

The pathophysiology of UVA-light induced hyperalgesia

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A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy.

Johannesburg, 2009

DECLARATION

The thesis is submitted in the optional format, approved by the Faculty of Health Sciences, University of the Witwatersrand, of published work and work submitted for publication with encompassing introduction and conclusion.

I declare that the work contained in this thesis is my own, unless otherwise stated.

This work has not been submitted before for any degree or examination at any other university.

Signed on the _____ day of _____, 2009

As part of my Declaration, contributions of each author to each of the published studies are outlined below.

Chapter 2.

Themistocleous A, Fick L, du Plessis I, Mitchell B and Mitchell, D. (2006). Exposure of the rat tail to ultraviolet A light produces sustained hyperalgesia to noxious thermal and mechanical challenges. *Journal of Neuroscience Methods* **152**, 267-273.

The idea for this study was my own. I prepared the equipment, collected and analysed the data, and wrote the manuscript. Linda Fick assisted with the data collection and reviewed the manuscript. Bridget Mitchell prepared and performed the histological analysis of the tissue samples taken from the rat tails. Irne du Plessis and Duncan Mitchell helped with interpretation of the data and reviewed the manuscript.

Chapter 3.

Themistocleous A, Kamerman P and Mitchell D. (2007). Pre-emptive ring-block with bupivacaine prevents the development of thermal hyperalgesia, but not sustained mechanical hyperalgesia, in rat tails exposed to ultraviolet A light. *The Journal of Pain* **8**, 208-214.

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Chapter 4.

Themistocleous A, Kamerman P and Mitchell D. (2008). Discharge patterns of nociceptive primary afferent fibres in the rat coccygeal nerve after UV_A-light exposure. Submitted to *Journal of Pain*.

The idea for this study was my own. I prepared the equipment, collected and analysed the data, and wrote the manuscript. Peter Kamerman and Duncan Mitchell helped with interpretation of the data and reviewed the manuscript.

The Animal Ethics Screening Committee of the University of the Witwatersrand approved all procedures performed in my experiments, and I have included a copy of the animal ethics clearance certificates in an appendix.

As supervisors of the candidate, we confirm that he has described the roles of his co-authors accurately and that he has functioned as principal investigator for all three studies.

Peter Kamerman: _____

Date: _____

Duncan Mitchell: _____

Date: _____

ABSTRACT

In this thesis I describe the development of an animal model of sustained hyperalgesia induced by exposure to ultraviolet (UV) A light to the rat's tail, and the role of the C-fibre barrage and peripheral afferent fibre sensitization in this model of hyperalgesia. Exposure of rats' tails to UV_A-light caused hyperalgesia to a noxious thermal challenge, immersion of the rats' tails into 49°C water, and a noxious mechanical challenge, application of a static force of 3.9N by a bar algometer onto the rats' tails. The hyperalgesia to the thermal challenge lasted eight days and hyperalgesia to the mechanical challenge continued for up to 16 days. Despite the sustained hyperalgesia, rats exposed to UV_A-light showed no overt signs of morbidity as they gained weight normally and were mobile throughout the study. Histological examination of rat tail tissue showed mild, chronic inflammation in rats exposed to UV_A-light and in rats that had their tails covered with a protective layer of aluminium foil during UV_A-light exposure. This inflammation was therefore not responsible for the behavioural hyperalgesia.

To investigate the role of C-fibre barrage in the development of hyperalgesia after UV_A-light exposure, I pre-emptively blocked C-fibre activation during UV_A-light exposure with the local anaesthetic bupivacaine. Injection of bupivacaine (1ml of 0.5%), into the base of the tail prevented the development of thermal hyperalgesia to tail immersion in 49°C water. However, it did not prevent the development of hyperalgesia to a noxious punctate challenge. Thus the sustained mechanical hyperalgesia did not depend on the activation of the C-fibre barrage, but thermal

hyperalgesia did depend on the activation of a C-fibre barrage during the conditioning event of UV_A-light exposure.

Lastly, in rats anaesthetised with enflurane, I examined the responses of coccygeal primary afferent fibres to noxious thermal and mechanical stimulation after UV_A-light exposure of their receptive fields on the tail. I investigated only pure nociceptive afferents and ignored those afferents that responded to challenges in the noxious and non-noxious ranges. The peak firing rates and areas under the curve of post-challenge histograms, a measure of neuronal firing over time, of A δ - and C-fibres were increased when noxious blunt and punctate challenges were applied to the rats' tails after UV_A-light exposure, showing that A δ - and C-fibres that encode for noxious mechanical challenges were sensitized. The peak firing rate of C-fibres that were responsive to noxious thermal challenges were not increased after UV_A-light exposure. Therefore, thermal hyperalgesia was probably mediated by sensitization of central nervous system neurones.

In summary, I developed a model of sustained mechanical and thermal hyperalgesia caused by UV_A-light exposure of the rat tail. The thermal hyperalgesia was initiated by the C-fibre barrage, while mechanical hyperalgesia did not depend on the C-fibre barrage and peripheral afferent sensitization of A δ - and C-fibres could account for the mechanical hyperalgesia.

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2. Themistocleous A, Kamerman P and Mitchell D. (2007). Pre-emptive ring-block with bupivacaine prevents the development of thermal hyperalgesia, but not sustained mechanical hyperalgesia, in rat tails exposed to ultraviolet A light. *The Journal of Pain* **8**, 208-214.

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LIST OF ABBREVIATIONS

AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
CINC	Cytokine-induced neutrophil chemoattractant
COX	Cyclooxygenase
CGRP	Calcitonin gene-related peptide
ICAM	Intracellular adhesion molecule
VCAM	Vascular cell adhesion molecule
IL	Interleukin
NK	Neurokinin
NMDA	N-methyl-D-aspartate
NGF	Nerve growth factor
PGE ₂	Prostaglandin E ₂
PGD ₂	Prostaglandin D ₂
PGF _{2α}	Prostaglandin F _{2α}
TNF	Tumour necrosis factor
UV	Ultraviolet
VRP	Ventral root potentials
WDR	Wide dynamic range

PREFACE

Chronic pain states are characterised by persistent pain, pain that persists longer than the temporal course of natural healing, enhanced pain sensation to noxious challenges (hyperalgesia) and the perception of non-noxious challenges as painful (allodynia). Although progress has been made over the last 25 years explaining the pathophysiological processes causing chronic pain, this increased knowledge has not translated adequately into clinical benefits. At best, chronic pain sufferers will experience sub-optimal pain relief through combination therapy of pharmacological agents and alternative treatments, such as acupuncture.

Inadequate therapeutic success may result partly from the paucity of suitable animal experimental models of chronic pain and hyperalgesia. The majority of current animal models are not ideal. For example, the duration of hyperalgesia in most models tends to be too short for antihyperalgesic agents to be administered after the hyperalgesia has developed, which is commonly the situation in the clinical setting, or they leave the experimental animals in persistent pain, which leads to a constitutive deterioration in their health and, in addition to being unethical, may confound their response to nociceptive testing. In addition, the majority of chronic pain models employ either damage to nerves or injection of a noxious agent into tissue. As a result, the main focus of experimental models of chronic pain are models that mimic neuropathic pain, or models that mimic arthritic pain.

I identified a model of hyperalgesia, previously described in the literature, caused by UV_A-light. After exposure of the rat hind paw to UV_A-light, rats developed

hyperalgesia to multiple noxious challenges for up to one week. Therefore, UV_A-light induced hyperalgesia would seem suitable for the testing of analgesic and anti-hyperalgesic agents, as the agents could be given after the conditioning event, thereby more closely mimicking the clinical setting. Although the behavioural characteristics of the hyperalgesia have been well described in animals, several important issues remained unclear. For example, UV_A-light caused chronic inflammation in human skin and it was assumed that the similar inflammation occurred in rats and was responsible for the hyperalgesia. Yet there are no reports of studies that have histologically examined rat, or any other animal, tissue after UV_A-light exposure to confirm the presence of inflammation, nor correlated the possible inflammatory changes with behavioural hyperalgesia. In addition, there are no reports of studies that assessed the health of rats to ensure no constitutive deterioration. Moreover, data from studies where UV_A-light was the conditioning event has been used interchangeably with data from studies using UV_B-light as the conditioning event. There was therefore an underlying assumption that UV_A- and UV_B- light caused hyperalgesia through the same mechanisms. I felt such an assumption was not justified as the two wavelengths of light differed remarkably from each other in their physical properties and in the manner they caused erythema. I therefore developed my own new model of UV_A-light induced hyperalgesia, and aimed to re-evaluate UV_A-light as a conditioning challenge, primarily if there were any histological changes in rat tissue, any constitutive deterioration in rat physical condition, and to explore relevant physiological mechanisms of how UV_A-light caused hyperalgesia.

My thesis is presented as a series of experimental papers. Chapter 2 and 3 have been published in peer-reviewed journals and chapter 4 has been submitted for publication.

The main focus of the my PhD thesis was validation of UV_A-light as a suitable conditioning challenge for causing sustained hyperalgesia, and description of the underlying pathophysiological mechanisms that caused the hyperalgesia. Chapter 1 is therefore an overview of UV-light and the proposed mechanisms for how UV-light causes hyperalgesia. I have not included a description of the basic mechanisms of nociception and hyperalgesia, as there are texts, such as Textbook of Pain, that provide a far more detailed and thorough description of such topics than I could do.

In the first experimental study of my thesis (Chapter 2) I aimed to establish an animal model of sustained hyperalgesia using both noxious thermal and mechanical challenges in rats by exposing their tails to UV_A-light and to correlate those findings with inflammation in the tail. My second aim was to ensure that the rats experienced no chronic stress, as determined by weight loss and altered behaviour during experimentation. I found that rats exposed to UV_A-light developed hyperalgesia to both noxious thermal and mechanical challenges, and that peripheral inflammation did not account for the sustained hyperalgesia. I therefore investigated the possible mechanisms that may account for the sustained hyperalgesia. These experiments are described in Chapter 3 and 4.

In Chapter 3 I described the experiment I undertook to explore the role of the C-fibre barrage in causing UV_A-light hyperalgesia. I administered a long-acting local anaesthetic into the base of the tail before UV_A-light exposure to prevent C-fibre activation during UV_A-light exposure. I found that thermal hyperalgesia, but not mechanical hyperalgesia was prevented by blocking the C-fibre barrage. This finding

was novel and showed that peripheral afferent sensitization did not account for the thermal hyperalgesia.

In my final experiment (Chapter 4) I sought to explore the firing properties of peripheral afferent fibres in the rat-tail after UV_A-light exposure. I examined the responses of coccygeal primary afferent fibres to noxious thermal and mechanical challenges after UV_A-light exposure of their receptive fields on the tail. I confirmed that the fibres' responses to noxious thermal challenges were unaltered by UV_A-light exposure but that the responses to noxious mechanical challenges were enhanced, which corroborated my findings in Chapter 3.

In Chapter 5 I have summarised the main findings and conclusions of my three studies and postulated on possible physiological mechanisms causing UV_A-light hyperalgesia and possible studies for the future.

CHAPTER 1

Introduction

1. Ultraviolet light and mechanisms of ultraviolet-light induced pain and hyperalgesia

1.1 Ultraviolet light

Ultraviolet (UV) light is electromagnetic radiation that is composed of a range of wavelengths between 100 and 400 nm and is divided into UV_A (320-400 nm), UV_B (280-320 nm) and UV_C (100-280 nm) light. UV_A-light is further subdivided into UV_{AI} (340-400 nm) and UV_{AII} (320-340 nm). UV-light constitutes approximately five percent of the energy of extraterrestrial radiation, and has numerous clinical effects, both in the short and long term. Acute effects include sunburn inflammation, tanning, local and systemic immunosuppression, vitamin D synthesis and thickening of the stratum corneum, epidermis and dermis of the skin. Chronic effects include photoaging and skin cancer. In this thesis I shall discuss the processes by which UV_A- and UV_B-light affect nociception and I shall not discuss UV_C-light, as it has not been used in models of acute nociception nor hyperalgesia.

In my thesis I shall focus only on studies that describe how UV_A- and UV_B-light cause pain and hyperalgesia at the site of exposure and in the central nervous system. I shall discuss all studies published describing UV_A- and UV_B-light hyperalgesia and possible pathophysiological mechanisms. However, very little work has been done exploring spinal cord changes after UV-light exposure, and similarly very few electrophysiological studies have been conducted using UV_A- and UV_B-light as conditioning challenges. I have included this information in order to provide as comprehensive overview as possible of UV-light induced hyperalgesia.

1.1.1 Mechanisms of UV-light induced injury

UV-light causes damage to skin through a cascade of reactions initiated by the absorption of UV-light energy by photoresponsive molecules in the skin called chromophores (Young, 1997). Each chromophore has a unique absorptive spectrum, a wavelength of light that has sufficient energy to initiate a chemical change in the chromophore. UV-light energy has the potential to initiate the biological response by either altering a target molecule, such as DNA, directly or to alter molecules indirectly through the generation of reactive oxygen species (Young, 1997). Chromophores in the epidermis and dermis that have absorptive spectra in the UV-light range include nucleic acids, urocanic acid, proteins such as collagen and elastin, melanins and their precursors (Hawk & Parrish, 1983; Young, 1997). Different wavelengths are absorbed by different chromophores, so the pattern of reaction occurring after exposure to UV-light radiation depends on the UV-light wavelength. For example, DNA absorbs UV_B-light and does not absorb UV_A-light. UV_A-light acts primarily on melanins, NADH and NADPH, and UV_B-light acts on nucleic acids, urocanic acid and proteins (Anderson & Parrish, 1981).

UV_A-light causes most of its injurious effects, in both humans and other animals, through generation of reactive oxygen species. UV_A-light upregulates xanthine oxidase with the subsequent production of reactive oxygen species, such as superoxide and hydrogen peroxide (Hruza & Pentland, 1993; Halliday, 2005). When cells' ability to neutralise oxygen radicals are exceeded cell damage occurs. Oxygen radicals cause significant damage to normal cellular processes by damaging DNA,

lipid peroxidation, and denaturation of proteins (Pathak *et al.*, 1999; Halliday, 2005). Further damage also can be caused by increased production of reactive nitrogen species. UV_A-light upregulates nitric oxide synthase with a subsequent increase in nitric oxide synthesis (Halliday, 2005). Nitric oxide combines with superoxide to produce a highly toxic reactive nitrogen species, peroxynitrite. Reactive nitrogen species damage DNA, alter enzyme activity, denature proteins, and initiate lipid peroxidation. Peroxynitrite-initiated DNA damage further interferes with cellular function by depleting ATP in cells. The enzyme poly (ADP-ribose) polymerase, activated after DNA damage by peroxynitrite, degrades NAD into its constituent components. Depletion of NAD causes a reduction in ATP formation with a subsequent decrease in cellular energy function that can lead to cell death (Halliday, 2005).

Unlike UV_A-light, UV_B-light causes most of its effects through direct damage of chromophores, such as DNA. However, UV_B-light also induces the production of reactive oxygen and nitrogen species that ultimately leads to inflammation. UV_B light causes DNA damage by directly altering DNA structure (Ichihashi *et al.*, 2003; Matsumura & Ananthaswamy, 2004). Absorption of UV_B-light by DNA causes the formation of mutagenic products called cyclobutane dimers, between adjacent thymine or cytosine residues, and pyrimidine dimers between adjacent pyrimidine residues. If these dimers are left unrepaired they can cause mutation in DNA sequences and subsequent carcinogenesis (Ichihashi *et al.*, 2003; Matsumura & Ananthaswamy, 2004). Alteration of DNA structure plays no role in UV_B-light induced inflammation.

1.1.2 Characteristics of UV_A- and UV_B-light induced injury

Exposure of skin, rodent and human, to UV-light causes erythema, increased tissue temperature, oedema, pain and pruritus, followed by tanning and epidermal thickening (Hruza & Pentland, 1993). Erythema as a marker of UV-light induced injury can be measured, usually with Laser Doppler, as a change in skin blood flow. The time course of erythema development, temporal pattern of erythema development (monophasic or biphasic), duration of erythema, and histological changes are dependent on the wavelength and dose of UV-light (Hruza & Pentland, 1993). The differences in erythema duration, time course of erythema and histological changes after UV_A- and UV_B-light irradiation are as a result of the differing penetration depths of the different wavelengths. UV_A-light penetrates to the deeper dermis, while UV_B-light penetrates through the epidermis into the upper dermis (Gilchrest *et al.*, 1981; Gilchrest *et al.*, 1983).

In humans, UV_A- and UV_B-light exposure causes a dose-dependant erythema i.e. the higher the dose the greater and longer the changes in skin blood flow (Bickel *et al.*, 1998; Hoffmann & Schmelz, 1999; Koppert *et al.*, 1999; Benrath *et al.*, 2001; Sycha *et al.*, 2003; Gustorff *et al.*, 2004a; Gustorff *et al.*, 2004b; Harrison *et al.*, 2004; Koppert *et al.*, 2004; Sycha *et al.*, 2005; Sycha *et al.*, 2006). However, the dose of UV_A-and UV_B-light required to cause similar effects differ significantly with UV_A-light requiring a dose a 1000 times greater than that of UV_B-light to cause similar effects (Hruza & Pentland, 1993). Low-dose UV_A-light irradiation of buttock skin caused an immediate erythema that peaked at 8 hours and lasted for 48 hours (Gilchrest *et al.*, 1983). However, at a higher dose the erythema peaked 48 hours after

UV_A-light exposure, and persisted for over 72 hours (Harrison *et al.*, 2004). Therefore, UV_A-light caused a monophasic erythema. In contrast, UV_B-light caused a biphasic erythema. UV_B-light irradiation of the forearm caused a dose-dependent increase of skin blood flow for more than 96 hours post-irradiation, with two peaks of blood flow, the first at 12 hours and the second at 36 hours after irradiation (Benrath *et al.*, 2001).

As with the erythema, histological changes occurred within human skin almost immediately after UV_A- and UV_B-light exposure. There were significant differences between the histological changes associated with UV_A- and UV_B-light, with UV_A-light causing predominantly dermal changes and UV_B-light causing both epidermal and dermal changes (McCregor & Hawk, 1999). Because of its low absorbance in the epidermis UV_A-light caused very little epidermal injury, with the injury that did occur being characterised by spongiosis and the absence of inflammatory cells (Soter, 1990). Within the deep dermis blood vessels were severely damaged by UV_A-light with endothelial swelling, extravasation of red blood cells, platelet aggregation, endothelial cell separation, extravascular fibrin deposition and partial occlusion of the vessels due to perivascular oedema (Kumakiri *et al.*, 1977). A mixed perivascular cellular infiltrate, consisting of predominantly monocytes and T lymphocytes with few neutrophils, encroached on the deeper dermal vessels (Kumakiri *et al.*, 1977; Gilchrest *et al.*, 1983). The histological features after UV_A-light exposure were consistent with a chronic inflammatory process.

The histological changes caused by UV_B-light exposure extend from the epidermis through to the dermis, and the higher the dose the more severe the inflammatory

changes. The first observable histological changes coincided with the first peak of erythema. Arterioles, capillaries and venules are dilated and there was a complete absence of inflammatory cells (Gilchrest *et al.*, 1981). The perivascular cellular infiltrate that eventually developed after UV_B-light exposure, coinciding with the second peak of erythema, consisted of neutrophils, T lymphocytes and mononuclear cells. Within the dermis, endothelial swelling and perivascular cellular infiltrate was present 24 hours to 72 hours after UV_B-light exposure (Gilchrest *et al.*, 1981). The histological features after UV_B-light exposure were consistent with an acute inflammatory response.

A proviso for the above histological findings was the suitability of controls used. Usually biopsies were taken from two sites. The first site was from skin that was irradiated with either UV_A- or UV_B-light, while tissue taken for comparison was removed from a site that was not irradiated. I believe they did not adequately control for all variables. Tissue exposed to UV-light increases in temperature, as a result of the UV-light and the heat generated from UV-light lamps. Therefore it was possible that an increase in tissue temperature and not the UV-light could have caused histological changes. A more suitable control would have been to expose all tissues taken for biopsy to UV-light, and have blocked the UV-light, but not the heat, over the control tissue.

