and **Miaron, J.O.O. (2009)**: Activation of mu, delta or kappa opioid receptors by DAMGO, DPDPE, U-50488 or U-69593 respectively causes antinociception in the formalin test in the naked mole-rat (*Heterocephalus glaber).* Pharmacology, Biochemistry and Behavior, doi, 10.1016/j.pbb.2008.09.011.

Tyres, M.B. (1980): A classification of opiate receptors that mediate antinociception in animals. British Journal of Pharmacology, **69**, 503-512.

Upton, N., Sewell, R.D.E. and **Spencer, P.S.J. (1982):** Differentiation of potent μ- and κ-opiate agonists using heat and pressure antinociceptive profiles and combined potency analysis. European Journal of Pharmacology, **78**:421-429.

Urca, G., Frank, H., Liebeskind, J.C. and **Taylor, A.N. (1977):**Morphine and enkephalin, analgesic and epileptic properties. Science, **197**:83-86.

Vander Wende, C. and **Margolin, S.** Analgesic tests based upon experimental induced acute abdominal pain in rats. Federation Proceedings, **15:**494.

Vane, J.R. and **Botting, R. (1987)**: Inflammation and the mechanism of action of anti-inflammatory drugs. FASEB Journal, **1**:89-96.

Vilaro, M.T., Wiederhold, K.H., Palacios, J.M.and **Mengod, G. (1992)**: Muscarinic M2 receptor mRNA expression and receptor binding in cholinergic and non-cholinergic cells in the rat brain: a correlative study using in situ hybridization histochemistry and receptor autoradiography. Neuroscience, **47**:367-393.

Vinegar, R., Truax, J.F. and **Selph, J.L. (1976):** Quantitative comparison of the analgesic and Anti-inflammatory activities of aspirin, phenacetin and acetaminophen in rodents. European Journal of Pharmacology, **37**:23-30.

Waelbroeck, M., Tastenoy, M., Camus, J. and **Christophe, J. (1990):** Binding of selective antagonists to four muscarinic receptors (M-1 to M-4) in rat forebrain. Molecular Pharmacology, **38**:267-273.

Wagman, I.H. and **Price, D.D. (1969):** Responses of dorsal horn cells of M. *mulatta* to cutaneous and sural nerve A and C fibre stimuli. Journal of Neurophysiology, **32**: 803-817.

Waldeyer, W. (1888): Das Gorilla-Rückenmark. Abhanglungen der Preussichen Akademie der Wisssenschaft en zu Berlin, **3**:1-147.

Walker, D.L., McGlynn, T., Grey, C., Ragozzino, M. and **Gold, P.E. (1991)**: Naloxone modulates the behavioral effects of cholinergic agonists and antagonists. Psychopharmacology, **105**:57-62.

Wall, P.D. (1967): The laminar organization of dorsal horn and effects of descending impulses. Journal of Physiology, **188**:403-423.

Wall, P.D. (1973): Dorsal horn electrophysiology. In Handbook of Sensory Physiology, **Vol. 2**. Iggo, A. (**ed.**) pp. 253-270. Springer Verlag, U.K.

Wall, P.D. (1983): Mechanisms of acute and chronic pain. In Advances in Pain Research and Therapy, **Vol. 6** Kruger, L. and Liebeskind, J.C. (**eds.**) pp. 95-104. Raven Press, New York, U.S.A.

Wall, P.D. and **Sweet W.H. (1967):** Temporary abolition of pain in man. Science, **155**: 108-109.

Wall, P.D., Merrill, E.G. and **Yaksh, T.L. (1979):** Responses of single units in Laminae II and III of cat spinal cord. Brain Research, **160**:245-260.

Wang, N., Orr-Urtreger, A., Chapman, J., Rabinowitz, R. and **Korczyn, A.D. (2004):** Nicotinic acetylcholine receptor 5 subunits modulate oxotremorine-induced salivation and tremor. Journal of Neurological Sciences, **222**:87-91.

Ward, S.J. and **Takemori, A.E. (1983):** Relative involvement of mu, kappa and delta receptor mechanisms in opiate-mediated antinociception in mice. Journal of Pharmacology and Experimental Therapeutics, **224**, 525-530.

Warncle, T., Stubhang, A. and **Jorum, E. (2000):** Pre-injury treatment with morphine or ketamine inhibits the development of experimentally induced secondary hyperalgesia in man. Pain, **86**:293-303.