In summary, in humans UV_A-light causes a monophasic erythema characterised by a chronic inflammatory process within the deeper dermis of the skin. UV_B-light causes a biphasic erythema characterised by an acute inflammatory process within the epidermis and superficial dermis of the skin. Typically the changes after UV_A-light

exposure are of a shorter duration and less severe than those after UV_B-light exposure. The differences in erythema duration, time course of erythema and histological changes after UV_A- and UV_B-light irradiation are as a result of the differing penetration depths of the different wavelengths, as UV_A-light penetrates to the deeper dermis while UV_B-light penetrates through the epidermis into the upper dermis.

1.1.3 Pro-inflammatory metabolites produced by UV-light irradiation

Numerous substances are produced within the skin and systemically after UV_A- and UV_B-light irradiation, such as prostaglandins, histamine, kinins, nitric oxide, interleukins, colony stimulating factors, growth factors, interferons, chemokines and neuropeptides (Kondo, 1999; Clydesdale *et al.*, 2001; Zegarska *et al.*, 2006). They each have a role in co-ordinating the multiple effects observed in the skin after UV-light irradiation. However, I am going to focus only on those substances that are involved in nociception and hyperalgesia.

After UV_A-light exposure, prostaglandin (PG) E₂, PGD₂, and PGF_{2α} are increased at the site of irradiation (Gilchrest *et al.*, 1981; Hawk *et al.*, 1983). Prostaglandins are synthesised by a constitutive cyclooxygenase (COX) enzyme, COX-1, and an inducible enzyme, COX-2. Their increase parallels the time course of erythema, such that they are produced immediately after UV_A-light exposure, their peak concentrations coincide with the peak period of erythema, and they return to pre-exposure levels one to three days after UV_A-light exposure of human skin (Gilchrest *et al.*, 1981; Hawk *et al.*, 1983). Nevertheless, the role of prostaglandins in UV_A-light induced erythema is unclear as topical or intradermal application of indomethacin, a

potent inhibitor of COX -1 and -2, has no effect on erythema caused by UV_A-light (Morrison *et al.*, 1977). Similarly to prostaglandins, histamine was increased after UV_A-light exposure of skin (Gilchrest *et al.*, 1981; Hawk *et al.*, 1983). Human mast cells degranulate and released histamine four hours after UV_A-light exposure. Histamine production peaked 9 to 15 hours after exposure and returned to baseline levels within 24 hours (Gilchrest *et al.*, 1981; Hawk *et al.*, 1983). However, antihistamines did not abolish UV_A-light induced erythema. Therefore, the exact role of histamine in UV_A-light induced erythema is unclear, and it has been proposed that histamine sensitizes irradiated tissue to multiple pro-inflammatory agonists such as prostaglandins and bradykinin, and facilitated the production of prostaglandins in the keratinocytes (Pentland & Jacobs, 1991). Contradictory findings have been reported regarding keratinocyte pro-inflammatory cytokine production after UV_A-light irradiation with some studies reporting an increase in pro-inflammatory cytokine production while others reporting no changes (Kondo, 1999). The reasons for these differences are unknown but it may be due to different doses of UV_A-light being used, or more likely different light sources and experimental protocols. After UV_B-light exposure, inflammation within skin consists of three overlapping phases. The first phase, early vasodilatory phase is mediated by PGE₂ and nitric oxide. The second phase is characterised by infiltration of inflammatory cells, namely neutrophils, T lymphocytes and mononuclear cells, and the release of multiple pro-inflammatory mediators. The last phase is the regressive phase, in which anti-inflammatory substances, such as interleukin (IL) -10, are released within the skin and the erythema and inflammation resolves (Terui & Tagami, 2000). My discussion will focus on the first two phases, because the pro-inflammatory mediators released in these phases are also those mediators that have pro-nociceptive and hyperalgesic effects.

After UV_B-light exposure, as with UV_A-light exposure, PGE₂, PGD₂, and PGF_{2α} are increased in the skin (Black *et al.*, 1978a; Black *et al.*, 1980). Their time course increase parallels the time course of erythema in exactly the same way as after UV_A-light exposure i.e. they are produced immediately after UV_A-light exposure, their peak concentrations coincide with the peak period of erythema, and they return to pre-exposure levels one to three days after UV_B-light exposure of human skin (Black *et al.*, 1978a; Black *et al.*, 1980). But unlike erythema caused by UV_A-light, UV_B-light induced erythema can be suppressed partially through the inhibition of eicosanoid synthesis by means of the administration of indomethacin either topically (Snyder & Eaglstein, 1974a; Rhodes *et al.*, 2001), intradermal injection (Snyder & Eaglstein, 1974b), or oral ingestion (Black *et al.*, 1978b). Similarly COX-2 inhibitors suppress UV_B-light induced erythema (Wilgus *et al.*, 2000; Wilgus *et al.*, 2002). A single dose of indomethacin, which inhibits mainly COX-1 isoenzymes, had no effect on erythema when administered 24 hours after UV_B-light exposure (Farr & Diffey, 1986), but repeated administration of indomethacin starting 24 hours after UV_B-light exposure did inhibit erythema induced by UV_B-light (Rhodes *et al.*, 2001). The partial suppression of erythema by COX inhibitors suggests that eicosanoids, although vital, are not the sole mediators of UV_B-light induced erythema, through prostaglandin production by constitutively expressed COX 1 and inducible COX 2 is essential in the onset and maintenance of UV_B-light induced erythema.

Another potential mediator of UV_B-light induced early vasodilatation is nitric oxide (Terui & Tagami, 2000; Rhodes *et al.*, 2001). Nitric oxide synthesis within the human skin was increased after UV_B-light irradiation (Rhodes *et al.*, 2001), and the early

vasodilatory phase was temporarily, but not permanently, blocked by the injection of a nitric oxide synthase inhibitor into the skin (Rhodes *et al.*, 2001). Therefore, it is possible that nitric oxide is continuously produced through the sustained activation of nitric oxide synthase. However, differing effects of nitric oxide on vasodilatation are seen at different UV_B-light doses. At lower doses of UV_B-light exposure, erythema can be abolished by the administration of a nitric oxide synthase inhibitor alone. However, at higher UV_B doses, erythema cannot be abolished with a nitric oxide synthase inhibitor alone, but it can be abolished in combination with indomethacin (Rhodes *et al.*, 2001). Taken together, it appears that prostaglandins and nitric oxide mediate the early vasodilatation of the first phase of the inflammatory response caused by UV_B-light.

In the second phase of the UV_B-light induced inflammatory process, pro-inflammatory cytokines IL-1 (Kupper *et al.*, 1987), IL-6 (Oxholm, 1992), IL-8 (Kondo *et al.*, 1993; Strickland *et al.*, 1997) and tumour necrosis factor (TNF)- α (Oxholm *et al.*, 1988; Kock *et al.*, 1990; Oxholm, 1992; Strickland *et al.*, 1997) are increased within the human epidermis after UV_B-light exposure and are expressed by keratinocytes (Kondo, 1999; Clydesdale *et al.*, 2001). Langerhans cell production of cytokines has not been as well investigated as keratinocyte production and the role of Langerhans cells in inflammation caused by UV_B-light is unclear (Clydesdale *et al.*, 2001). UV_B-light exposure also caused an increase in circulating pro-inflammatory cytokines in humans (Urbanski *et al.*, 1990) and mice (Saade *et al.*, 2000). TNF- α and IL-1 suppress keratinocyte and melanocyte proliferation, regulate collagen and collagenase synthesis, activate and stimulate the migration of lymphocytes in the affected area, induce the production of other cytokines and PGE₂, and promote the

upregulation of the cellular glycoproteins intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial adhesion molecules (E selectin) (Clydesdale *et al.*, 2001). The cellular glycoproteins orchestrate the recruitment and activation of neutrophils, monocytes and T lymphocytes from the blood to cutaneous tissues (Kondo, 1999). IL-1 also stimulates the release of histamine from mast cells (Kondo, 1999). IL-6 induces proliferation and chemotaxis of keratinocytes, sustains the viability and function of Langerhans cells, activates T lymphocytes, inhibits melanocyte proliferation and prolongs the survival of mast cells (Kondo, 1999). IL-8 is chemotactic for both neutrophils and T lymphocytes and induces proliferation and chemotaxis of keratinocytes (Kondo, 1999; Terui & Tagami, 2000).

As with pro-inflammatory cytokines vasoactive neurokinins contribute to peripheral inflammation after UV_B-light irradiation. Substance P and calcitonin gene-related peptide (CGRP) are vasoactive neurokinins that are found in the branches of primary afferent neurones that end near blood vessels and can be released from the peripheral terminals of these nerves (Zegarska *et al.*, 2006). Substance P and CGRP content in skin nerve fibres, in the rat hind paw, are increased after UV_B-light exposure of the rat hind paw (Benrath *et al.*, 1995; Eschenfelder *et al.*, 1995). When released peripherally, as occurs in a variety of inflammatory models, substance P enhances vascular permeability and blood flow, and CGRP causes long-lasting vasodilatation (Zegarska *et al.*, 2006). Substance P also stimulates macrophages to produce PGE₂, superoxide ions, and thromboxane B₂, regulates the expression of adhesion molecules, stimulates keratinocytes to produce pro-inflammatory cytokines and is chemotactic for leukocytes (Zegarska *et al.*, 2006). When neurokinin, substance P

receptor, antagonists are administered either systemically, via subcutaneous injection, or topically, UV_B-light induced erythema and oedema were reduced in rats (Benrath *et al.*, 1995; Eschenfelder *et al.*, 1995). CGRP receptor antagonists injected intradermally predictably had no effect on UV_B-light induced oedema, but did reduce UV_B-light induced erythema (Benrath *et al.*, 1995). Although, substance P and CGRP contribute to peripheral inflammation after UV_B-light irradiation, their role in UV_B-light induced hyperalgesia is less clear. The end-points measured in the above studies by Benrath and colleagues were markers of inflammation and not hyperalgesia. Therefore, the role of substance P and CGRP in hyperalgesia still is unclear.

Neurokinin content within the central nervous system also was altered after UV-light exposure of the rat hind paw (Gillardon *et al.*, 1991; Gillardon *et al.*, 1992a; Polgar *et al.*, 1998). Substance P content within the spinal cord at L4 and L5 segments, bilaterally, was decreased after UV_A-light exposure of the rat hind paw (Polgar *et al.*, 1998). In contrast to the lumbar segments, substance P content was increased on the contralateral side of the midthoracic segments (Polgar *et al.*, 1998) UV_B-light irradiation of the rat hind paw caused an increase of intracellular CGRP content in the spinal cord (Gillardon *et al.*, 1992a) with a decrease in CGRP messenger RNA in the L4 and L5 dorsal root ganglia (Gillardon *et al.*, 1991). Since no further experiments have been published exploring altered central nervous system neurokinin content, the significance of the above findings is unknown.

In summary, UV_A- and UV_B-light cause an increase in prostaglandins and histamine in skin. Their increase parallels the time course of erythema, such that they are produced immediately after UV-light exposure, their peak concentrations coincide

with the peak period of erythema, and they return to pre-exposure levels as the erythema resolves. However, the administration of suitable antagonists does not reverse nor attenuate UV_A-light induced erythema. Therefore the role, if any, of pro-inflammatory mediators in UV_A-light induced erythema is not known. In contrast, UV_B-light induced erythema is reversed by the administration of suitable antagonists. Furthermore, UV_B-light, but not UV_A-light, causes an increase in pro-inflammatory cytokines and neurokinins. Therefore, UV_B-light induces a typical inflammatory milieu within irradiated tissue and UV_A-light does not.

1.2 Ultraviolet-light induced hyperalgesia

Perkins and Campbell first described UV-light as a conditioning event for the development of hyperalgesia in 1992, and subsequently used pharmacological interventions, immunohistochemistry and electrophysiology to explore the physiological mechanisms underlying the UV-light induced hyperalgesia. In the following sections of this chapter I describe key studies, in detail, to highlight major contributions to the field.

1.2.1 Time-course of UV-light induced hyperalgesia: behavioural studies

UV_A-light and UV_B-light cause hyperalgesia in a variety of animals (Table 1). Exposure of the rat hind paw to UV_A light causes thermal hyperalgesia of the irradiated paw that can last for 10 to 13 days (Perkins *et al.*, 1992; Perkins *et al.*, 1993; Perkins & Kelly, 1993; Davies *et al.*, 2005). There was up to a 70% reduction in response latencies to a noxious thermal challenge of the irradiated paw on the first day after exposure to UV_A-light, with peak hyperalgesia being reached three days after exposure to UV_A-light. The hyperalgesia was constant for five to seven days, with full resolution of hyperalgesia 12 to 13 days after UV_A-light exposure. Hyperalgesia also developed in the hind paw that was not exposed to UV_A-light, but was present for only seven days and was not as severe as in the paw exposed to UV_A-light (Perkins *et al.*, 1992). The presence of hyperalgesia in non-exposed areas implies that either central sensitization had occurred or peripheral nociceptors were upregulated.

Table 1: UV-light induced hyperalgesia

Species	Light wavelength	Site irradiated	Noxious challenge	Duration of hyperalgesia	Reference
Rats	UV _A	Hindpaw	Radiant heat Randall-Sellito test	7 days	(Perkins <i>et al.</i> , 1992)
	UV _A	Hindpaw	Radiant heat	10 days	(Perkins <i>et al.</i> , 1993)
	UV _A	Hindpaw	Radiant heat	≥ 4 days	(Perkins & Kelly, 1993)
	UV _A	Hindpaw	Radiant heat Randall-Sellito test	≥ 6 days 4 days	(Thompson <i>et al.</i> , 1994)
	UV _A	Hindpaw	Von Frey hairs Randall-Sellito test	≥ 4 days	(Boxall <i>et al.</i> , 1998)
	UV _A	Hindpaw	Pin prick Radiant heat Acetone drop test Von Frey hairs	No hyperalgesia 2 days 5 days 5 days	(Davies <i>et al.</i> , 2005)
	UV _B	Hindpaw	Von Frey hair Water bath	10 days 7 days	(Bishop <i>et al.</i> , 2007)
Mice	UV _B	Dorsal surface of body	Tail flick test	4 days	(Saade <i>et al.</i> , 2000)
			Hot plate test	3 days	

Species	Light wavelength	Site irradiated	Noxious challenge	Duration of hyperalgesia	Reference
Pig	UV _B	Not stated	Von Frey hairs Heat pad	≥ 2 days ≥ 2days	(Rukwied <i>et al.</i> , 2008)
Humans	UV _A	Thigh	Radiant heat Impact	Not present Not present	(Hoffmann & Schmelz, 1999)
	UV _A	Buttock	Radiant heat Impact	≥ 3 days ≥ 3 days	(Harrison <i>et al.</i> , 2004)
	UV _B	Thigh	Radiant heat Impact	4 days 5 days	(Hoffmann & Schmelz, 1999)
	UV _B	Forearm	Thermode Mechanical	3 days 5 days	(Benrath <i>et al.</i> , 2001)
	UV _B	Upper leg	Electrical Thermal Mechanical	≥ 2days ≥ 2days ≥ 2days	(Gustorff <i>et al.</i> , 2004a)

Hyperalgesia after UV_A-light irradiation was not confined to noxious thermal challenges. In their initial description of the UV_A model, Perkins and Campbell (1992), concentrated on describing the thermal hyperalgesia and only briefly mentioned that mechanical hyperalgesia was present (Perkins *et al.*, 1992). However, others have shown that mechanical hyperalgesia and allodynia are induced by exposure of the rat hindpaw to UV_A-light (Boxall *et al.*, 1998; Davies *et al.*, 2005).

Mechanical hyperalgesia developed within 2 to 12 hours, after UV_A-light exposure, and resolved within four to five days (Boxall *et al.*, 1998; Davies *et al.*, 2005). However, the onset and duration of allodynia was different to those of hyperalgesia (Davies *et al.*, 2005). von Frey hairs generating 8g and 15g of force applied to rat hindpaws evoked hyperalgesia, in UV_A-light exposed paws, within 12 hours of UV_A-light exposure, with the hyperalgesia lasting five days. In contrast allodynia, in UV_A-light exposed paws, to von Frey hairs generating 2g and 4g of force appeared only 36-48 hours after UV_A irradiation, and lasted for four days. There was no explanation as to why there should be such discrepancies in the rats' response to the mechanical challenges. Cold allodynia also was present after UV_A-light exposure of the rat hind paw (Davies *et al.*, 2005). Cold allodynia, as assessed by the acetone drop test, presented within six hours and lasted for three days after UV_A-light exposure (Davies *et al.*, 2005). Therefore in rats, exposure of the rat hindpaw to UV_A-light causes a reliable and easily reproducible thermal hyperalgesia, mechanical hyperalgesia, mechanical allodynia and cold allodynia, and the duration and peak of hyperalgesia depended on the noxious challenge modality used.

In humans, UV_A-light induced thermal and mechanical hyperalgesia has been described only once (Harrison *et al.*, 2004). Exposure of buttock skin produced thermal and mechanical hyperalgesia that peaked two days after UV_A-light exposure and had resolved by three days (Harrison *et al.*, 2004). However, most studies have found that UV_A-light caused erythema and tanning but not hyperalgesia in humans (Bickel *et al.*, 1998; Hoffmann & Schmelz, 1999).

UV_B-light also has been used as a conditioning event in behavioural studies of nociception in rats (Hamilton *et al.*, 1999; Bishop *et al.*, 2007), mice (Saade *et al.*, 2000) and humans (see below). In rats, UV_B-light exposure of rat hind paw dorsal and plantar surfaces caused thermal hyperalgesia and mechanical allodynia in the paw exposed to UV_B-light (Hamilton *et al.*, 1999; Bishop *et al.*, 2007). The duration and severity of the thermal hyperalgesia and mechanical allodynia were dose dependant, with higher doses causing longer and more severe thermal hyperalgesia and mechanical allodynia. However, the peak severity of thermal hyperalgesia and mechanical allodynia, at all doses tested, was reached after two days (Bishop *et al.*, 2007). Thermal hyperalgesia and mechanical allodynia lasted for seven and ten days respectively, after exposure to UV_B-light at the highest dose (Bishop *et al.*, 2007).

UV_B-light irradiation of the backs of the mice, as opposed to rats, produced thermal hyperalgesia at anatomical sites distant from the site of UV_B-light exposure, and a differing time course of hyperalgesia (Saade *et al.*, 2000). After the backs of mice were irradiated with UV_B-light at different doses, there was a dose-dependant decrease in the paw hot-plate test and tail-flick test response latencies. The time-course of the hyperalgesia displayed a biphasic response, as opposed to a monophasic

response that occurred after UV_B-light exposure of the rat hind paw (Hamilton *et al.*, 1999; Bishop *et al.*, 2007), with an initial phase that peaked at 3-6 hours after exposure, and a late phase that peaked 48-96 hours after exposure.

UV_B-light irradiation of human skin causes a dose-dependant hyperalgesia to noxious mechanical and thermal challenges (Bickel *et al.*, 1998; Hoffmann & Schmelz, 1999; Koppert *et al.*, 1999; Benrath *et al.*, 2001; Sycha *et al.*, 2003; Gustorff *et al.*, 2004a; Gustorff *et al.*, 2004b; Harrison *et al.*, 2004; Koppert *et al.*, 2004; Sycha *et al.*, 2005; Sycha *et al.*, 2006). Hyperalgesia to noxious mechanical and thermal challenges develops in a dose-dependant manner, with higher doses of UV_B-light causing more severe and prolonged hyperalgesia. The hyperalgesia for the thermal and mechanical challenge modalities lasted from four to nine days, depending on the body area irradiated, with a peak reached between one and two days post-irradiation (Hoffmann & Schmelz, 1999; Benrath *et al.*, 2001).

Therefore, in rodents and humans, exposure to UV_B-light caused thermal and mechanical hyperalgesia and allodynia. Exposure of the rat hindpaw caused thermal hyperalgesia and mechanical allodynia that lasted for seven and ten days respectively. In mice, exposure of their dorsal surface caused a secondary thermal hyperalgesia. The time course of the hyperalgesia was biphasic, with an initial phase, which peaked at 3-6 hours after exposure, and a late phase, which peaked 48-96 hours after UV_B-light exposure. In humans thermal and mechanical hyperalgesia peaked three days after to UV_B-light exposure and lasted for four to nine days. In human studies the peak and duration of hyperalgesia differed due to variation in dose and body sites used.

1.2.2 Pharmacology of UV-light induced hyperalgesia

Different pharmacological agents have been used in an attempt to characterise hyperalgesia caused by UV_A-light and UV_B-light (Table 2). I have divided the agents under separate headings and highlighted the major findings for each agent.

1.2.2.1 Opioids

Morphine, the gold-standard analgesic, injected subcutaneously produced a dose-related, and naloxone-reversible, increase in response latencies to a noxious thermal one and five days after UV_A-light exposure of rat hind-paws (Perkins *et al.*, 1992). The investigators did not measure motor responses of the rats at the doses of morphine they used, and it was unclear whether the increase in response latencies was due to analgesia or sedation of the rats (Cartmell *et al.*, 1991). Morphine also significantly reversed, in a dose-dependant manner, thermal and mechanical hyperalgesia after UV_B-light exposure of the rat hind paw (Bishop *et al.*, 2007). Loperamide, a peripheral μ opioid receptor agonist that does not cross the blood brain barrier, reversed thermal and mechanical hyperalgesia after UV_B-light exposure of the rat hind paw, in a dose-dependant manner (Bishop *et al.*, 2007). Therefore, morphine's analgesia may have been mediated solely through activation of peripheral, and not central, μ opioid receptors.

Table 2: Effect of pharmacological interventions on UV-light induced hyperalgesia

Pharmacological intervention	Effect on hyperalgesia	Species	Light wavelength	Site irradiated	Route of administration	Noxious challenge	Reference
Systemic morphine	Reduced	Rats	UV _A	Hindpaw	Subcutaneous	Thermal	(Perkins <i>et al.</i> , 1993)
	Reduced	Rats	UV _B	Hindpaw	Not stated	Thermal Mechanical	(Bishop <i>et al.</i> , 2007)
Regional morphine	Reduced	Humans	UV _B	Upper leg	Intravenous	Thermal Mechanical	(Koppert <i>et al.</i> , 1999)
Remifentanyl	Reduced	Humans	UV _B	Upper leg	Intravenous	Thermal Mechanical	(Gustorff <i>et al.</i> , 2004b)
κ -agonist (EMD61753)	No effect	Humans	UV _B	Upper leg	Oral	Thermal	(Bickel <i>et al.</i> , 1998)
Loperamide – peripheral μ opioid receptor agonist	Reduced	Rats	UV _B	Hindpaw	Not stated	Thermal Mechanical	(Bishop <i>et al.</i> , 2007)

Pharmacological intervention	Effect on hyperalgesia	Species	Light wavelength	Site irradiated	Route of administration	Noxious challenge	Reference
Systemic ibuprofen-non selective COX inhibitor	Reduced	Rats	UV _B	Hindpaw	Subcutaneous	Thermal	(Perkins <i>et al.</i> , 1993)
	Reduced	Rats	UV _B	Hindpaw	Not stated	Thermal Mechanical	(Bishop <i>et al.</i> , 2007)
	Reduced	Humans	UV _B	Upper leg	Oral	Thermal Mechanical	(Bickel <i>et al.</i> , 1998)
	Reduced	Humans	UV _B	Upper leg	Oral	Thermal	(Sycha <i>et al.</i> , 2003)
Topical ibuprofen	Reduced	Rats	UV _B	Hindpaw	Gel on hindpaw	Thermal Mechanical	(Bishop <i>et al.</i> , 2007)
Rofecoxib-COX 2 inhibitor	Reduced	Humans	UV _B	Upper leg	Oral	Thermal Mechanical	(Sycha <i>et al.</i> , 2005)

Pharmacological intervention	Effect on hyperalgesia	Species	Light wavelength	Site irradiated	Route of administration	Noxious challenge	Reference
Bradykinin agonists							
Bradykinin	Enhanced	Rats	UV _A	Hindpaw	Intravenous	Thermal	(Perkins & Kelly, 1993)
B1 receptor	Enhanced	Rats	UV _A	Hindpaw	Intravenous	Thermal	(Perkins & Kelly, 1993)
	Enhanced	Rats	UV _A	Hindpaw	Intravenous	Thermal	(Perkins <i>et al.</i> , 1993)
	Enhanced	Humans	UV _B	Thigh	Intracutaneously	Pain intensities	(Eisenbarth <i>et al.</i> , 2004)
B2 receptor	Enhanced	Humans	UV _B	Thigh	Intracutaneously	Pain intensities	(Eisenbarth <i>et al.</i> , 2004)
Bradykinin antagonists							
B1 receptor	Reduced	Rats	UV _A	Hindpaw	Intraplantar	Thermal	(Perkins <i>et al.</i> , 1993)
B2 receptor	No effect	Rats	UV _A	Hindpaw	Subcutaneous	Thermal	(Perkins <i>et al.</i> , 1993)

Pharmacological intervention	Effect on hyperalgesia	Species	Light wavelength	Site irradiated	Route of administration	Noxious challenge	Reference
ATP	Enhanced	Rats	UV _B	Hindpaw	Intraplantar	Thermal	(Hamilton <i>et al.</i> , 1999)
	Enhanced	Humans	UV _B	Forearm	Intracutaneously	Pain intensities	(Hamilton <i>et al.</i> , 2000)
IL-10 and IL-13	Reduced	Mice	UV _B	Dorsal surface	Intra-peritoneal	Thermal	(Saade <i>et al.</i> , 2000)
Lidocaine-sodium channel antagonist	Reduced	Humans	UV _B	Upper leg	Intravenous	Mechanical	(Koppert <i>et al.</i> , 2004)
Gabapentin	No effect	Humans	UV _B	Upper leg	Oral	Thermal Mechanical	(Gustorff <i>et al.</i> , 2004b)
Botulinum toxin	No effect	Humans	UV _B	Upper leg	Intracutaneously	Thermal Mechanical	(Sycha <i>et al.</i> , 2006)

In humans, opioid agonists administered systemically or regionally, reversed UV_B-light induced thermal hyperalgesia (Koppert *et al.*, 1999; Gustorff *et al.*, 2004b). When administered regionally the extremity, usually the arm, is elevated and exanguinated by tightly wrapping bandages in a proximal to distal direction. The opioid agonist is then injected intravenously to anaesthetise the arm. Remifentanyl, a potent synthetic opioid agonist, injected intravenously reversed thermal hyperalgesia and secondary mechanical hyperalgesia after UV_B-light exposure of the upper leg (Gustorff *et al.*, 2004b). Morphine injected locally, via intravenous regional anesthesia, after UV_B-light irradiation of the upper leg, abolished thermal hyperalgesia, but not mechanical hyperalgesia at UV_B-light exposed areas (Koppert *et al.*, 1999). As plasma levels of morphine were very low, morphine probably attenuated thermal hyperalgesia, but not mechanical hyperalgesia, by a peripheral mode of action, which implies inhibition of effector pathways leading to heat, but not mechanical, sensitization. Therefore, UV_B-light may sensitize peripheral afferent neurons to noxious thermal but not mechanical challenges. Morphine probably acted through μ opioid receptors, as oral administration of κ -selective agonists did not reduce thermal or mechanical hyperalgesia in humans after UV_B-light irradiation of the upper leg (Bickel *et al.*, 1998).

1.2.2.2 Eicosanoids

Eicosanoids are arachadonic acid metabolites that include prostaglandins, prostacyclins, thromboxanes and leukotrienes. They sensitize nociceptors to endogenous and exogenous compounds (McMahon *et al.*, 2005). Prostaglandins sensitize nociceptors by reducing the activation threshold of voltage sensitive

tetrodotoxin-resistant sodium channels specific to nociceptors, increasing intracellular cyclic AMP levels, and increasing the excitability of nociceptive neurones (McMahon *et al.*, 2005).

Ibuprofen, a non-selective COX-inhibitor, reversed thermal hyperalgesia when injected subcutaneously one day after UV_A-light exposure of the rat hind-paw, but was significantly less effective when given five days after exposure (Perkins *et al.*, 1992). Therefore, prostaglandins may be responsible for initiating hyperalgesia but not maintaining hyperalgesia after UV_A-light exposure. Such a finding contrasts with the lack of efficacy of COX inhibitors in erythema induced by UV_A-light. It is possible that prostaglandins are involved in hyperalgesia but not erythema after UV_A-light exposure. Since COX-2 inhibitors have not been used in models of UV_A-light induced hyperalgesia, no information was available as to whether COX-1 or COX-2 (or both) are responsible for prostaglandin synthesis after UV_A-light exposure.

Systemic and locally applied ibuprofen also reversed thermal hyperalgesia and mechanical allodynia after UV_B-light exposure of the rat hindpaw (Bishop *et al.*, 2007). When administered two days after UV_B-light exposure, systemic and local administered ibuprofen produced a dose dependant reversal of thermal hyperalgesia and mechanical allodynia (Bishop *et al.*, 2007). Ibuprofen, ingested before UV_B-light exposure of human skin, also significantly decreased heat and mechanical hyperalgesia caused by UV_B-light exposure, but it had no effect on responses obtained from applying the same noxious challenge to skin not exposed to UV_B-light (Bickel *et al.*, 1998; Sycha *et al.*, 2003).

The majority of COX inhibitors inhibit both COX-1 and COX-2. Therefore, unless selective inhibitors are used, it is difficult to determine the relative importance of each enzyme in hyperalgesia caused by UV-light. Rofecoxib, a COX-2 selective inhibitor, taken orally before UV_B-light exposure, significantly reduced thermal hyperalgesia, superficial skin blood flow and the area of secondary hyperalgesia caused by UV_B-light irradiation of human skin (Sycha *et al.*, 2005). Hence, the study suggests that COX-2, not COX-1, was responsible for prostaglandin synthesis after human UV_B-light exposure. Injury after UV_B-light exposure was consistent with an inflammatory model of hyperalgesia, whereby COX enzymes are upregulated and prostaglandins synthesized in response to an inflammatory conditioning event. Since rofecoxib and ibuprofen were administered systemically, their site of action in the neuraxis was unknown, but since rofecoxib reduced the area of secondary hyperalgesia, it was possible that rofecoxib altered prostaglandin production within the central nervous system. However, it could be that inhibiting peripheral prostaglandin production prevented central sensitization.

1.2.2.3 Bradykinin

Bradykinin is an endogenous algogen that is released after tissue injury. It can activate nociceptors directly and sensitize nociceptors through activation of phospholipase C, protein kinase C, production of arachadonic acid and modulation of TRPV1 channels (McMahon *et al.*, 2005). Bradykinin exerts its action through B₁ and B₂ receptors (McMahon *et al.*, 2005). B₁ receptors are expressed during inflammation and injury, and B₂ receptors are constitutively expressed. However, B₂ receptors do play a role in pain during inflammation (McMahon *et al.*, 2005).

B₁ receptors, not B₂ receptors, play a significant role in thermal hyperalgesia after UV_A-light exposure in rats (Perkins *et al.*, 1993; Perkins & Kelly, 1993). The B₁-receptor agonist des-Arg⁹-BK, and, surprisingly bradykinin, injected subcutaneously into the rat hind paw, had no effect on withdrawal latency to a noxious heat challenge. They did not, therefore, sensitize nociceptors to a noxious thermal challenge (Perkins & Kelly, 1993). However, when given after UV_A-light exposure both agents exacerbated thermal hyperalgesia in the exposed paw (Perkins & Kelly, 1993). In addition, polymodal C-fibre receptor activity was increased when bradykinin was administered after UV-light (no wavelength stated) irradiation of the rabbit ear (Szolcsanyi, 1987). Therefore, UV-light sensitized animal tissue to the effects of bradykinin receptor agonists. The hyperalgesic actions of des-Arg⁹-BK and bradykinin, after UV_A-light exposure of the rat hindpaw, were reduced by the B₁ receptor antagonist des-Arg⁹, Leu⁸-BK, but not by the B₂ receptor antagonist HOE 140 (Perkins & Kelly, 1993). In another study, B₁ and B₂ receptor antagonists attenuated thermal hyperalgesia after UV_A-light irradiation of the rat hindpaw. However, there was a clear difference in the efficacy of the two antagonists with des-Arg⁹, Leu⁸-BK, a B₁ receptor antagonist, being more effective than HOE 140, a B₂ receptor antagonist. Only repeated administrations of HOE 140 was effective and HOE 140 increased the paw withdrawal latencies by 35%, while des-Arg⁹, Leu⁸-Bk increased paw withdrawal latencies by up to 85% (Perkins *et al.*, 1993). The reduction of des-Arg⁹-BK induced hyperalgesia by a B₁ receptor, but not a B₂ receptor antagonist implies that des-Arg⁹-BK was activating B₁ receptors that were not normally present as des-Arg⁹-BK was inactive in naïve animals. Therefore, UV_A-light exposure induced B₁ receptor expression but not B₂ receptor expression.

In humans, only one study has investigated the role of bradykinin receptors in hyperalgesia induced by UV_B-light (Eisenbarth *et al.*, 2004), and their role in hyperalgesia induced by UV_A-light in humans has not been investigated. The B₁ receptor agonist, des-Arg¹⁰-kallidin, and bradykinin, presumed to be a B₂ receptor agonist, were injected into the dermis before and after UV_B-light irradiation of ventral thigh skin, and pain, vasodilatation, protein extravasation, and axon reflex vasodilatation were measured (Eisenbarth *et al.*, 2004). UV_B-light exposure of ventral thigh skin enhanced des-Arg¹⁰-kallidin and bradykinin mediated pain, but UV_B-light exposure enhanced only des-Arg¹⁰-kallidin mediated local vasodilatation and had no effect on bradykinin mediated local vasodilatation (Eisenbarth *et al.*, 2004). Protein extravasation was not enhanced by UV_B-light exposure, so neuronal and vascular responses were differentially sensitized. Although a novel study, there were anomalies in the study that precluded definitive interpretation of the study's findings. First, bradykinin was used as a B₂ receptor agonist, but bradykinin acts at both B₁ and B₂ receptors. Secondly, it was stated that the pain responses to bradykinin injection were elevated significantly; but post-hoc statistical tests did not indicate significance.

1.2.2.4 ATP

After tissue injury ATP is released into the extracellular tissues and activates C-fibre nociceptors, but it does not sensitize nociceptors to thermal nor mechanical challenges, and activates only capsaicin-sensitive neurones (Hamilton *et al.*, 1999; Hamilton *et al.*, 2000). Receptors for ATP have been found on primary sensory afferents both on the dorsal root ganglion and in the periphery. ATP activates

nociceptive neurones in normal skin via the $P2X_3$ receptor and the heteromeric $P2X_2$ - $P2X_3$ receptor (McMahon *et al.*, 2005).

In rats (Hamilton *et al.*, 1999) and humans (Hamilton *et al.*, 2000) intradermal injection of ATP caused nociceptive responses (rats) and pain (humans). Intradermal injection of ATP and its analogues into the hindpaw of rats, in the absence of inflammation, produced a dose-dependant increase in hindpaw lifting and a decrease in withdrawal latencies to thermal challenge (Hamilton *et al.*, 1999). Therefore, in the absence of inflammation ATP is a potent algescic compound (Hamilton *et al.*, 1999). In the presence of inflammation, caused by UV_B-light exposure, or intradermal injection of carrageenan or PGE₂, animals' nociceptive responses to ATP and its analogues were augmented (Hamilton *et al.*, 1999). Similarly, in humans intradermal injection of ATP caused moderately severe pain (Hamilton *et al.*, 2000), and pre-treatment of the skin with UV_B-light doubled the pain felt after ATP intradermal injection, an effect that lasts for 24 hours (Hamilton *et al.*, 2000). The mechanisms for this enhanced response to ATP in the presence of inflammation were unclear, but it may be through the upregulation of the $P2X_3$ receptor.

1.2.2.5 Nerve growth factor and pro-inflammatory cytokines

Nerve growth factor (NGF) and pro-inflammatory cytokines contribute to inflammatory hyperalgesia through direct and indirect mechanisms (Poole *et al.*, 1999; Sommer, 2001; Koltzenburg, 2004; Sommer & Kress, 2004). NGF contributes to inflammatory hyperalgesia by sensitizing peripheral nociceptors to noxious thermal challenges, with a subsequent increase in spontaneous polymodal receptor activity

and increased nociceptor discharge to noxious thermal challenges (Koltzenburg, 2004). There is no evidence that NGF sensitizes peripheral nociceptors to noxious mechanical challenges. NGF indirectly contributes to inflammatory hyperalgesia by causing the release of serotonin and bradykinin from mast cells in the periphery and altering sensitivity of neurons to other algogenic agents such as bradykinin and capsaicin (Koltzenburg, 2004). The pro-inflammatory cytokines TNF- α , IL-1 β , IL-6, and cytokine-induced neutrophil chemoattractant (CINC) -1 are upregulated in rat models of inflammatory pain and neuropathic pain (Poole *et al.*, 1999; Sommer, 2001; Lorenzetti *et al.*, 2002; Sommer & Kress, 2004). The cytokines are upregulated both at the site of injury and within the spinal cord (Sommer, 2001). Upregulated pro-inflammatory cytokines mediate hyperalgesia by either directly sensitizing nociceptors or activating a cytokine cascade (Poole *et al.*, 1999; Sommer, 2001; Sommer & Kress, 2004). TNF- α induces the production of IL-6 which in turn induces IL-1 β , with IL-1 β activating COX-2 dependant prostaglandin release (Poole *et al.*, 1999). TNF- α also induces CINC-1 production, which in turn causes the release of catecholamines from sympathetic fibres (Poole *et al.*, 1999).

NGF and pro-inflammatory cytokines are increased after UV_B-light exposure (Gillardon *et al.*, 1995; Saade *et al.*, 2000). NGF and pro-inflammatory cytokines have not been measured after exposure to UV_A-light. UV_B-light exposure of the rat hind paw (Gillardon *et al.*, 1995) and the dorsal surface of mice (Saade *et al.*, 2000) caused an accumulation of nerve growth factor within the epidermis. NGF was concentrated within epidermal keratinocytes and in the cells of the underlying dermis (Gillardon *et al.*, 1995). UV_B -light irradiation of the back of mice at different doses caused a dose-dependant decrease in response latencies in hot plate and tail-flick tests

(Saade *et al.*, 2000). Coinciding with this secondary hyperalgesia, exposure to the highest dose of UV_B-light caused an increase in the pro-inflammatory cytokines IL-1 β , TNF- α , and NGF. Daily treatment of mice with intra-peritoneal injections of the anti-inflammatory cytokines IL-10 and IL-13 caused a dose-dependant attenuation of the UV_B-light induced secondary thermal hyperalgesia, with a parallel decrease in the concentrations of IL-1 β , TNF- α and NGF (Saade *et al.*, 2000). Similarly, systemic sequestration of NGF through subcutaneous injection of TrkAd5 decreased primary and secondary thermal hyperalgesia and mechanical allodynia after UV_B-light irradiation of the rat hind paw (Bishop *et al.*, 2007).

Therefore, NGF and pro-inflammatory cytokines are involved in secondary thermal hyperalgesia and NGF was further involved in mechanical allodynia caused by UV_B-light exposure. Since secondary thermal hyperalgesia and mechanical allodynia are produced by central sensitization, it was possible that pro-inflammatory cytokines and NGF were acting within the central nervous system to produce their effects.

Up to this point I have focused on the peripheral changes that occur locally at the site of UV-light exposure. I have included a brief section describing all the studies that include investigations exploring receptors within the spinal cord. Because of the paucity of studies, the following sections provide a comprehensive overview of changes within the spinal cord after UV-light exposure.

1.2.2.6 Glutamate receptors

Metabotropic glutamate receptors (mGluRs) are a family of G-protein coupled receptors that modulate nociceptive processing within the peripheral and central nervous system (for an extensive review see Neugebauer 2002). There are eight subtypes of metabotropic glutamate receptors, mGluR 1-8, divided into three groups, group I (mGluR 1,5), group II (mGluR 2,3) and group III (mGluR 4,6,7,8), and receptors from different groups have a different role in nociceptive processing. Activation of group I mGluRs contribute to peripheral and central sensitization, while activation of group II mGluRs reduces peripheral and central sensitization (Neugebauer, 2002). The role of group III mGluRs in nociceptive transmission remains unclear.