Wieselfield-Hallin, Z., Hockfelt, T., Lundberg, J.M., Forssmann, W.G., Reinecke, M., Tschopp, F.A. and **Fischer, J.A. (1984):** Immunoreactive calcitonin gene-related peptide and substance P coexist in sensory neurones of the spinal cord and interact in spinal behavioral responses of the rat. Neuroscience Letters, **52**:199-204.

Willis, W.D. Jr. (1985): The Pain System. New York, Karger.

Willis, W.D. and **Coggeshall, R.E. (1978):** Sensory mechanisms of the spinal cord. Willis, W.D. and Coggeshall, R.E. (**eds.**) Plenum Press, New York.

Willis, W.D. and **Coggeshall, R.E**. (**1991**): Sensory mechanisms in spinal cord, 2nd ed., **Vol. 3**. New York, NY, Plenum Press.

Willis, W.D. and **Westlund, K.N. (1997):** Neuroanatomy of the pain system and of the pathways that modulate pain. Journal of Clinical Neurophysiology, **14**:2-31.

Wilson, P.R. and **Yaksh, T.L. (1978):** Baclofen is antinociceptive in the spinal intrathecal space of animals. European Journal of Pharmacology. **51**:323-330.

Wood, P.L. and **Stotland, L.M. (1980)**: Actions of enkephalin, μ- and partial agonist analgesics on acetylcholine turnover in rat brain. Neuropharmacology, **19**:975-982.

Woolf, C.J. and **Chong, M.S. (1983):** Preemptive analgesia-treating postoperative pain by preventing the establishment of central sensitization. Anesthesia Analgesia, **77**:362-379.

Woolfe, G. and **MacDonald, A.O. (1944):** The evaluation of the analgesic action of pethidine hydrochloride (Demerol). Journal of Pharmacology and Experimental Therapeutics, **120**:52-57.

Xu, J.Y. and **Tseng, L.F. (1994):** Role of nitric oxide/cyclic GMP in i.c.v. administered β-endorphin-and (+)-cis-dioxolane-induced antinociception in the mouse. European Journal of Pharmacology, **262**:223-231.

Xu, Z., Tong, C. and **Eisenach, J.C. (1996):** Acetylcholine stimulates the release of nitric oxide from rat spinal cord. Anesthesiology, **85**:107-111.

Xu, Z., Tong, C., Pan, H.L., Cerda, S.E. and **Eisenach, J.C. (1997):** Intravenous morphine increases release of nitric oxide from spinal cord by an α-adrenergic and cholinergic mechanism. Journal of Neurophysiology, **78**:2072-2078.

Xu, Z., Chen, S.R., Eisenach, J.C. and **Pan, H.L. (2000):** Role of spinal muscarinic and nicotinic receptors in clonidine-induced nitric oxide release in a rat model of neuropathic pain. Brain Research, **861**:390-398.

Yael, V-R., Jane, G., Gregory, W.T. and **Charles, C. (2000):** Mu opioid analgesia and analgesic tolerance in two mouse strains: C57BL/6 and 129/SVJ. Proceedings of West Pharmacological Society, **43**:15-17.

Yaksh, T.L. (1979): Direct evidence that spinal serotonin and noradrenaline terminals mediate the spinal antinociceptive effects of morphine in the periaqueductal gray. Brain Research, **160:**180-185.

Yaksh, T.L. (1987a): Opioid receptor systems and endorphins: a review of their spinal organization. Journal of Neurosurgery, **64**:157-176.

Yaksh, T.L. (1987b): Spinal opiates: a review of their effect on spinal function with emphasis on pain processing. Anaesthesiologica Scandanavica, **31**:25-37.

Yaksh, T.L. (1991): Tolerance: Factors involved in changes in dose-effect relationship with chronic drug exposure. In Towards a New Pharmacotherapy of Pain. Basbaum, A.I. and Besson J.M. (**eds**.), pp. 157-179. John Wiley and Sons Ltd., U.S.A.

Yaksh, T.L., Dirksen, R. and **Harty, G.J. (1985):** Antinociceptive effects of intrathecally injected cholinomimetic drugs in the rat and the cat. European Journal of Pharmacology, **117**:81-88.

Yaksh, T.L. and **Rudy, T.A. (1977):** Studies on the direct spinal action of narcotics in the production of analgesia in the rat. Journal of Pharmacology and Experimental Therapeutics, **202**:411-428.