UV_A-light exposure of the rat hind paw caused a significant increase in the expression of mGluR 3 mRNA within the cells of the dorsal laminae in both sides of the lumbar spinal cord (Boxall *et al.*, 1998). The increase was most pronounced in laminae II, III and IV, and gradually decreased and disappeared by the third day after UV_A-light irradiation. In parallel with these changes in mRNA, behavioural experiments revealed mechanical hyperalgesia in both hindpaws after UV_A-light exposure. No changes were detected in the levels of expression of mGluR 1,2,4,5,7 subtype mRNA in spinal cords taken from hyperalgesic animals and no changes were noted in mRNA for any of the glutamate receptors in thoracic segments (Boxall *et al.*, 1998). The significance of this finding is unclear, as mGluR mRNA increase does not necessarily mean an increase of mGluR within cell membranes, nor does it prove an increase in neuronal hyperexcitability within the spinal cord. Furthermore, correlating mRNA

changes with behavioural changes does not prove causality. A reversal of behavioural hyperalgesia with a concomitant reversal of mRNA expression would have proven more definitive. The absence of an increase in mRNA expression for the other mGluR does not preclude their role in UV_A-light induced hyperalgesia. Thus the study by Boxall et al. (1988) does not provide any definitive answers for the role of mGluRs in UV_A-light induced hyperalgesia.

1.2.2.7 Proto-oncogenes

The proto-oncogenes *c-fos* and *junD* produce immunologically detectable nuclear proteins Fos and JUND. Different spatial and temporal patterns of proto-oncogene expression, especially *c-fos* and its product Fos, in spinal neurones have been reported in response to various peripheral noxious and inflammatory stimuli (Coggeshall, 2005). Fos and *c-fos* are expressed rapidly in central nervous system neurones after noxious challenges and their expression identifies those neurones activated by a nociceptive challenge (Coggeshall, 2005). The physiological functions of these proto-oncogenes and their products are unknown. Their main contribution is as an efficient tool to delineate anatomical organisation of nociceptive systems (Coggeshall, 2005). In addition, changes in noxious challenge intensity and duration alter Fos expression, thus Fos can be used to assess functioning of dorsal horn neurones, particularly in response to pharmacological agents (Coggeshall, 2005).

UV_B-light exposure of the rat hind paw did not increase *c-fos* expression in the spinal cord laminae I and II (Bishop *et al.*, 2007). However, thermally evoked *c-fos* expression within laminae I and II was enhanced after UV_B-light exposure (Bishop *et*

al., 2007). The absence of an increase in basal *c-fos* expression after UV_B-light exposure implies that UV_B-light exposure does not cause ongoing afferent activity within the irradiated area, but that UV_B-light exposure may sensitize peripheral nociceptors such that there was increased activation of nociceptive pathways in response to noxious thermal challenge after UV_B-light exposure. Since this postulate requires electrophysiological recordings to confirm its veracity, any conclusions reached from *c-fos* and Fos expression regarding neurophysiological changes within the spinal cord after UV_B-light irradiation are speculative.

UV_B-light exposure of the right hind paw increased the levels of *junD* mRNA in the ipsilateral half of the lumbar spinal cord (Gillardon *et al.*, 1992b). The increase in *junD* mRNA was paralleled by an increase in JUND protein levels in ipsilateral dorsal root ganglion neurones. Increased labelling of *junD* mRNA occurred in large and small diameter neurones (Gillardon *et al.*, 1995). As with *c-fos* and Fos the physiological relevance of an increase of *junD* mRNA and JUND in contributing to UV_B-light induced hyperalgesia is unknown.

In summary, significantly fewer studies have investigated the biochemical changes, related to nociception, after UV_A-light than after UV_B-light exposure. The systemic administration of morphine, ibuprofen, a COX inhibitor, and bradykinin antagonists reversed UV_A-light induced hyperalgesia. It seems that prostaglandins are involved in initiating but not maintaining UV_A-light induced hyperalgesia. In addition, bradykinin B₁ receptors are upregulated and sensitized after UV_A-light exposure. Metabotropic glutamate receptor mRNA in spinal cord lamina was increased after UV_A-light exposure. However, there are no data that demonstrate an increase in glutamate

receptor number or altered sensitivity to glutamate, and the physiological significance of increased metabotropic glutamate receptor mRNA is thus unknown.

As with UV_A-light exposure, morphine reversed hyperalgesia after UV_B-light exposure, and morphine's analgesia may have been mediated through activation of peripheral, and not central, μ opioid receptors. In addition, regional administration of opioids abolished thermal hyperalgesia but not mechanical hyperalgesia after UV_B-light exposure, which suggests that UV_B-light sensitizes peripheral afferent neurones to noxious thermal, but not mechanical, challenges. The administration of COX-1 and COX-2 inhibitors reversed hyperaemia, and primary and secondary hyperalgesia after UV_B-light exposure. Therefore, injury after UV_B-light irradiation was consistent with an inflammatory model of hyperalgesia, whereby COX enzymes are upregulated and prostaglandins synthesized in response to an inflammatory conditioning event. UV_B-light exposure enhanced nociceptive responses to ATP and its analogues. The mechanisms for this enhanced response to ATP were unclear, but it may be through the upregulation of the ATP receptor *P2X₃*. UV_B-light caused an increase in the synthesis the proto-oncogenes *c-fos* and *junD* mRNA and their protein products Fos and JUND. The physiological relevance of an increase of proto-oncogenes and their protein products in contributing to UV_B-light induced hyperalgesia is unknown. NGF and pro-inflammatory cytokines are increased after UV_B-light exposure, and UV_B-light hyperalgesia can be reduced by anti-inflammatory cytokines. NGF and pro-inflammatory cytokines contribute to secondary thermal hyperalgesia, and NGF further contributes to mechanical allodynia after UV_B-light exposure. Since secondary thermal hyperalgesia and mechanical allodynia are produced by central sensitization it is possible that pro-inflammatory cytokines and NGF act within the central nervous

system to produce their effects. There is therefore ample biochemical evidence that UV_B -light causes an inflammatory hyperalgesia, with a paucity of evidence that UV_A -light causes an inflammatory hyperalgesia.

1.2.3 Electrophysiology of UV-induced hyperalgesia

Polymodal receptors, which are quiescent in normal skin became spontaneously active after UV-light (spectrum not stated) exposure of rabbit ears (Szolcsanyi, 1987) and UV_A-light exposure of rat hind paws (Andreev *et al.*, 1994). Also, capsaicin-induced activation of polymodal receptors was enhanced after UV_A-light pre-treatment. Direct administration of morphine, DAGOL (a μ receptor agonist) and U-69593 (a κ receptor agonist) but not DPDPE (a δ receptor agonist) to the receptive field of UV_A-light irradiated neurones produced a concentration-related and naloxone-reversible suppression of spontaneous firing in polymodal receptors of UV_A-light exposed skin (Andreev *et al.*, 1994). Therefore, polymodal receptors changed their activity and sensitivity to exogenous opioids after the induction of peripheral hyperalgesia by UV_A-light exposure, and this hypersensitivity was attenuated by μ and κ opioid receptor antagonists (Andreev *et al.*, 1994). Increased peripheral neuronal hyperexcitability occurs after exposure of rat hind paw (Eschenfelder *et al.*, 1995) and pig skin (Rukwied *et al.*, 2008) to UV_B-light. UV_B-light exposure of rat hind paw, with recordings from C-fibres innervating the hindpaw skin showed long-lasting low-frequency (0.8-1.25 Hz) spontaneous activity, and the number of spontaneously-active C-fibres increased from 0% to 35.3% of the total population of C-fibres 72 hours after UV_B-light exposure. This rise in the number of spontaneously-active C-fibres was evident 24 hours after UV_B-light exposure (Eschenfelder *et al.*, 1995).

Coinciding with the peripheral neuronal hyperexcitability are changes within the central nervous system. For example, the proportion of spontaneously-active wide

dynamic range (WDR) neurones was increased after UV_A-light exposure of the rat hindpaw, and the degree of spontaneous activity was enhanced during the time course of the hyperalgesia (Urban *et al.*, 1993). The WDR neurones showed a significant increase in the total number of spikes and the duration of responses when evoked by von Frey stimulation at 15mN, noxious pinch, noxious radiant heat and water heated to 52°C, when tested one to three days, and five to seven days after UV_A-light irradiation (Urban *et al.*, 1993). In addition, the threshold temperature was significantly decreased and the average sizes of the receptive fields were expanded after UV_A-light exposure. To distinguish between possible central and peripheral components of the hyperactivity of WDR cells, dorsal rhizotomies were performed during the recordings. Cutting L2-3 dorsal roots evoked a larger and more prolonged discharge in WDR cells in the rats hindpaws exposed to UV_A-light (Urban *et al.*, 1993). Spontaneous activity over one to three days after UV_A-light exposure was reduced after the dorsal rhizotomy. However, dorsal rhizotomy had no effect on the spontaneous activity of WDR neurones five to seven days after UV_A-light exposure. Responses to supramaximal electrical stimulation of the rat hind paws also were enhanced in the UV_A-light exposed rats and once again dorsal rhizotomy reduced the number of evoked spikes one to three days after UV_A-light exposure but not five to seven days after UV_A-light exposure. Therefore, UV_A-light exposure caused prolonged excitability in spinal WDR neurones with enhanced input from primary afferents contributing to this increased excitability for the first three days after exposure to UV_A-light (Urban *et al.*, 1993)

To measure neuronal hyperexcitability within the spinal cord, A- and C-fibre evoked VRP (ventral root potentials) were measured in isolated spinal cord preparations that

were taken from young rats in which behavioural hyperalgesia (thermal and mechanical) had been induced by UV_A-light irradiation of the hind paw (Thompson *et al.*, 1994). The duration and amplitude of both the A- and C-fibre evoked VRP were increased significantly in UV_A-light exposed rats. In rats exposed to UV_A-light, a NMDA receptor antagonist and neurokinin 1 and neurokinin 2 receptor antagonists significantly reduced the prolonged VRP evoked by C-fibre stimulation. However, in animals not exposed to UV_A light, the prolonged VRP evoked by a single shock C-fibre stimulation was significantly antagonised by a NMDA receptor antagonist and a neurokinin 2 receptor antagonist, but not by a neurokinin 1 receptor antagonist, implying that neurokinin 1 receptors were altered after UV_A-light exposure. Thus, enhanced receptor sensitivity of ventral root neurones may contribute to central sensitization and may be directly related to behavioural hyperalgesia after UV_A-light exposure (Thompson *et al.*, 1994).

Another possible mechanism for UV_B-light induced hyperalgesia is the activation of silent nociceptors (Koppert *et al.*, 2004; Rukwied *et al.*, 2008). The neurogenic axon reflex in human skin is mediated by mechanically-insensitive or silent nociceptors, and when phasic mechanical stimulation was compared in non-exposed and UV_B-light exposed skin, the neurogenic flare reaction was present in the irradiated skin but not in the non-irradiated skin (Koppert *et al.*, 2004). Systemic lidocaine administration suppressed the mechanically induced flare and the mechanical hyperalgesia in UV_B-light exposed skin, while leaving the impact induced ratings in normal skin unchanged (Koppert *et al.*, 2004). A similar change of neurogenic axon reflex has been observed after UV_B-light exposure of pig skin (Rukwied *et al.*, 2008). In pigs, C-fibre activation was assessed indirectly by axon reflex erythema or the flare response

in response to noxious mechanical challenges, von Frey filament exerting 100mN and 600 mN of pressure, and, noxious thermal challenges, 45°C and 47°C. After UV_B-light exposure of the pig skin, the area of axon reflex erythema was increased in response to the various noxious challenges thereby suggesting that peripheral C-fibres were sensitized (Rukwied *et al.*, 2008). Data from the above two studies were consistent with the sensitization of silent nociceptors.

In summary, UV_A-light exposure of rat tissue altered both peripheral nociceptive receptors and WDR neurones of the spinal cord. UV_A-light increased the spontaneous activity of polymodal receptors and spinal WDR neurones, enhanced the response of spinal WDR neurones to peripheral noxious challenges, and increased the amplitude and duration of VRP. Therefore, UV_A-light caused both peripheral nociceptor sensitization, increased activation of peripheral nociceptors, and central sensitization, enhanced response of dorsal horn cell to peripheral input. UV_B-light activated silent nociceptors and caused peripheral sensitization. However, no studies have explored changes within the central nervous system after UV_B-light exposure.

1.3 Thesis aims

UV-light induced hyperalgesia is a unique model of hyperalgesia in that it causes peripheral and central sensitization through the release of endogenous compounds, without necessitating the injection of algogenic substances into animal tissue, such as carrageenan into rat hind paw or joints (Hargreaves *et al.*, 1988; Radhakrishnan *et al.*, 2003), or directly damaging animal tissue, such as nerve ligation or incising rat skin (Choi *et al.*, 1994; Brennan *et al.*, 1996; Weber *et al.*, 2005). Almost all current animal models of pain and hyperalgesia rely on the above two techniques and, although extremely important in the study of chronic pain, are associated with a number of problems. For example, the duration of the hyperalgesia is too short for anti-hyperalgesic agents to be adequately tested. Ideally anti-hyperalgesics should be administered after the hyperalgesia has developed in order to mimic the clinical situation, in which anti-hyperalgesics are given only after the patient complains. In animal models of sustained hyperalgesia, the experimental animals usually are in persistent pain, which in turn causes a constitutive deterioration in their health that confounds their responses to nociceptive testing (Blackburn-Munro, 2004). In addition, almost none of the procedures can be used in human subjects. The conditioning challenges used generally cause irreversible damage to tissue, such as nerve ligation, or the conditioning challenge does not cause hyperalgesia in human subjects, such as reperfusion hyperalgesia (unpublished observations).

I believe UV-light induced hyperalgesia offers a solution for the above-mentioned problems, in that, in animal models, experimental hyperalgesia is of duration sufficient to test anti-hyperalgesic agents without the presence of persistent pain, and

UV-light has been used successfully to generate experimental hyperalgesia in human subjects. UV_B-light causes an inflammatory hyperalgesia that has been successfully translated from animal subjects to human subjects (see Table 1 and 2). UV_B-light causes an increase of pro-inflammatory mediators such as prostaglandins (Black *et al.*, 1978a; Black *et al.*, 1980), nitric oxide (Rhodes *et al.*, 2001), and cytokines (Oxholm *et al.*, 1988; Kock *et al.*, 1990; Oxholm, 1992; Kondo *et al.*, 1993; Strickland *et al.*, 1997; Saade *et al.*, 2000) in exposed tissue, and the histological features after UV_B-light exposure are consistent with an acute inflammatory response (Gilchrest *et al.*, 1981). In addition, hyperalgesia and erythema can be reversed successfully through the administration of suitable antagonists, such as COX-1 (Snyder & Eaglstein, 1974b, a; Black *et al.*, 1978b) and COX-2 (Wilgus *et al.*, 2000; Wilgus *et al.*, 2002) inhibitors and nitric oxide synthase inhibitor (Rhodes *et al.*, 2001). However, the evidence that proves that UV_A-light causes a classic inflammatory hyperalgesia is not as convincing.

In rats, UV_A-light causes an increase in prostaglandins and histamine in skin (Gilchrest *et al.*, 1981; Hawk *et al.*, 1983) and the hyperalgesia to UV_A-light can be reversed with the administration of morphine, ibuprofen (a COX inhibitor), only demonstrated in one study (Perkins *et al.*, 1992), and bradykinin antagonists (Perkins *et al.*, 1993). Therefore, prostaglandins and bradykinins (both components of the inflammatory soup) it would seem are involved in UV_A-light induced hyperalgesia. It has been assumed therefore that UV_A-light causes an inflammatory hyperalgesia in rats. However, no studies have examined animal tissue histologically after UV_A-light exposure to confirm the presence of inflammation. In addition, after exposure to UV_A-light, human tissue exhibits only a mild chronic inflammatory infiltrate within the

dermis, very unlike the classic acute inflammatory response observed after UV_B-light exposure (Kumakiri *et al.*, 1977; Gilchrest *et al.*, 1981). It is therefore possible that UV_A-light may be causing prolonged hyperalgesia through an inflammation-independent pathway. If so, UV_A-light may offer completely new mechanisms to explain prolonged hyperalgesia. I therefore decided to focus my thesis on UV_A-light hyperalgesia in order to explore whether UV_A-light does cause inflammation in rats, and to answer several issues that I believe have not been adequately addressed in the literature.

In order to study UV_A-light induced hyperalgesia, I had to develop my own animal model. I have outlined my exposure protocol, and the duration and severity to noxious thermal and mechanical challenges in Chapter 2. Although a number of studies have been published describing the pathophysiology UV_A-light, several issues have not been adequately addressed. For example, in none of the studies published was the UV-light spectrum of the UV lamps independently verified. As the nature of light changes both with the duration the UV lamps are activated and over the lifespan of the UV lamp, it is entirely possible that unwanted light wavelengths may have contaminated the UV_A-light during animal exposures in previous studies. I commissioned experts at the Council for Scientific and Industrial Research, Tshwane, South Africa, to measure the spectrum of UV-light emitted by my lamp and I have described my findings in Chapter 2. In addition, no studies have examined animal tissue to confirm the presence, or absence, of inflammation, and whether inflammation does in fact correlate with the behavioural hyperalgesia observed after UV_A-light exposure. I commissioned a qualified histopathologist to examine, histologically, skin tissue removed from the dorsal surface of the rat tail after UV_A-

light exposure to determine the presence of inflammation, and whether inflammation correlated with the duration and severity of hyperalgesia. I have described my findings in Chapter 2. After I established my model of UV_A-light hyperalgesia I investigated the neurophysiological basis for the sustained hyperalgesia that UV_A-light caused.

UV_A-light increases the spontaneous activity of polymodal receptors and spinal WDR neurons (Andreev *et al.*, 1994), enhances the response of spinal WDR neurones to peripheral noxious challenges (Urban *et al.*, 1993) and increases the amplitude and duration of VRP with possible altered NMDA and neurokinin receptor sensitivity (Thompson *et al.*, 1993a). Changes within the WDR neurones may account for UV_A-light induced thermal and mechanical hyperalgesia. The altered WDR activity may result from either plasticity in spinal neuronal circuitry after a peripheral insult or from changes in primary afferent fibre impulses converging on those spinal neurones or both. To explore the above question I blocked the C-fibre barrage, and also recorded the responses of peripheral afferent fibres after UV_A-light exposure.

Activation of WDR neurones by C-fibres (C-fibre barrage) during a conditioning challenge, causes persistent changes in WDR neurones that far outlasts the duration of the conditioning challenge and the C-fibre barrage (Dickenson & Sullivan, 1987; Woolf, 1989; Thompson *et al.*, 1993b; Vattne *et al.*, 1998; Ji *et al.*, 2003). The C-fibre barrage, as the first step in central sensitization, causes a cascade of reactions that ultimately lead to altered excitability of ion channels and membrane-bound receptors in WDR cells (Julius & Basbaum, 2001). Consequently, blocking the C-fibre barrage, through the administration of a regional local anaesthetic, has attenuated the severity

and duration of hyperalgesia in certain animal models of chronic pain (Gonzalez-Darder *et al.*, 1986; Seltzer *et al.*, 1991). Blocking the C-fibre barrage decreased autotomy after sciatic nerve transection (Gonzalez-Darder *et al.*, 1986), and post-incisional allodynia in rats (Duarte *et al.*, 2005). Since no experiments have investigated the role of the C-fibre barrage in UV_A-light induced hyperalgesia, I decided to block the C-fibre barrage before UV_A-light exposure, and record any changes in nociceptive testing. I injected bupivacaine, a long-acting local anaesthetic, into the base of the rat tail before UV_A-light exposure, and then measured the behavioural response of animals to noxious thermal and mechanical challenges. I have described my findings in Chapter 3. To determine whether sensitization of primary afferent nociceptive fibres contributed to mechanical and thermal hyperalgesia after UV_A-light exposure, I measured the responses of fibres in the rat coccygeal nerve, which convey the afferent neuronal activity from the tail, to noxious thermal challenges and noxious mechanical challenges after UV_A-light exposure of the rat tail. I have described my findings in Chapter 4.