Yaksh, T.L. and **Wilson, P.R. (1979):** Spinal serotonin terminal system mediates antinociception. Journal of Pharmacology and Experimental Therapeutics, **208**:446-453.

Zhuo, M. and **Gebhart, G.F. (1991):** Tonic cholinergic inhibition of spinal mechanical transmission. Pain, **46**:211-222.

Zimmerman, M. (1983): Ethical guidelines for investigation of experimental pain in conscious animals. Pain, **16**:109-110.

Zotterman, Y. (1939): Touch, pain and tickling: an electrophysiological investigation on cutaneous sensory nerves. Journal of Physiology, **95**:1-28.

APPENDICES:

APPENDIX 1: TIME-COURSE (SECONDS) OF PAIN BEHAVIOUR FOLLOWING INTRADERMAL INJECTION OF TWENTY MICROLITRE OF 10% FORMALIN OR 0.9% SALINE INTO THE DORSAL RIGHT HIND PAW.

: TIME SPENT IN PAIN BEHAVIOUR FOLLOWING FORMALIN ADMINISTRATION														
AL	SEX	WT (gms)	0-5 min	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45	45-50	50-55	55-60
	F	32.8	72.11	0	0	0	0	4.37	8 67	10 24	34 77	46 12	96 19	62 13
	F	30.2	96 34	0	0	0	0	5.22	0	12 71	37 67	59 56	132 97	97 31
	F	34.3	121.23	0	0	0	0	0	0	102 44	77.39	67 56	93 02	78 24
	F	34.1	33.81	0	0	0	0	0	0	8 88	33.88	18 51	25 09	15 41
	M	30.1	75.13	0	0	0	15 29	22.48	33.87	40 99	64 65	137 77	133 33	111 03
	M	31.4	96.21	0	0	0	46 03	4 62	128 57	119 04	78 66	104 29	80 82	116 34
	F	33.9	88.08	0	0	0	3.76	5.77	8 34	37 91	28 14	27 22	45 99	76 62
	M	31.6	116.08	0	0	0	0	0	3 77	23 25	59 87	110 06	59 23	52 25
N			82.47	0	0	0	10.22	6.115	28.518	49.035	51 8788	72 302	93 57	80 065
			9.802726	0	0	0	5.72784	2 6083	15 60687	15 16202	7.29127	14 955	13.75597	11.757

A: TIME SPENT IN PAIN BEHAVIOUR FOLLOWING SALINE ADMINISTRATION														
MAL	SEX	WT (gms)	0-5 min	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45	45-50	50-55	55-60
9	F	31 7	0	0	0	0	1.9	0	0	0	0	0	0	0
10	M	32.2	0	0	0	25.71	7.44	0	0	6 54	0	17.47	0	0
11	F	33.3	1 72	1.35	4 07	0	0	0	0	0	13 28	0	0	0
12	F	29.7	0	0	0	0	0	0	0	0	0	0	0	0
13	M	31.3	0	0	0	0	0	0	0	0	0	0	0	0
14	F	30.8	0	0	0	0	0	0	0	0	0	0	0	0
15	F	32.8	0	0	0	0	0	1 38	0	0	0	0	0	0
16	F	30.2	0	0	0	0	0	0	0	0	0	0	0	0
AN			0.215	0 169	0 5088	3.21375	1 1675	0.1725	0	0.8175	1 66	2 18375	0	0
M			0.215	0 169	0 5088	3.21375	0.92639	0 1725	0	0.8175	1 66	2 18375	0	0

tment/Time rval	0-5 min	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45	45-50	50-55	55-60
alin 10%	82.47	0	0	0	10.22	6 115	28 52	49 035	51 879	72 302	93 57	80 07
ne	0.215	0.169	0.5088	3.2138	1.1675	0.1725	0	0.8175	1 66	2 1838	0	0

APPENDIX 2: TIME (SECONDS) SPENT IN PAIN BEHAVIOUR IN THE FORMALIN TEST.