CHAPTER 2

Exposure of the rat tail to ultraviolet A light produces sustained hyperalgesia to noxious thermal and mechanical challenges

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Exposure of the rat tail to ultraviolet A light produces sustained hyperalgesia to noxious thermal and mechanical challenges

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Abstract

We aimed to establish whether exposing the tails of rats to ultraviolet A (UV_A) light generated sustained hyperalgesia to noxious thermal and mechanical challenges. The tails of 21 rats underwent eight 40 s exposures of UV_A light, with 260 s between each exposure. As a control procedure, during UV_A-light exposure the tails of 11 of those rats were shielded with aluminium foil. Thermal hyperalgesia was assessed by immersing the rat tail in 49 °C water (modified tail flick test). Mechanical hyperalgesia was assessed by applying a bar algometer onto the tail and timing the escape response. Exposure to direct UV_A light produced hyperalgesia for 8 days to the noxious thermal challenge ($P < 0.05$, two-way ANOVA, Tukey post hoc tests) and at least 16 days to the noxious mechanical challenge ($P < 0.05$, two-way ANOVA, Tukey post hoc tests). They gained mass throughout the study at the same rate as the control rats. The control rats did not develop thermal nor mechanical hyperalgesia. The tails of a further 20 rats were exposed similarly, and tail tissue examined histologically. Both exposed and control rats developed mild chronic inflammation unrelated to the hyperalgesia.

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Keywords: Hyperalgesia; Ultraviolet A light; Rat; Inflammation

1. Introduction

Hyperalgesia, which is hypersensitivity to a painful challenge, has been generated experimentally, in laboratory animals, with two primary objectives. The first is to explore possible mechanisms of clinical hyperalgesia, and the second is to provide an animal assay for antihyperalgesic agents. Current experimental hyperalgesias are not ideal. They tend either to have a duration that is too short for antihyperalgesic agents to be administered after the hyperalgesia has developed (see, for example, Gelgor et al., 1992) where clinically antihyperalgesics normally would be administered only after the patient complains, or they leave the experimental animals in persistent pain (Blackburn-Munro, 2004). Not only does the persistent pain present ethical problems, but also animals in pain show a constitutive deterioration in their health that may confound their response to nociceptive testing (Blackburn-Munro, 2004).

There are some animal procedures that generate hyperalgesia of more than 2-day duration, without the animals suffering from persistent pain. Two such procedures are skin incisions (Brennan et al., 1996; Weber et al., 2005) and ultraviolet (UV) light irradiation of skin (Perkins et al., 1993; Perkins and Kelly, 1993; Saade et al., 2000). Exposure of the rat hind paw to UV_A light produced thermal hyperalgesia for 7 days (Perkins et al., 1993), and exposure of the mouse dorsal surface to UV_B light produced thermal hyperalgesia for 3 days (Saade et al., 2000). A similar duration of thermal hyperalgesia has been observed after skin burns and surgical incision (Perkins et al., 1993; Brennan et al., 1996; Walker et al., 1999). Incision of the rat hind paw (Brennan et al., 1996) and rat tail (Weber et al., 2005) produced mechanical hyperalgesia for 7 days. Although UV irradiation, and in particular UV_A light irradiation, does not mimic sunburn in humans (Benrath et al., 2001) UV_A-induced hyperalgesia provides the possibility for experimental hyperalgesia of duration sufficient to test antihyperalgesic agents without the presence of persistent pain.

Despite hyperalgesia to a thermal challenge after UV_A-light exposure being well described in animals (Perkins et al., 1993;

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Perkins and Kelly, 1993), the duration of the UV_A-light induced hyperalgesia to a mechanical challenge in rats has not been reported, so it is not known whether UV_A-induced hyperalgesia is confined to noxious thermal challenge. The site of UV_A light irradiation conventionally has been the rat hind paw (Perkins et al., 1993; Perkins and Kelly, 1993). A sustained potentially painful intervention is less likely to compromise the well-being of experimental animals if applied to the tail rather than the load-bearing foot, and is less likely to confound responses to a mechanical challenge. Also, although UV-light exposure induced local inflammation in human subjects (Hruza and Pentland, 1993), it is not known whether UV_A irradiation in rats induces an inflammatory or non-inflammatory hyperalgesia, a distinction important in assays of antihyperalgesic agents. We therefore attempted to induce hyperalgesia to noxious thermal and mechanical challenges in rats by exposing their tails to UV_A light, to assess potential distress in the rats by monitoring mass loss and behaviour during experimentation, and to examine the irradiated tissue histologically for signs of inflammation.

2. Materials and methods

2.1. Animals

Forty-one male Sprague–Dawley rats with an initial body mass of 200–225 g were used. Rats were housed two per cage at an ambient temperature of 21–25 °C, and a 12/12 h light/dark cycle, with lights on at 07:00. Rats had free access to water and food. All procedures were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (clearance certificate 2002/89/3) and complied with the recommendations of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (Zimmermann, 1983).

2.2. UV_A light source

A double-ended high-pressure metal halide UV_A lamp (HPM 13, Philips, Belgium) was used as the source of UV_A light. We commissioned the South African Council for Scientific and Industrial Research, Pretoria, to measure the energy spectrum of the lamp. It had a spectral irradiance of 37 mW cm⁻², with 60% of the energy emitted in the UV_A range, 30% in the UV_B range and 10% in the UV_C range, in the steady state. The UV_B irradiance began to appear only from 30 s after the lamp was turned on. Since we limited individual exposures to a maximum of 40 s (see experimental procedure), the lamp emitted predominantly UV_A light during each exposure. The UV_A lamp was housed in a rig (Fig. 1) that irradiated the dorsal surface of the rat tail of two rats simultaneously, with the lamp positioned 350 mm above the tails.

2.3. Tail skin temperature

Tail skin temperature was measured, as an indicator of potential cutaneous hyperaemia after UV_A-light exposure, with an

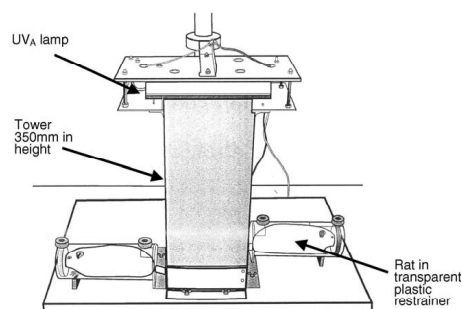


Fig. 1. Diagram of the UV_A-light rig. The construction of the rig prevented exposure of the eyes of the rats, and of the observers, to direct or reflected UV_A light.

infrared thermometer (Genius®, First Temp Model 3000 A, Intelligent Medical Systems, California, USA). The thermometer was aimed directly at the base of the tail that was outside the area of direct irradiation. Tail skin temperature was measured immediately after the final UV_A-light exposure and every 15 min for 90 min thereafter. The thermometer was calibrated, by aiming it at a blackened re-entrant cone submerged in a temperature-controlled waterbath, against a quartz-crystal thermometer (Quat 100, Heraeus, Germany), to an accuracy of 0.1 °C.

2.4. Noxious thermal challenge

The rats' responses to a noxious thermal challenge were measured using the modified tail flick test described by Gelgor et al. (1986). Rats' tails were immersed in 29 °C water for 30 min before the first challenge was made, and between challenges, to maintain a constant tail skin temperature and to prevent the confounding effects that variations in tail skin temperature have on tail flick latency (Tjolsen et al., 1989; Hole and Tjolsen, 1993). To apply the noxious thermal challenge, we immersed the whole tail into 49 °C water, and measured the time taken from immersion of the tail until the first co-ordinated motor response of the tail with a stopwatch. The average of three measurements, taken at least 1 min apart, was recorded as the tail flick latency. To avoid possible tissue damage, immersion of rats' tails in the 49 °C water was limited to 30 s.

2.5. Noxious mechanical challenge

We used a bar algometer to apply a noxious mechanical challenge to the tail. The bar algometer consisted of a spring-loaded force gauge (Halda, RS, Sweden), connected, via a lever, to a 20 mm long steel bar with a diameter of 1.2 mm. The steel bar was placed transversely across the tail, at its midpoint and at two separate sites 10 mm proximal and distal to the tail midpoint, and a suprathreshold noxious static force of 3.9 N applied and maintained by hand (Vidulich and Mitchell, 2000). The bar algometer was applied to three different sites on the

tail to prevent the possible confounding effects of repetitive testing. The time from application of the noxious mechanical challenge until the first sign of aversive behaviour (pulling away, looking back, tucking in the head, vigorous grooming and jumping) was measured with a stopwatch. The average of three measurements, taken at least a 1 min apart, was recorded as the escape latency. To avoid tissue damage the bar algometer was removed from the tail if the rat failed to respond within 30 s.

2.6. Experimental procedure

UV_A-light exposure and all nociceptive testing took place with the rats restrained in transparent plastic restrainers that allowed exposure and movement of the tail, but restricted movement of the body. Before the start of the experiment, rats were habituated to the restrainers for two hours a day on three consecutive days. On experimental days, the rats were placed in the restrainers 30 min before the start of experimentation. For three consecutive days before we exposed the tails to UV_A light, we measured response latencies to the noxious thermal and mechanical challenges. On the day of UV_A-light exposure, the dorsal surface of rats' tails was exposed to the light eight times for 40 s each, with 260 s between the exposures. During UV_A-light exposure, the tails of 11 rats were covered with aluminium foil (to shield the tails from the light), and ten rats' tails were left uncovered. If any rats displayed any aversive behaviour (pulling away, looking back, tucking in the head, vigorous grooming and jumping) to the UV_A-light exposure, the number of exposures required to cause the first aversive response was recorded. Nociceptive testing started 24 h after UV_A-light exposure, and continued, at the same time each day (in the rats' normal inactive period between 9:00 and 12:00) for 16 days. The same investigator (AT) performed all the nociceptive testing. The measurements were terminated after 16 days, when the rats became too large for the restrainers.

2.7. Histology

A separate group of 20 male Sprague–Dawley rats had their tails exposed to the UV_A light (same protocol as experimental procedure), but did not undergo nociceptive challenges. Instead they had skin tissue removed from the dorsal surface of the tail for histological assessment. On each of days 1, 3, 6 and 9 after UV_A-light exposure, three rats of which the tails had not been covered with foil and two rats the tails of which had been covered with foil were euthanased by intraperitoneal injection of sodium pentobarbital (Euthanase, Kyron Labs, South Africa), and 10 mm² of tail skin was excised from a site 60 mm from the base of the tail. The tissue samples were fixed in 10% formalin, embedded in paraffin, stained with haematoxylin and eosin and examined for inflammation under light microscopy by a qualified histopathologist. Any inflammation was classified as acute if neutrophils were present, or chronic if lymphocytes and plasma cells were present. The severity was graded as mild, moderate, or severe depending on the number of inflammatory cells present (Kumar et al., 2005).

2.8. Data analysis

No animal reached the 30 s cut-off time during nociceptive testing, allowing the use of parametric statistical analysis of response latencies. To determine whether UV_A-light exposure induced statistically significant hyperalgesia, the tail flick latencies and escape latencies for the exposed and shielded rats after UV_A-light exposure were compared to each other and to their pre-exposure latencies with a two-way ANOVA with treatment (exposed and shielded) and time after UV_A-light exposure as the main effects, followed by Tukey post hoc tests if differences were found. To determine whether UV_A-light exposure caused statistically significant cutaneous hyperaemia, tail skin temperatures for the exposed and shielded rats after UV_A-light exposure were analyzed similarly, with a two-way ANOVA followed by Tukey post hoc tests. To detect whether UV_A light was noxious during the exposure itself, we compared the number of exposures required to cause the first aversive response by means of the Mann–Whitney test. The rates at which the rats with exposed and shielded tails gained mass over the course of the experiment were compared with an unpaired Student's *t*-test. The severity of tissue inflammation was scored as one for mild inflammation, two for moderate inflammation and three for severe inflammation and the average scores were compared, on days 1, 3, 6 and 9 after UV_A-light exposure, between rats of which the tails were covered with foil during UV_A-light exposure and rats the tails of which were not covered with foil during UV_A-light exposure, by means of the Mann–Whitney test. Data are expressed as mean (S.D.).

3. Results

3.1. Responses during UV_A-light exposure

None of the rats with foil shielding their tails displayed aversive responses to UV_A light, but those with unshielded tails displayed defecation, tail withdrawal and vocalisation. The median number of exposures required to elicit the first aversive response in the rats with tails not covered with foil was three ($P < 0.001$, Mann–Whitney test). However, the rats never were so distressed that we considered it necessary to curtail the UV_A exposures.

Immediately after the barrage of UV_A-light exposures, there was reddening of the distal third of the tail in the rats with tails exposed, but not in the tails of rats with their tails shielded. Exposure to UV_A light did not cause blistering or visible swelling of the tails in any rats. After UV_A-light exposure, all the rats groomed normally, and exhibited no signs of distress when handled or after being restored to their home cages.

3.2. Changes in tail skin temperature after UV_A-light exposure

The effect that the UV_A light had on rat tail skin temperature after exposure is shown in Fig. 2. Pre-exposure tail skin temperature was $26.4 \pm 1.3^\circ\text{C}$ for the group destined for UV_A-light exposure, and $26.3 \pm 1.0^\circ\text{C}$ for the group destined to be shielded. Their pre-exposure tail skin temperatures did

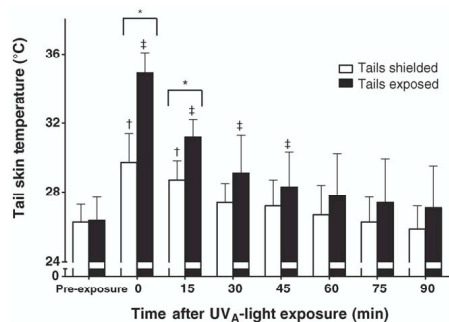


Fig. 2. Mean (S.D.) tail skin temperatures (°C) before and after UV_A-light exposure in rats with tails exposed (closed bars, $n=10$) or shielded (open bars, $n=11$) during UV_A-light exposure. The tail skin temperatures of the exposed rats were significantly higher than the shielded group for 15 min after UV_A-light exposure ($P<0.05$, two-way ANOVA with Tukey post hoc test). The tail skin temperature of the rats with tails exposed was increased significantly above pre-exposure tail skin temperature for 75 min ($P<0.05$, two-way ANOVA with Tukey post hoc tests). The tail skin temperature of the rats with tails shielded was increased significantly above pre-exposure tail skin temperature for 15 min ($P<0.05$, two-way ANOVA with Tukey post hoc tests) (* indicates significant difference between exposed and shielded rats, $P<0.05$; † indicates significant difference from pre-exposure temperatures for shielded rats $P<0.05$; ‡ indicates significant difference from pre-exposure temperatures for exposed rats, $P<0.05$).

not differ significantly ($P=0.99$, two-way ANOVA with Tukey post hoc test). The main effects of UV_A-light exposure with or without shielding ($F_{1,19}=9.71$, $P<0.001$, two-way ANOVA) and time after UV_A-light exposure ($F_{7,133}=64.36$, $P<0.001$, two-way ANOVA), as well as their interaction ($F_{7,133}=9.85$, $P<0.001$, two-way ANOVA) were statistically significant. In rats with tails exposed to UV_A light tail skin temperatures (34.9 ± 1.2 °C) were significantly increased compared to the rats with shielded tails (29.7 ± 1.7 °C) immediately after UV_A-light exposure ($P<0.001$, two-way ANOVA with Tukey post hoc test). Thirty minutes after exposure the tail skin temperatures were no longer different ($P=0.69$, two-way ANOVA with Tukey post hoc test). The tail skin temperatures of the rats with shielded tails were significantly higher than their pre-exposure skin temperatures for 45 min after exposure ($P<0.05$, two-way ANOVA with Tukey post hoc tests), and the tail skin temperatures of the rats with shielded tails were significantly higher than their pre-exposure skin temperatures for 15 min after exposure ($P<0.001$, two-way ANOVA with Tukey post hoc tests).

3.3. Response to a noxious thermal challenge

The effect of UV_A-light exposure on the subsequent tail flick latencies to a 49° thermal challenge is shown in Fig. 3. Pre-exposure tail flick latencies were 5.8 ± 0.9 s for the group destined for UV_A-light exposure, and 6.3 ± 0.7 s for the group destined to be shielded. Their pre-exposure latencies did not

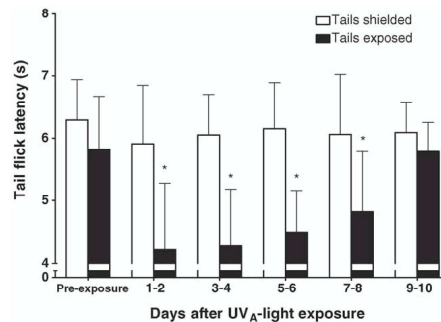


Fig. 3. Mean (S.D.) tail flick latencies (s) before and after UV_A-light exposure, for 10 days after exposure, for rats with tails exposed (closed bars, $n=10$) and shielded (open bars, $n=11$) during UV_A-light exposure. The data has been grouped into 2-day epochs. The tail flick latencies of rats with tails exposed during UV_A-light exposure, were lower than their pre-exposure tail flick latencies and the tail-flick latencies of rats with tails shielded ($P<0.05$, two-way ANOVA with Tukey post hoc tests) for 8 days after UV_A-light exposure. There were no significant changes from pre-exposure tail flick latencies for the rats with shielded tails (* indicates significant difference from pre-exposure and from the rats with shielded tails, $P<0.05$).

differ significantly ($P=0.31$, two-way ANOVA, Tukey post hoc test). The main effects of UV_A-light exposure with or without shielding ($F_{1,19}=15.2$, $P=0.0095$, two-way ANOVA) and time after UV_A-light exposure ($F_{5,95}=19.4$, $P<0.001$, two-way ANOVA), as well as their interaction ($F_{5,95}=11.9$, $P<0.001$, two-way ANOVA) were statistically significant. In rats with tails exposed to UV_A light, tail flick latencies were significantly decreased compared to pre-exposure latencies and to latencies of the rats with shielded tails for 8 days ($P<0.001$, two-way ANOVA with Tukey post hoc tests). The tail flick latencies increased progressively with time after exposure, and by day 9 after UV_A-light exposure, the tail flick latencies were not significantly different from pre-exposure latencies and the latencies of the rats with shielded tails ($P<0.05$, two-way ANOVA with Tukey post hoc tests). The tail flick latencies of the rats with shielded tail never were significantly different from their pre-exposure latencies ($P>0.05$, two-way ANOVA).

3.4. Response to a noxious mechanical challenge

The effect of UV_A-light exposure on the subsequent escape latencies to noxious mechanical challenge is shown in Fig. 4. Pre-exposure tail flick latencies were 6.2 ± 1.7 s for the group destined for UV_A-light exposure, and 5.3 ± 1.7 s for the group destined to be shielded. Their pre-exposure latencies did not differ significantly ($P=0.31$, two-way ANOVA, Tukey post hoc test). The main effects of UV_A-light exposure with or without shielding ($F_{1,18}=14.78$, $P=0.0039$, two-way ANOVA) and time after UV_A-light exposure ($F_{4,72}=25.85$, $P<0.001$, two-way ANOVA), as well as their interaction ($F_{4,72}=11.86$, $P<0.001$,

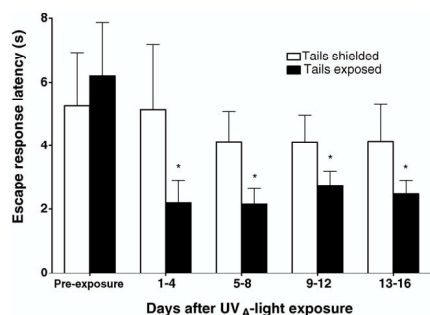


Fig. 4. Mean (S.D.) escape latencies (s), before and after UV_A-light exposure for 16 days after exposure, for rats with tails exposed (closed bars, $n = 10$) and shielded (open bars, $n = 11$) during the exposure. The data has been grouped into 4-day epochs. The escape latencies of rats with tails exposed during UV_A-light exposure, were lower than their pre-exposure escape latencies and the escape latencies of rats with tails shielded ($F_{4,72} = 11.9$, $P < 0.05$, two-way ANOVA with Tukey post hoc tests) for 16 days after UV_A-light exposure. There were no significant changes from pre-exposure tail flick latencies for the rats with shielded tails (*) indicates significant difference from pre-exposure and from the rats with shielded tails, $P < 0.05$).

two-way ANOVA) were significantly different. In rats with tails exposed to UV_A light, tail flick latencies were significantly decreased compared to pre-exposure latencies and to latencies of the rats with shielded tails for 16 days ($P < 0.001$, two-way ANOVA with Tukey post hoc tests). The escape latencies did not increase significantly with time after exposure. The tail flick latencies of the rats with shielded tail never were significantly different from their pre-exposure latencies ($P > 0.05$, two-way ANOVA).

3.5. Body mass

Rats from both groups gained body mass over the course of the experiment at similar growth rates. The initial body mass of the rats destined for UV_A exposure was 211 ± 15 g, and that of the rats to be shielded was 213 ± 11 g. The mean weights did not differ significantly ($P = 0.793$, unpaired t -test). At 16 days after UV_A light exposure, the body mass of the rats with tails exposed had increased by 34% to 282 ± 14 g, that of the rats with tails shielded increased by 32% to 285 ± 9 g. The final mass of the two groups were also not significantly different ($P = 0.531$, unpaired t test).

3.6. Histology

Histological examination of the tail skin revealed signs of mild chronic inflammation, with a few scattered chronic inflammatory cells, namely lymphocytes and plasma cells. Notably, though, there was no difference, on days 1, 3, 6 and 9, in the severity of the inflammation between the rats with tails exposed to UV_A light and those with tails shielded from it ($P = 0.486$, Mann–Whitney test).

4. Discussion

We set out to investigate whether UV_A irradiation of the rats' tail would produce hyperalgesia of sufficient duration potentially to allow the testing of antihyperalgesic drugs, but without subjecting the rats to ongoing pain or distress during the period of hyperalgesia. UV_A-light exposure of the rats' tail indeed produced a sustained hyperalgesia to both noxious thermal and noxious mechanical challenges. The hyperalgesia to the noxious thermal challenge lasted for 8 days after UV_A-light exposure and to the noxious mechanical challenge for at least 16 days. The amplitude of the hyperalgesia was substantial: the response latency to the noxious thermal challenge was shortened by nearly a third in the hyperalgesic state and that to the mechanical challenge by over half. Nevertheless, despite being demonstrably hyperalgesic for over 2 weeks after UV_A-light exposure, the rats did not exhibit signs of chronic distress, such as abnormal gait, reduced mobility, or reduced grooming when handled, and they gained body mass over the duration of the experiment at a rate similar to that of the control rats. Our study is the first to have demonstrated mechanical hyperalgesia in rats after UV_A-light exposure. It also is the first to have explored the site of UV_A irradiation for inflammation. We discovered mild chronic inflammation at the site, but it was present in the control rats too. The control rats never were hyperalgesic, so the sustained hyperalgesia we observed was unlikely to have been caused or maintained by the mild inflammation. Whatever the events were which led to the hyperalgesia, they were not visible, under light microscopy, in the irradiated tissue.

It was events associated with the UV_A light irradiation itself, though, which were responsible for the hyperalgesia. When a test for experimental hyperalgesia requires the use of repetitive noxious challenges, as ours did, there always is a risk that those challenges induce or exacerbate hyperalgesia. The 49 °C water-immersion challenge we employed does so, if the challenges are not spaced sufficiently (Gelgor et al., 1986). The noxious mechanical challenge we employed also does so, if applied to the tail during reperfusion after transient ischaemia (Vidulich and Mitchell, 2000). However, our control rats, which were subjected to the same series of noxious challenges, did not develop hyperalgesia.

The ethical advantages of generating sustained hyperalgesia without, apparently, ongoing pain or distress in the rats would be negated if the conditioning event itself induced unacceptable (and particularly inescapable) pain or distress. The absence of blistering of the skin, and indeed the absence of acute inflammation, implies that UV_A light did not seriously damage the tissue. The rats did exhibit aversive behaviour, beginning, on average, at the third of the eight 40 s exposures, but not to a degree which would have necessitated discontinuation of the exposures. In an earlier pilot experiment, we had discovered that continuous, rather than intermittent, exposure to the same UV_A irradiation, in our opinion, could not be applied ethically for a duration that would induce subsequent hyperalgesia.

Our control rats were subjected to the same sequence of UV_A-light exposures which induced hyperalgesia in their counterparts, but we shielded their tails from direct exposure using

aluminium foil. That control procedure successfully prevented subsequent hyperalgesia, but it did not influence the degree of inflammation produced, and though it reduced the subsequent rise in tail skin temperature (Fig. 2), it did not eliminate the hyperaemia which the rats exhibited. We believe that the mild chronic inflammation, and part of the hyperaemia, may have been the result of the tail hyperthermia. The air in the UV_A light rig reached a non-noxious peak temperature of about 45 °C during triggering of the UV_A lamp. The hyperthermia, if it was indeed the cause of the unexpected reactions of the control rats, did not induce hyperalgesia, and so did not confound our main results. It could be avoided in future by constructing a lightproof but ventilated rig.

Another unexpected outcome of our study was that the mechanical hyperalgesia induced by UV_A-light exposure had not resolved by 16 days after exposure, when we had to stop testing for hyperalgesia because the rats had outgrown their restrainers. Since no-one previously had investigated noxious mechanical hyperalgesia in rats subject to UV_A light, we had no prior evidence that the hyperalgesia would last that long. The ultimate duration of the hyperalgesia will need to be determined in a future modified protocol.

Although UV_A-induced mechanical hyperalgesia was a novel finding, and, indeed, we have been the first to explore UV_A-induced hyperalgesia to any challenge in the rat tail, others have reported thermal hyperalgesia induced by UV_A-light exposure of the rat hind-paw (Perkins et al., 1993; Perkins and Kelly, 1993). The duration of thermal hyperalgesia in the tails of our rats was comparable to the 10 days of hyperalgesia elicited by Perkins et al. (1993) in the rat hind paw, but twice as long as the thermal hyperalgesia elicited by exposure of the skin of mice to UV_B light (Saade et al., 2000). UV light is a well described cause of inflammation (Hruza and Pentland, 1993), but, in the skin of rats and mice at least, it appears to be wavelengths in the UV_B, and not in the UV_A, range which are responsible. UV_B irradiation of mouse skin caused an increase in the pro-inflammatory cytokines interleukin (IL)-1 β , tumour necrosis factor- α , nerve growth factor and administration of the anti-inflammatory cytokines IL-10 and IL-13 attenuated the UV_B-

light induced hyperalgesia (Saade et al., 2000). However, UV_A light has not been shown to increase tissue cytokine production, nor does it produce a tissue inflammatory response that parallels behavioural hyperalgesia in the rat hind paw (Perkins et al., 1993; Perkins and Kelly, 1993). Also, hyperaemia persists in inflammatory hyperalgesia (Benrath et al., 2001) and it did not do so in our rats.

When hyperalgesia is induced experimentally by some other conditioning events, an inflammatory response does parallel the duration of hyperalgesia to either thermal or mechanical challenges (Vinegar et al., 1987; Diehl et al., 1988; Weber et al., 2005). Carrageenan injected into the paw of rats and cats produced an acute inflammation that persisted for the duration of the hyperalgesia (Vinegar et al., 1987; Diehl et al., 1988). Histopathological evidence of inflammation after experimental incision of the rat tail correlated well with the development of mechanical hyperalgesia: at the time when the mechanical hyperalgesia was at its peak, acute inflammation was present, and as the acute inflammatory tissue was replaced by granulation tissue, the mechanical hyperalgesia resolved (Weber et al., 2005). During the hyperalgesia to a noxious thermal challenge which occurs after transient ischaemia of the rat's tail (Gelgor et al., 1992), however, as in our UV_A-induced hyperalgesia, there was no histopathological evidence for inflammation. Experimental hyperalgesia without inflammation provides an advantage for testing of putative antihyperalgesic agents, because agents which relieve such hyperalgesia must act through antinociceptive rather than anti-inflammatory mechanisms.

Our UV_A-induced hyperalgesia had another advantage, too, as the basis of a prolonged assay for antihyperalgesic agents, namely that it lasted for 8 days, as assessed by enhanced responses to two kinds of noxious challenge, and at least a further 8 days when assessed by a noxious mechanical challenge. Table 1 shows the duration of some comparable experimental hyperalgesias reported in the literature. There are experimental hyperalgesias which last for weeks to months, which may simulate the clinical hyperalgesia associated with conditions like rheumatoid arthritis, osteo-arthritis and fibromyalgia (Calvino et al., 1987; Colpaert, 1987; Sluka et al., 2001; Radhakrishnan

Table 1
Duration of experimental hyperalgesia induced by different conditioning events

Conditioning event	Site	Duration of hyperalgesia		Reference
		Thermal challenge	Mechanical challenge	
Capsaicin injection	Rat hind paw	45 min	3 h	Gilchrist et al. (1996)
Ischaemia	Rat tail	90 min	–	Gelgor et al. (1986), Vidulich and Mitchell (2000)
Nitroglycerin injection	Rat hind paw	4 h	–	Tassorelli et al. (2003)
Carrageenan injection	Rat hind paw	24 h	–	Hargreaves et al. (1988)
Bee venom injection	Rat hind paw	48 h	24 h	Chen et al. (1999)
UV _B light	Mouse dorsum	3 days	–	Saade et al. (2000)
Skin incision	Rat hind paw	7 days	7 days	Brennan et al. (1996), Zahn and Brennan (1999)
Skin incision	Rat tail	–	7 days	Weber et al. (2005)
UV _A light	Rat tail	8 days	>16 days	This study
UV _A light	Rat hind paw	10 days	–	Perkins et al. (1993), Perkins and Kelly (1993)
Carrageenan injection	Rat joint + muscle	7–8 weeks	7–8 weeks	Radhakrishnan et al. (2003)
Spinal nerve ligation	Rat L5-L6	16 weeks	>20 weeks	Choi et al. (1994)
Acidic saline injection	Muscle	–	30 days	Sluka et al. (2001)

et al., 2003). However, the conditioning events necessary to induce ultra long-lasting hyperalgesias leave the experimental animals in persistent pain, which not only confounds responses to evoked pain, but which also compromises the health of the animals, which characteristically lose mass, showed an abnormal gait, reduced mobility, reduced grooming and disturbed sleep (Blackburn-Munro, 2004). Ours is the longest-lasting experimental hyperalgesia reported so far during which the experimental animals gain weight, and appear to be healthy and pain-free. Not only were they free of impaired mobility associated with long lasting hyperalgesias, but because the hyperalgesia was produced in the tail, not the hind paw, they also were free of the impaired mobility associated with any lesions of the foot, including those induced in previous studies of UV_A-induced hyperalgesia.

We were not the first to employ UV_A irradiation to induce experimental hyperalgesia (Perkins et al., 1993; Perkins and Kelly, 1993). What we have done is to show that the hyperalgesia is evident to a mechanical noxious challenge, that the hyperalgesia to the mechanical challenge lasts substantially longer than that to the thermal challenge, and that the hyperalgesia is not the consequence of local inflammation. We also have transferred the site of hyperalgesia from the hind paw to the tail, where it interferes less with the mobility of the animal. UV_A-induced hyperalgesia of the rat tail provides the basis for an assay of antihyperalgesic agents, testing their antinociceptive rather than anti-inflammatory capacity, and lasting sufficiently long for a course of agents to be administered after the hyperalgesia has developed.

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CHAPTER 3

Pre-emptive ring-block with bupivacaine prevents the development of thermal hyperalgesia, but not sustained mechanical hyperalgesia, in rat tails exposed to ultraviolet A light

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Pre-Emptive Ring-Block With Bupivacaine Prevents the Development of Thermal Hyperalgesia, but not Sustained Mechanical Hyperalgesia, in Rat Tails Exposed to Ultraviolet A Light

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Abstract: We investigated the role of the C-fiber barrage in the development of hyperalgesia in rat tails exposed to ultraviolet A (UVA)-light exposure by pre-emptively blocking C-fiber activation with the local anesthetic bupivacaine. Thirty minutes before UVA-light exposure, male Sprague-Dawley rats were given subcutaneous injections, in the base of the tail, of either saline or bupivacaine (1 mL of .5%). Thermal hyperalgesia was assessed daily from day 1 to day 10 after UVA-light exposure by measuring response latency to noxious heat (tail immersion in 49°C water). Injection of bupivacaine completely prevented the development of thermal hyperalgesia ($P < .05$, main effect of group, 2-way analysis of variance). Primary mechanical hyperalgesia was assessed daily from day 1 to day 14 after UVA-light exposure by measuring aversive behavior responses to a punctate pressure applied to the tail with a von Frey anesthesiometer. The rats given bupivacaine developed hyperalgesia to the mechanical challenge that persisted for 14 days ($P < .05$, main effect of time, 2-way analysis of variance) and was identical to the hyperalgesia developed by the rats given saline. We concluded that the C-fiber barrage is involved in the development of thermal hyperalgesia but not sustained primary mechanical hyperalgesia, induced by exposing rats' tails to UVA light.

Perspective: UVA-light exposure of the rat tail causes prolonged hyperalgesia to noxious thermal and mechanical challenges. We have demonstrated that the C-fiber barrage is involved in the development of sustained thermal hyperalgesia, but not mechanical hyperalgesia, caused by exposure of the rat tail to UVA light.

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Key words: UVA-light hyperalgesia, local anaesthetic, bupivacaine, pre-emptive analgesia, thermal and mechanical hyperalgesia.

Hyperalgesia after ultraviolet A (UVA)-light exposure relies on sensitization of spinal wide dynamic range (WDR) neurons. The sensitized WDR neurons show increased spontaneous activity, reduced

thresholds to non-noxious thermal challenges, increased responses to noxious mechanical and thermal challenges, and expanded receptive fields.³⁵ Similar spinal neuroplasticity has been implicated in the generation of sustained hypersensitivity and persistent pain and involves activation of WDR neurons by C fibers (C-fiber barrage) during a conditioning stimulus.^{12,22,33,38} This C-fiber barrage causes persistent changes in WDR neurons that far outlast the duration of the conditioning stimulus and C-fiber barrage³⁶ and is therefore not dependent on sustained C-fiber input. C-fiber barrage causes recruitment of N-methyl-D-aspartate (NMDA) re-

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ceptors^{11,38} and activation of intracellular second messenger systems, which cause long-term changes in gene expression, and ultimately alters the excitability of ion channels and membrane-bound receptors in WDR cells.²³ Accordingly, blocking the C-fiber barrage attenuates the severity and duration of hyperalgesia in certain animal models of chronic pain.^{17,29} For example, administration of a regional local anesthetic, which preferentially blocks C-fiber transmission, decreases autotomy after sciatic nerve transection¹⁷ and postincisional allodynia^{13,18} in both rats¹³ and human beings.¹⁸ Whether preferential C-fiber block similarly attenuates the hyperalgesia induced by UVA-light exposure has not been investigated.

Recently, we described persistent noninflammatory mechanical and thermal hyperalgesia after brief exposure of the rat tail to UVA light.³¹ To determine whether C-fiber barrage during UVA-light exposure has a role in the development of this hyperalgesia, we blocked the C-fiber barrage before UVA-light exposure by using the long-acting local anesthetic bupivacaine and then measured the behavioral response of animals to noxious thermal and mechanical challenges for the subsequent 2 weeks.

Materials and Methods

Animals

Twenty male Sprague-Dawley (Harlan, Indianapolis, IN) rats with an initial mass of 200 to 225 g were used. Rats were housed 2 per cage at an ambient temperature of 21°C to 25°C and a 12:12-hour light/dark cycle, lights on at 7:00 AM. Rats had free access to tap water and food. All procedures were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (clearance certificate 2004/39/3) and complied with the recommendations of the Committee for Research and Ethical Issues of the International Association for the Study of Pain.⁴⁰

UVA Light Source

A double-ended high-pressure metal halide UVA lamp was used as a source of UVA light. The lamp had a spectral irradiance of 37 mW/m², with 60% of the energy emitted in the UVA range, 30% in the UVB range, and 10% in the UVC range. The UVB irradiance began to increase only from 30 seconds after the lamp was turned on.³¹ Because the maximum time the lamp was turned on while exposing the rats' tails was 40 seconds, the tail was exposed predominantly to UVA light. The UVA lamp was housed in a rig and positioned 350 mm above the tail.

Noxious Thermal Challenge

The rats' responses to a noxious thermal challenge were measured by using the modified tail-flick test described by Gelgor et al.¹⁶ Rats' tails were immersed in 29°C water for 30 minutes before the first challenge was made and between challenges to maintain a constant tail-skin temperature and prevent the confounding ef-

fects that variations in tail-skin temperature have on tail-flick latency.^{21,34} To apply the noxious thermal challenge, we immersed the whole tail into 49°C water and measured the time taken from immersion of the tail until the first coordinated motor response of the tail with a stopwatch. The average of 3 measurements, taken at least 1 minute apart, was recorded as the tail-flick latency. To avoid possible tissue damage, immersion of rats' tails in the 49°C water was limited to 30 seconds.

Noxious Mechanical Challenge

An electronic von Frey anesthesiometer was used to apply a noxious mechanical challenge to the tail. The von Frey filament was applied at 3 separate sites, 50, 55, and 60 mm from the base of the tail, by one investigator (A.T.) with uniformly increasing force until the rat exhibited aversive behaviors (eg, pulling away, looking back, tucking in the head, vigorous grooming, and jumping), when the pressures were recorded. The mean of these 3 measurements, taken with at least a 1-minute recovery period between successive measurements, was designated the noxious aversive pressure. To avoid tissue damage, the von Frey filament was removed if the pressure reached 400 g without the rat responding.

Analgesic Efficacy of Bupivacaine

One milliliter of .5% bupivacaine (Macaine; Adcock Ingram, Bryanston, South Africa) was injected into the base of the rat tail, producing a ring-block. In a pilot study to establish the analgesic efficacy and duration of action of bupivacaine, we injected 3 rats with bupivacaine and 2 rats with saline and measured the rats' response to a noxious thermal challenge (49°C water)¹⁶ 20, 40, and 90 minutes after the injection and 24 hours later.

Experimental Procedure

UVA-light exposure, bupivacaine injection, and all nociceptive testing took place on rats restrained in polymethyl methacrylate restrainers that allowed movement and UVA-light exposure of the tail but restricted movement of the body. Before the start of the experiment, rats were habituated to the restrainers for 2 hours a day on 3 consecutive days. On experimental days, the rats were placed in the restrainers 30 minutes before the start of experimentation.

For 3 consecutive days before exposure to UVA light, pre-exposure responses to the noxious thermal and mechanical challenges were measured. Twenty minutes before UVA-light exposure, 8 animals were injected with bupivacaine and 7 rats with saline. Immediately before the rats were exposed to UVA light, responses to noxious thermal challenge of the tail were measured. The rats' tails then were exposed to UVA light 8 times, with each exposure lasting 40 seconds and with 260 seconds between the exposures. Any aversive behaviour during the UVA-light exposure was noted, and the number of exposures required to cause the first aversive response, if any, was recorded. Starting 24 hours after UVA-light exposure, responses to the noxious challenges were measured

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daily for up to 14 days. The same experimenter (A.T.) performed all nociceptive testing. The experimenter was blinded to the bupivacaine/saline treatment. A laboratory technician drew up the saline and bupivacaine into their respective syringes, and the respective agents were revealed to A.T. only at the end of the experiment.