DRUG	DOSE (µg/kg or mg/kg)	n	EARLY PHASE (0-5 minutes)	LATE PHASE (35-60 minutes)
			MEAN ± S.E.M	MEAN ± S.E.M
Controls	0	8	87.37 ± 9.80	60.43 ± 11.13
Oxotremorine	10 µg/kg	6	70.36 ± 7.57	49.33 ± 4.01
	20 µg/kg	6	45.37 ± 6.41	31.13 ± 8.15
	50 µg/kg	6	27.85 ± 4.58	26.63 ± 13.85
	100 µg/kg	6	24.56 ± 4.00	22.76 ± 5.36
Atropine	2.5 mg/kg	6	83.13 ± 5.48	56.82 ± 3.64
Naloxone	2.5 mg/kg	6	85.83 ± 5.42	55.53 ± 4.99
Oxotremorine plus Atropine	20 µg/kg + 2.5 mg/kg	6	70.41 ± 3.99	50.08 ± 2.36
Oxotremorine plus Naloxone	20 µg/kg + 2.5 mg/kg	6	9.02 ± 1.90	1.86 ± 1.63
Epibatidine	0.5 µg/kg	6	76.21 ± 9.13	42.39 ± 8.75
Epibatidine	1 µg/kg	6	57.68 ± 2.77	12.76 ± 4.92
Epibatidine	2 µg/kg	6	44.30 ± 3.08	6.66 ± 3.71
Epibatidine	3 µg/kg	6	31.67 ± 3.14	0.41 ± 0.23
Mecamylamine	50 µg/kg	6	72.54 ± 2.48	52.48 ± 3.50
Epibatidine plus Mecamylamine	2 µg/kg + 50 µg/kg	6	83.32 ± 2.11	51.36 ± 1.84
Epibatidine plus Naloxone	2 µg/kg + 2.5 mg/kg	6	21.42 ± 2.29	0.93 ± 0.61

APPENDIX 3: RESPONSE LATENCIES (SECONDS) IN THE HOT PLATE TEST.

Drug/Dose	n	Hot plate response latency
Controls (0)	8	5.29 ± 0.10
Oxotremorine (10 μg/kg)	6	5.70 ± 0.17
Oxotremorine (20 μg/kg)	6	9.81 ± 0.72
Oxotremorine (50 μg/kg)	6	14.38 ± 1.05
Oxotremorine (100 μg/kg)	6	18.88 ± 0.78
Atropine (2.5 mg/kg)	6	5.65 ± 0.34
Naloxone (2.5 mg/kg)	6	5.96 ± 0.16
Oxotremorine (20 μg/kg) plus Atropine (2.5 mg/kg)	6	6.02 ± 0.02
Oxotremorine (20 μg/kg) plus Naloxone (2.5 mg/kg)	6	11.99 ± 0.75
Epibatidine 0.5 μg/kg	6	5.96 ± 0.09
Epibatidine 1 μg/kg	6	7.73 ± 0.23
Epibatidine 2 μg/kg	6	9.12 ± 0.75
Epibatidine 3 μg/kg	6	14.99 ± 0.72
Mecamylamine 50μg/kg	6	6.44 ± 0.46
Epibatidine 2 μg/kg plus Mecamylamine 50μg/kg	6	5.65 ± 0.14
Epibatidine 2 μg/kg plus Naloxone 2.5 mg/kg	6	11.34 ± 0.67

APPENDIX 4: RESPONSE LATENCIES (SECONDS) IN THE TAIL-FLICK TEST.

Drug/Dose	n	Tail-flick latency
Controls (0)	8	5.48 ± 0.23
Oxotremorine10 μg/kg	6	5. 54 ± 0.15
Oxotremorine 20 μg/kg	6	6.62 ± 0.30
Oxotremorine 50 μg/kg	6	8.03 ± 0.12
Oxotremorine 100 μg/kg	6	8.82 ± 0.15
Atropine 2.5 mg/kg	6	6.17 ± 0.12
Naloxone 2.5 mg/kg	6	5.91 ± 0.25
Oxotremorine 20 μg/kg plus Atropine 2.5 mg/kg	6	5.66 ± 0.31
Oxotremorine 20 μg/kg plus Naloxone 2.5 mg/kg	6	8.89 ± 0.14
Epibatidine 0.5 μg/kg	6	5. 57 ± 0.41
Epibatidine 1 μg/kg	6	6.88 ± 0.49
Epibatidine 2 μg/kg	6	7. 58 ± 0.35
Epibatidine 3 μg/kg	6	8.43 ± 0.11
Mecamylamine 50μg/kg	6	6.39 ± 0.34
Epibatidine 2 μg/kg plus Mecamylamine 50μg/kg	6	5.77 ± 0.34
Epibatidine 2 μg/kg plus Naloxone 2.5 mg/kg	6	9.22 ± 0.27