Data Analysis

No animals reached the 30-second cutoff time for tail noxious heat or the 400-g cutoff pressure for noxious pressure during nociceptive testing, allowing the use of parametric statistical analysis. To determine whether bupivacaine prevented UVA-light-induced hyperalgesia, latencies and aversive pressures for rats injected with saline and bupivacaine were compared with each other and with pre-exposure values with a 2-way analysis of variance (ANOVA), with treatment (saline and bupivacaine) and time after UVA-light exposure as the main effects. Student-Newman-Keuls post hoc tests were performed if significant main effects or interactions were found. The pre-exposure latencies and aversive pressure of the 2 groups of rats were compared with unpaired *t* test. To determine the analgesic efficacy and duration of action of bupivacaine, we compared percentage changes in tail-flick latencies to a noxious thermal challenge 20, 40, and 90 minutes and 24 hours after the injection of bupivacaine with those animals injected with saline, with zero (pre-injection) with a 2-way ANOVA, followed by a Student-Newman-Keuls post hoc test if differences were detected. We used percentage change instead of response latency, because reaction times differed between rats in our pilot study. To detect whether UVA light was noxious during the exposure itself, we compared between those rats that were injected with saline and those injected with bupivacaine the number of exposures before rats started to display aversive responses to UVA light by means of the Fisher exact test.

Results

Analgesic Efficacy of Bupivacaine

Fig 1 shows that the ring-block of bupivacaine increased tail-flick latencies for 90 minutes after injection, but by 24 hours after bupivacaine injection, tail-flick latencies had returned to preinjection values ($F_{4,12} = 4.8$, $P = .02$, 2-way ANOVA). Therefore, bupivacaine will have produced analgesia for the time for which rats' tails were exposed to the UVA light but could have had no effect in responses to challenges applied 24 hours later.

Aversive Responses During UVA-Light Exposure

Rats injected with bupivacaine exhibited no aversive behaviors during UVA-light exposure. Rats injected with saline exhibited aversive behavior from the third UVA-light exposure to the eighth UVA-light exposure ($P < .001$, Fisher exact test). Therefore, ring-block of the tail with bupivacaine prevented aversive behaviors during the UVA-light exposure.

Pre-Emptive Ring Block Prevents UVA-Light Hyperalgesia

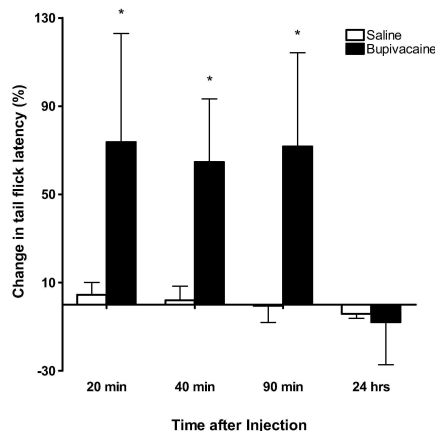


Figure 1. Mean (standard deviation) percentage change in response latencies to tail immersion in 49°C water, after ring block of the tail with saline (open bars, $n = 2$) and bupivacaine (closed bars, $n = 3$). The bupivacaine tail block significantly increased the tail-flick latencies 20, 40, and 90 minutes after injection ($P < .05$, 2-way ANOVA), whereas the saline had no effect at any time period. The response latencies of the rats injected with bupivacaine returned to preinjection values 24 hours after injection. (*Significant difference from preinjection tail-flick latencies and between groups, $P < .05$).

Response to Noxious Thermal Challenge After UVA-Light Exposure

Fig 2 shows that injection of bupivacaine prevented the decrease of response latencies to a noxious thermal challenge, otherwise evident after UVA-light exposure. Pre-exposure response latencies were 5.9 ± 1.2 seconds for the group destined for saline injection and 6.0 ± 0.7 seconds for the group destined for bupivacaine injection, and they did not differ significantly ($P = .78$, unpaired *t* test). Significant main effects of saline or bupivacaine injection ($F_{1,12} = 6.4$, $P = .03$) and time after UVA-light exposure ($F_{5,60} = 10.5$, $P < .05$), as well as interaction ($F_{5,60} = 8.4$, $P < .05$), were detected. A significant decrease from pre-exposure response latencies developed in rats injected with saline for 8 days after UVA-light exposure ($P < .05$, 2-way ANOVA). The decrease was greatest during the first 2 days after UVA-light exposure (3.9 ± 1.2 seconds), with a gradual return to pre-exposure values during a period of 8 days. Response latencies of the bupivacaine group remained unchanged from their pre-exposure tail-flick latencies.

Response to Noxious Mechanical Challenge

Fig 3 shows that injection of bupivacaine before UVA-light exposure did not prevent a decrease in aversive pressure thresholds. Pre-exposure pressures were 229 ± 60 g for the group destined for saline injection and $214 \pm$

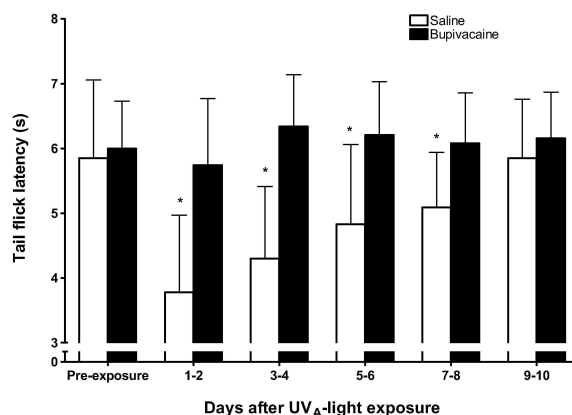


Figure 2. Mean (standard deviation) response latencies to tail immersion in 49°C water before and after UVA-light exposure in rats injected with saline (open bars, $n = 7$) before UVA-light exposure and in rats injected with bupivacaine (closed bars, $n = 8$). There was a significant decrease in response latencies after UVA-light exposure for the rats injected with saline; the greatest decrease occurred during the first 2 days, with a gradual recovery during the next 6 days ($P < .05$, 2-way ANOVA with Student-Newman-Keuls post hoc test). There were no significant changes from pre-exposure response latencies in the rats injected with bupivacaine. The response latencies of the bupivacaine and saline groups differed from day 1 after UVA-light exposure until day 8 after UVA-light exposure ($P < .05$, 2-way ANOVA with Student-Newman-Keuls post hoc test). (*Significant difference from pre-exposure tail-flick latencies and from rats injected with bupivacaine, $P < .05$).

38 g for the group destined for bupivacaine injection, and they did not differ significantly ($P = .56$, unpaired t test). The main effect of time was statistically significant ($F_{7,91} = 31.9$, $P < .05$, 2-way ANOVA), whereas the main

effect of saline or bupivacaine injection ($F_{1,13} = .02$, $P = .88$, 2-way ANOVA), as well as main effects interaction ($F_{7,91} = .78$, $P = .61$, 2-way ANOVA), were not statistically significant. The pressure threshold required to elicit an

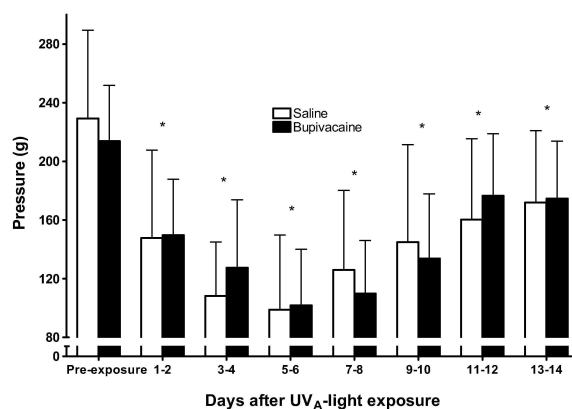


Figure 3. Mean (standard deviation) aversive pressures (g) during noxious punctate challenge to the tail, before and after UVA-light exposure, in rats injected with saline (open bar, $n = 7$) before UVA-light exposure and in rats injected with bupivacaine (closed bar, $n = 8$). The aversive pressure of the rats injected with both saline and bupivacaine differed significantly from their pre-exposure pressures for the 14 days measured after UVA-light exposure, with the greatest change during day 3 to day 6 ($P < .05$, 2-way ANOVA with Student-Newman-Keuls post hoc test). The aversive pressures exhibited by the groups did not ever differ significantly from each other. (*Significant difference from pre-exposure aversive pressures for both the saline- and bupivacaine-injected groups, $P < .05$).

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aversive behavior was significantly reduced from pre-exposure pressures in animals injected with both saline and bupivacaine for 14 days after UVA-light exposure ($P < .05$, 2-way ANOVA with Student-Newman-Keuls post hoc test). The greatest decrease in aversive pressure occurred from 3 to 6 days after UVA-light exposure ($P < .05$, 2-way ANOVA with Student-Newman-Keuls post hoc tests). The aversive pressures of the saline and bupivacaine groups did not differ with each other during the 14 days ($F_{7,91} = .78$, $P = .61$, 2-way ANOVA). Although neither the bupivacaine nor the saline group reached pre-exposure pressures by day 14, pressure thresholds gradually increased from day 6 after UVA-light exposure and reached a plateau from 10 to 14 days ($P < .05$, 2-way ANOVA with Student-Newman-Keuls post hoc tests).

Discussion

We set out to investigate what effect pretreatment blocking of C-fiber activity during UVA-light exposure would have on the development of UVA-induced hyperalgesia to noxious thermal and punctate mechanical challenges. Bupivacaine injection into the base of the rat tail before UVA-light exposure prevented the development of hyperalgesia to a subsequent noxious thermal challenge, but it did not affect at all the development of primary hyperalgesia to a noxious punctate mechanical challenge. The absence of thermal hyperalgesia in bupivacaine-treated animals cannot be explained by continued anesthetic nerve block by bupivacaine, because bupivacaine-induced analgesia had abated by the time at which nociceptive testing took place. Therefore, adequate afferent neuronal activity during UVA-light exposure is critical for the development of thermal but not mechanical hyperalgesia.

Previous studies have shown that perineural bupivacaine blocks C-fiber afferent activity in rats,^{1,13,17,18} and our bupivacaine ring-block did render our rats insensitive to the noxious effects of repeated UVA-light exposure of the rat tail, which evoked aversive responses by the third exposure in rats not given bupivacaine. However, some neuronal afferent activity must have survived the ring-block, because the ring-block extended the latency of, but did not abolish, the response of rats to immersion of their tails in 49°C water. We believe that the reduced activity would have been in C fibers, because they are thought to be responsible for the thermal nociception induced by 49°C water,^{2,3,5,6} but they might have also been in Aδ fibers; resolving which fibres survived would require electrophysiologic measurement of fiber activity above the ring-block. Importantly, the residual activity neither made the rats sentient to UVA-light exposure of the rat tail, nor did it induce the plasticity in the central nervous system that presumably led to the sustained thermal hyperalgesia in rats that had not received the ring-block. It would seem that it did not require total abolition of C-fiber afferent input to ward off hyperalgesia.

A possible confounding factor in our study would be the systemic absorption of bupivacaine and a subsequent

Pre-Emptive Ring Block Prevents UVA-Light Hyperalgesia

systemic analgesic effect. However, for local anesthetics to have a significant nontoxic systemic analgesic effect, administration of the local anesthetic would have to be intravenous. It is possible that some of the locally administered bupivacaine entered the plasma, but at the dose of bupivacaine we used, the plasma concentration would have been low, and consequently the amount of drug that would reach the central nervous system probably would have been negligible. We therefore believe that although we cannot prove the absence of a systemic bupivacaine effect, we do not think that the dose we injected would have caused a systemic analgesic effect.

Thermal hyperalgesia might be mediated both by sensitization of cutaneous thermal nociceptors and by hypersensitivity of interneurons within the spinal dorsal horn, following C-fiber barrage.^{24,35} Previous studies have shown that the hyperalgesia resulting from exposure of rats to UVA light involves sensitization of WDR neurons³⁵ but also can be abolished by using peripherally administered bradykinin receptor antagonists.^{27,28,35} Because our ring blocked animals did not develop thermal hyperalgesia at any time, we conclude that the local nerve block interfered with all mechanisms by which UVA-light exposure induced thermal hyperalgesia in the control animals. The block successfully intercepted the C-fiber barrage during UVA-light exposure. If local neurochemical changes capable of sensitizing nociceptors were evoked, they no longer were operative after the ring-block wore off, because if they were, thermal hyperalgesia would have developed on the second or later days. Centrally mediated thermal hyperalgesia after UVA-light exposure might be mediated by the removal of Mg^{2+} blockade of NMDA receptors. Similar to UVA light, subcutaneous bee venom injection causes a centrally mediated thermal hyperalgesia with an associated primary mechanical hyperalgesia.^{7,8,10} Intraplantar injection of bee venom causes a contralateral thermal hyperalgesia in the absence of contralateral mechanical hyperalgesia, and the contralateral thermal hyperalgesia is a centrally mediated phenomenon that can be blocked through the systemic administration of NMDA receptor antagonist.^{7,9}

Despite preventing the development of thermal hyperalgesia, ring-block did not interfere with the hyperalgesia to the punctate mechanical noxious challenge. It might be that the mechanical hyperalgesia depended on sensitization processes activated by myelinated afferent pathways,²⁵ which were not disabled by the local anesthesia and which did not contribute to the sensation the rats experience during the actual UVA-light exposure. It is more likely, however, that sustained peripheral neuronal sensitization was responsible for the mechanical hyperalgesia. Indeed, Urban et al³⁵ observed continuous low-threshold mechanoreceptor input to the dorsal horn during the first 3 days after UVA-light exposure of the rat hind paw. Subsequently, the same group³² showed enhanced summation of ventral root potentials, a measure of windup, in response to C- and A-fiber stimulation. Therefore in our animals, continuous input from low-threshold mechanoreceptor after UVA-light exposure might have led to progressive windup in dorsal horn neu-

rons²⁰ and therefore central sensitization, independent of an initial C-fiber barrage during UVA-light exposure. It remains unknown what would cause sustained discharge in afferent mechanoreceptive pathways after UVA-light exposure. It is unlikely that the release of inflammatory mediators at the exposure site causes continued mechanical hyperalgesia because we have shown histologically that there is no inflammation.³¹

Although we did not observe signs of inflammation on the histologic sections of our rats' tails, inflammatory mediators such as histamine and prostaglandins still might be involved in sensitizing nociceptive pathways during UVA-light exposure or maintaining the hyperalgesia after UVA-light exposure. Dissociation between histologic findings and the involvement of inflammatory mediators has been reported previously for a model of reperfusion hyperalgesia in which pre-emptive administration of cyclooxygenase inhibitors, NMDA receptor antagonists, and antihistamines reversed acute hyperalgesia produced by ischemia of the rat tail, yet histologic evidence of inflammation was absent.¹⁴⁻¹⁶ Another possible explanation for our data is that UVA light through its effects on chromophores causes permanent changes within peripheral and central nociceptive pathways. Chromophores such as DNA, proteins, amino acids like tryptophan and tyrosine, melanins, and urocanic acid are molecules in skin that absorb UV light and are responsible for initiating the biologic response to UV light.³⁹ Energy from UV light either directly alters the biologic activity of chromophores (eg, DNA), or chromophores might affect the biologic activity of other molecules. For example, porphyrins cause damage to molecules such as DNA and proteins through the generation of oxygen radicals. Therefore, UV light acting through chromophores might produce permanent changes within the peripheral nervous system. These changes might have caused the change in the sensitivity of peripheral nociceptors in our rats in the absence of overt histologic signs of inflammation and thereby account for an undetected peripheral mechanism for the sustained hyperalgesia we observed. The development of primary hyperalgesia to noxious challenges of one modality but not others is not unique to the conditioning event of UVA-light hyperalgesia.

As mentioned above, subcutaneous bee venom injection causes a centrally mediated thermal hyperalgesia with an associated primary mechanical hyperalgesia.^{7,8,10} Intraplantar injection of bee venom causes a contralateral thermal hyperalgesia in the absence of contralateral mechanical hyperalgesia.^{7,9} Nitroglycerin and carrageenan injected into the rat hind paw cause an acute thermal hyperalgesia, yet a comparable mechanical hyperalgesia has not been described.^{19,30} In addition, incision of the rat tail caused an 8-day primary mechanical hyperalgesia without an accompanying thermal hyperalgesia.³⁷ What could cause primary mechanical hyperalgesia in the absence of thermal hyperalgesia is unclear.

Primary hyperalgesia is caused by sensitization of nociceptors located on the terminals of peripheral afferent nociceptive fibers, whereby the properties of the receptors and their second messenger systems are altered. At the water temperature our rats' tails were exposed to, thermal nociception is transduced into neural signals by the vanilloid receptor TRPV1.⁴ The receptors for mechanical transduction have not been identified,²⁶ but candidate receptors include TRPA1 or TRPN1 receptors. It therefore is possible that receptors specific for mechanotransduction are altered by exposure to UVA light; but TRPV1 receptors are unaffected. This postulate contradicts the established paradigm that thermal hyperalgesia is a peripherally mediated phenomenon. However, as different models of hyperalgesia are developed and their mechanisms investigated, we do not think it is implausible that novel mechanisms causing hyperalgesia will be revealed.

In conclusion, we have demonstrated that the C-fiber barrage is involved in the development of sustained thermal hyperalgesia but not mechanical hyperalgesia caused by exposure of the rat tail to UVA light. To resolve the mechanisms contributing to the development and maintenance of the mechanical hyperalgesia, we will undertake neurophysiologic investigations of the primary afferent and dorsal horn neurons that innervate the rat tail after UVA-light exposure.

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CHAPTER 4

**Discharge patterns of nociceptive primary afferent fibres in
the rat coccygeal nerve after ultraviolet A light exposure**

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Abstract

We have shown that administration of the local anaesthetic bupivacaine into the base of rats' tails prevents the development of hyperalgesia induced by UV_A-light to a noxious thermal challenge, but not to noxious mechanical challenges. The aim of this study was to determine whether exposure of rats' tails to UV_A-light altered the responses of A δ - and C-fibres innervating the tail to noxious mechanical and noxious thermal challenges. We dissected the right ventral coccygeal nerve in Sprague Dawley rats that had had their tails exposed to, or shielded from, UV_A-light 24-hours earlier. Single afferent A δ - and C-fibre receptive fields were located and the fibres response to noxious blunt (3.9N bar algometer) and punctate (rat toothed forceps) mechanical challenges, as well as a noxious thermal (49°C) challenge was measured. In rats exposed to UV_A-light, the peak firing rates and areas under the curve of post-challenge histograms of A δ - and C-fibres were increased when noxious blunt and punctate challenges were applied to the rats' tails. However, the peak firing rate and areas under the curve of post-challenge histograms of C-fibres, which were responsive to noxious thermal challenges, were not increased after UV_A-light exposure. We have shown that the A δ - and C-fibres that encode for noxious punctate and blunt challenges were sensitized after UV_A-light exposure, but C-fibres responsible for encoding noxious thermal challenges were not sensitized by UV_A-light exposure.

Perspectives: Exposure of rats' tails to UV_A-light altered the responses of A δ - and C-fibres innervating the tail to noxious mechanical and noxious thermal challenges. A δ - and C-fibres that encode for noxious punctate and blunt challenges were sensitized after UV_A-light exposure, but C-fibres responsible for encoding noxious thermal challenges were not sensitized by UV_A-light exposure.

Introduction

Ultraviolet (UV)-light exposure of skin causes peripheral and central neuronal hyperexcitability. Polymodal receptors, which are quiescent in normal skin became spontaneously active after UV-light (spectrum not stated) exposure of rabbit ears³³ and UV_A-light exposure of rat hind paws¹. Similarly to UV_A-light, UV_B-light exposure of the rat hind paw¹¹ and pig skin²⁷ caused peripheral neuronal hyperexcitability. UV_B-light exposure of rat hind paw caused long-lasting low-frequency spontaneous activity, and increased number of spontaneously-active fibres in recordings from C-fibres innervating the hindpaw skin. This rise in the number of spontaneously active C-fibres was evident 24 hours after UV_B-light exposure and lasted for 72 hours¹¹. In pigs, C-fibre activation, as assessed by axon reflex erythema, was increased to noxious thermal and mechanical challenges in skin that had been irradiated with UV_B-light²⁷. In humans, as in pigs, UV_B-light activated silent nociceptors²¹. The neurogenic axon reflex in human skin was present in UV_B-light irradiated skin but not in the non-irradiated skin²¹, and systemic lidocaine administration suppressed the mechanically induced flare and the mechanical hyperalgesia in sunburnt skin, while leaving the impact induced ratings in normal skin unchanged²¹.

Coinciding with peripheral neuronal hyperexcitability following UV-light exposure are changes within the central nervous system^{37,40,41}. UV_A-light caused numerous changes within wide dynamic range (WDR) neurones of the spinal cord. The sensitized WDR neurones showed increased spontaneous activity, reduced thresholds to non-noxious thermal challenges, increased responses to noxious mechanical and

thermal challenges, and expanded receptive fields ⁴⁰. Input from primary afferents contributed to the increased WDR excitability for the first three days after exposure to UV_A-light, thereafter WDR neuronal hyperexcitability was entrenched and independent of peripheral neuronal input ⁴⁰. In addition, the duration and amplitude of both the A- and C-fibre evoked ventral root potentials (VRP) were increased significantly in UV_A-light exposed rats ³⁷. In rats exposed to UV_A-light, a NMDA receptor antagonist, and neurokinin 1 and neurokinin 2 receptor antagonists significantly reduced the prolonged VRP evoked by C-fibre stimulation. However, in animals not exposed to UV_A light, the prolonged VRP evoked by a single shock C-fibre stimulation was significantly antagonised by a NMDA receptor antagonist and a neurokinin 2 receptor antagonist, but not by a neurokinin 1 receptor antagonist, implying that functioning or expression of neurokinin 1 receptors were altered after UV_A-light exposure ³⁷.

The above-mentioned changes within the peripheral and central nervous system may account for the behavioural thermal and mechanical hyperalgesia present after UV_A-light exposure of the rat hind paw and rat tail ^{23,25,35,36,43}. The altered WDR neurone activity after UV-light exposure may result from plasticity in spinal neuronal circuitry following peripheral insult ^{37,40,42}, or from changes in primary afferent fibre impulses converging on those spinal neurons ³⁹, or both.

We recently demonstrated that stopping C-fibre activation by administering local anaesthetic into the base of the rat tail during UV_A-light exposure of the tail, prevented the development of thermal but not mechanical hyperalgesia ³⁶. A possible explanation for our findings is that central changes within the WDR neurones of the

dorsal horn are responsible for thermal hyperalgesia following UV_A-light exposure, while peripheral neural changes mediate the mechanical hyperalgesia. To determine whether sensitization of primary afferent nociceptive fibres contributes to mechanical and thermal hyperalgesia after UV_A-light exposure, we investigated the responses of fibres in the rat coccygeal nerve, which conveys the afferent neuronal activity from the tail, to noxious thermal challenges and noxious mechanical challenges after UV_A-light exposure of the rat tail. We measured the behavioural response of the animals to the noxious challenges before we dissected and recorded from the rat coccygeal nerve to ensure that hyperalgesia was present in rats exposed to the UV_A-light.

A previous study has shown that rat coccygeal nerve contains four groups of afferent fibres. A β -fibres that respond to both brush and pinch of their receptive fields, A δ -fibres that respond to pinch, polymodal C-fibres that respond to noxious heat (49°C) and pinch, and another group of C-fibres, that are chemosensitive and show increased spontaneous activity in the presence of ischaemia and reperfusion. We focused our investigation on only those fibres that would most likely encode for challenges to which we subjected our rats, namely the A δ -fibres and the polymodal C-fibres. We did not investigate the responses of the A β -fibres because they are predominantly responsible for encoding information in the non-noxious range and we have not yet established the presence of allodynia, enhanced response to non-noxious challenges, in our model of UV_A-light induced pain.

Materials and Methods

Animals

Thirty-five male Sprague-Dawley rats with an initial mass of 200-225g were used. Rats were housed two per cage at an ambient temperature of 21-25°C, and a 12:12 hour light/ dark cycle, lights on at 07:00. Rats had free access to tap water and standard rat chow. All procedures were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (clearance certificate 2005/07/3) and complied with the recommendations of the Committee for Research and Ethical Issues of the International Association for the Study of Pain ⁴⁴.

UV_A- light source

A double-ended high-pressure metal halide UV_A lamp was used as a source of UV_A light. The lamp had a spectral irradiance of 37 mW.cm⁻², with 60 % of the energy emitted in the UV_A range, 30% in the UV_B range and 10% in the UV_C range. The UV_B irradiance only began to increase from 30 seconds after the lamp was turned on. Since the maximum time the lamp was turned on while exposing the rats' tails was 40 seconds (see experimental procedure), the tail was exposed predominantly to UV_A-light. The UV_A-light lamp was housed in a rig and positioned 350mm above the tail.

Noxious thermal challenge

The modified tail flick test described by Gelgor et al.^{13,14} was used for behavioural measurements undertaken to confirm the presence of hyperalgesia to a noxious thermal challenge in rats exposed to UV_A-light. Rats' tails were immersed in 29°C water for 30 minutes before the first challenge was made, and between challenges, to maintain a constant tail-skin temperature and prevent the confounding effects variations in tail skin temperature have on tail flick latency^{19,38}. To apply the noxious thermal challenge, we immersed the whole tail into 49°C water, and measured the time taken, from immersion of the tail until the first co-ordinated motor response of the tail, with a stopwatch^{35,36}. The average of three measurements, taken at least one-minute apart, was recorded as the tail-flick latency. To avoid possible tissue damage, immersion of rats' tails in the 49°C water was limited to 30 seconds.

To apply a thermal challenge during electrophysiological recordings in anaesthetised rats, a copper thermode heated to 49°C was used to apply the noxious thermal challenge. The copper electrode temperature was maintained by placing the electrode in a hot water bath at a fixed temperature of 49°C. During electrophysiological recordings the copper thermode was placed lightly against the rat tail, and to ensure no mechanoreceptors were activated by this light touch, we applied an innocuous challenge (brushing the tail with a camel hair brush, with repeated strokes, for a 10 sec period) to the same area before applying the thermode.

Noxious punctate mechanical challenge

For behavioural measurements undertaken to confirm the presence of hyperalgesia to a punctate mechanical challenge in rats exposed to UV_A-light, an electronic von Frey anaesthesiometer was used to apply a noxious mechanical challenge to the tail, as we have described previously³⁶. The von Frey filament was applied 50, 55 and 60mm from the base of the tail with uniformly increasing force until the rat exhibited aversive behaviours (e.g. pulling away and jumping). At least a one-minute period of recovery for the animal was taken between successive measurements. The mean of these three measurements was recorded as the noxious aversive pressure. To avoid tissue damage the von Frey filament was removed when the pressure reached 400g.

During electrophysiological recordings in anaesthetised rats a rat toothed forceps was used to apply the noxious punctate challenge, and we pinched the receptive field with serrated forceps (force of 1.7N) for 10 seconds²⁹⁻³¹.

Noxious blunt mechanical challenge

For behavioural measurements undertaken to confirm the presence of hyperalgesia to a blunt mechanical challenge in rats exposed to UV_A-light, we used a bar algometer to apply the noxious mechanical challenge to the tail. The bar algometer consisted of a spring-loaded force gauge (Halda, RS, Sweden), connected, via a lever, to a 20 mm long steel bar with a diameter of 1.2 mm. The steel bar was placed transversely across the tail, at its midpoint and at two separate sites 10mm proximal and distal to the tail midpoint, and at a static force of 3.9N. The time from application of the noxious

mechanical challenge until the first sign of aversive behaviour (pulling away and jumping) was measured with a stopwatch. To avoid tissue damage the bar algometer was removed from the tail if the rat failed to respond within 30 seconds. During electrophysiological recordings in anaesthetised rats the bar algometer, generating a static force of 3.9N, was applied to the receptive field for 10 seconds.

Experimental procedure

UV_A-light exposure and all nociceptive testing took place with the rats restrained in transparent plastic restrainers that allowed exposure and movement of the tail, but restricted movement of the body. Before the start of the experiment, rats were habituated to the restrainers for two hours a day on three consecutive days. On experimental days, the rats were placed in the restrainers 30 minutes before the start of experimentation. For three consecutive days before we exposed the rats' tails to UV_A light, we measured response latencies to the noxious thermal and mechanical challenges. On the day of UV_A-light exposure, the dorsal surface of the rats' tails was exposed to the light eight times for 40 seconds each, with 260 seconds between the exposures^{35,36}. During UV_A-light exposure, the tails of 16 rats were covered with aluminium foil (to shield the tails from the light), and left that tails of 19 rats uncovered. Nociceptive testing was repeated 24 hours after exposure to UV_A-light, but before animals were anaesthetised for electrophysiological recordings, to confirm whether exposure to the UV_A-light had caused mechanical and thermal hyperalgesia. A single investigator (AT) performed the behavioural and electrophysiological experiments, and he was blinded as to whether the rats were shielded or exposed to the UV_A-light.

Electrophysiological recording

Anaesthesia in the rats was induced with xylazine-ketamine (1:4, 1 ml.kg⁻¹ I.M.), and maintained with 1.5% enflurane (Ethrane, Abbott Laboratories) in 33% oxygen/67% nitrous oxide, which was administered via a face mask. The rats breathed spontaneously throughout the experiments. Core body temperature was maintained at 38°C by means of a heating blanket. The right ventral coccygeal nerve was exposed close to the tail base and protected from drying in a pool of mineral oil. To facilitate dissection a black plastic plate was placed beneath the nerve. Under a dissecting microscope, the epi-and perineural sheaths were slit lengthwise, individual fibre bundles were cut proximally and small filaments further divided with micro-dissection scissors (Trident) and insect pins. The small filaments were placed on a 0.2 mm diameter platinum wire recording electrode (a silver reference electrode was placed in the surrounding tissue), and further dissected until impulses from individual fibres could be identified by electrical stimulation of the coccygeal nerve. Electrical stimulation (Digitimer DS9A) was via two L-shaped stainless-steel needle electrodes, which were inserted subcutaneously along the path of the nerve, approximately 40 mm distal to the recording site. Typical challenges used for searching for single fibres were 1 Hz pulses of 0.04-0.06 ms duration at 1-7 V for A fibres and 1 Hz pulses 0.5-0.7 ms duration at 10-40 V for C fibres. Filaments containing fibres that could not be distinguished easily by difference in spike amplitude were rejected, as were filaments containing more than five active fibres. Spikes were amplified (Digitimer NL104), filtered through a bandpass of 200-1500 Hz, and discriminated using a spike

processor (Digitimer D130) connected to a laboratory interface (CED 1401, Cambridge Electronic Design) attached to a microcomputer.

Once a single afferent fibre had been isolated with the electrical search challenge, we located its receptive field and determined its response to the noxious challenges. We located the receptive field depending on the nature of the fibre examined. If it was an A δ -fibre we located the receptive field with either the noxious pinch challenge or the noxious blunt challenge, and if it was C-fibre we located the receptive field with the noxious thermal challenge. If we had not located the receptive field after several attempts the procedure was abandoned to prevent damaging the tail through repeated noxious challenges. We did not delineate the extent of the receptive field as repeated noxious challenges irreversibly damaged the rat tail tissue and the nature of challenges applied did not allow us to describe the true extent of the receptive field. We then determined the afferent fibre's responses to noxious thermal and mechanical challenges. Noxious thermal stimulation was achieved by applying a copper thermode heated to 49°C, noxious punctate challenge was achieved by pinching the receptive field with serrated forceps, and noxious blunt challenge was achieved by applying a bar algometer to the receptive field for 10 seconds. We took care not to flex the tail during the mechanical challenges, because of the profusion of flex-sensitive fibres in the nerve ²². Only one of us (AT) applied the challenges, so they were consistent throughout the procedure. Areas under the curve and peak firing rates were calculated from post challenge time histograms, produced by the computer analysis software, that were generated after each noxious challenge. The area under the curve is a measure of firing rate over time and was calculated as the total area covered by the post challenge histograms. Conduction velocities were also measured from the post

challenge time histograms following suprathreshold electrical stimulation of the fibre. In some preparations several afferent fibres could be discharged within the same filament of nerve, and their responses were characterised individually on the basis of their conduction velocities, amplitude and shape of their spikes.

Data analysis

No animals reached the 30-second cut-off time for tail-flick latencies and aversive response latencies or the 400g cut-off pressure for the aversive behaviour responses during nociceptive testing, allowing the use of parametric statistical analysis. To determine whether UV_A-light caused hyperalgesia, tail-flick latencies, aversive response latencies and aversive pressures, for shielded and exposed rats, were compared to each other using a two-way ANOVA and Tukey post-hoc tests. Neural responses, peak firing rates and areas under the curve, to noxious thermal, punctate and blunt challenges were compared with unpaired t-tests.

Results

Behavioural response to noxious thermal challenge

The effect of UV_A-light exposure on tail flick latencies to a 49° thermal challenge is shown in Figure 1a. Pre-exposure tail flick latencies were 6.2 ± 1.0 s for the group destined for UV_A-light exposure, and 6.5 ± 1.2 s for the group destined to be shielded and their pre-exposure latencies did not differ significantly ($P > 0.05$, two-way ANOVA). In the rats with tails that had been exposed to UV_A light, one day after UV_A light exposure tail flick latencies were significantly decreased from their pre-exposure tail flick latencies by 25%, to 4.9 ± 1.3 s ($P < 0.001$, two-way ANOVA with Tukey post-hoc test). Their tail flick latencies also were significantly lower than the latencies of the rats exposed to the UV_A-light but with tails shielded, 7.2 ± 1.5 s ($P < 0.001$, two-way ANOVA with Tukey post-hoc test).

Behavioural response to noxious punctate mechanical challenge

The effect of UV_A-light exposure on aversive pressure thresholds is shown in Figure 1b. Pre-exposure pressures were 265 ± 32 g for the group destined for UV_A-light exposure, and 274 ± 46 g for the group destined to be shielded. Their pre-exposure pressures did not differ significantly ($P > 0.05$, two-way ANOVA). In the rats with tails that had been exposed to UV_A-light one day after UV_A-light exposure aversive pressure thresholds were significantly decreased from their pre-exposure aversive pressure thresholds by 51% to 128 ± 43 g ($P < 0.001$, two-way ANOVA with Tukey post-hoc test). Their aversive pressure thresholds also were significantly lower than the thresholds of the rats exposed to the same UV_A-light but with tails shielded, 257 ± 40 g ($P < 0.001$, two-way ANOVA with Tukey post-hoc test).

Behavioural response to noxious blunt mechanical challenge

The effect of UV_A-light exposure on aversive escape latencies to a blunt mechanical challenge is shown in Figure 1c. Pre-exposure aversive escape latencies were 11.3 ± 4.6 s for the group destined for UV_A-light exposure, and 11.7 ± 3.0 s for the group destined to be shielded and their pre-exposure latencies did not differ significantly ($P > 0.05$, two-way ANOVA). In the rats with tails that had been exposed to UV_A light one day after UV_A-light exposure aversive escape latencies were significantly decreased from their pre-exposure aversive escape latencies, by 51%, to 5.3 ± 2.8 s ($P < 0.001$, two-way ANOVA with Tukey post-hoc test). Their aversive escape latencies also were significantly lower than the latencies of the rats exposed to the same UV_A-light but with tails shielded, 10.6 ± 3.0 s ($P < 0.001$, two-way ANOVA with Tukey post-hoc test).

Electrophysiological recordings

Conduction velocities

The conduction velocities of the A δ -fibres were 3.4 ± 1.2 m/s. The conduction velocities of the C-fibres were 0.7 ± 0.3 m/s. Figures 2 and 3, show examples of recordings from two A δ -fibres and two C-fibres, respectively.

Neural response to noxious thermal challenge

The effect of UV_A-light exposure on C-fibre response to noxious heat is shown in Figure 4 and Figure 5. We identified C-fibres that were responsive to a noxious thermal challenge in nine exposed animals and 11 shielded animals. Peak firing rate of C-fibres for the tails of rats that had been shielded was 47 ± 12 spikes.s⁻¹ and 46 ± 19 spikes.s⁻¹ for those rats the tails of which had been exposed. Their peak firing rates were not significantly different ($P = 0.76$, unpaired t-test). Area under the curve of C-fibres for the tails of rats that had been shielded was 146 ± 44 spikes and 157 ± 56 spikes for those rats the tails of which had been exposed. The areas under the curve were not significantly different between groups ($P = 0.72$, unpaired t-test).

Neural response to noxious punctate mechanical challenge

The effect of UV_A-light exposure on A δ - and C-fibre responses to a noxious punctate challenge is shown in Figure 6 and 7. . We identified 7 A δ -fibres and 6 C-fibres that were responsive to a punctate mechanical challenge in rats exposed to UV_A-light, and 8 A δ -fibres and 8 C-fibres that were responsive to a punctate mechanical challenge in rats shielded from UV_A-light. Only one type of each fibre was identified in each animal. The peak firing rate of A δ -fibres for the tails of rats which had been shielded were 25 ± 14 spikes.s⁻¹ and 48 ± 12 spike.s⁻¹ for those rats the tails of which had been exposed. Their peak firing rates were significantly different ($P = 0.003$, unpaired t-test). The peak firing rate of C-fibres for the tails of rats which had been shielded was 19 ± 8 spikes.s⁻¹ and 43 ± 11 spikes.s⁻¹ for those rats the tails of which had been exposed. The peak firing rates were significantly different ($P = 0.0004$, unpaired t-

test). The area under the curve of A δ -fibres for the tails of rats that had been shielded was 50 ± 22 spikes and 180 ± 61 spikes for those rats the tails of which had been exposed. Their areas under the curve were significantly different ($P < 0.0001$, unpaired t-test). The area under the curve of C-fibres for the tails of rats which had been shielded was 56 ± 20 spikes and 166 ± 49 spikes for those rats the tails of which had been exposed. Their areas under the curve were significantly different ($P = 0.0001$, unpaired t-test).

Neural response to noxious blunt mechanical challenge

The effect of UV_A-light exposure on A δ - and C-fibre responses to a noxious blunt challenge is shown in Figure 8 and Figure 9. We identified 7 A δ -fibres and 6 C-fibres that were responsive to a blunt mechanical challenge in rats exposed to UV_A-light, and 8 A δ -fibres and 10 C-fibres that were responsive to a blunt mechanical challenge in rats shielded from UV_A-light. Only one type of each fibre was identified in each animal. The peak firing rate of A δ -fibres for the tails of rats which had been shielded was 31 ± 6 spikes.s⁻¹ and 44 ± 14 spikes.s⁻¹ for those rats the tails of which had been exposed. Their peak firing rates were significantly different ($P = 0.02$, unpaired t-test). The peak firing rate of C-fibres for the rats the tails of which had been shielded was 25 ± 12 spikes.s⁻¹ and 46 ± 25 spikes.s⁻¹ for those rats the tails of which had been exposed. Their peak firing rates were significantly different ($P = 0.04$, unpaired t-test). The area under the curve of A δ -fibres for the tails of rats which had been shielded was 67 ± 22 spikes and 185 ± 47 spikes for those rats the tails of which had been exposed. Their areas under the curve were significantly different ($P < 0.0001$, unpaired t-test). The peak firing rate of C-fibres for the rats the tails of which had been shielded was

53 ± 18 spikes and 149 ± 40 spikes for those rats the tails of which had been exposed.

Their areas under the curve were significantly different ($P = 0.005$, unpaired t-test).

Discussion

We investigated what effect UV_A-light exposure of the rats' tails would have on the sensitivity of nociceptive primary afferent fibres that innervate the rat tail. We did not investigate those afferents that responded to challenges both in the noxious and non-noxious challenge ranges. After UV_A-light exposure, we assessed A δ - and C-fibre sensitivity by measuring area under the curve, a measure of firing rate over time, and peak firing rates. Both the area under the curve and the peak firing rates for the A δ - and C-fibres were increased when the noxious blunt and punctate challenges were applied to the rat tail. We have therefore shown that the A δ - and C-fibres that encoded for noxious punctate and blunt challenges were sensitized after UV_A-light exposure. The areas under the curve and the peak firing rate of C-fibres that were responsive to noxious thermal challenges were not increased after UV_A-light exposure, that is, C-fibres responsible for encoding noxious thermal challenges were not sensitized by UV_A-light exposure.

The aim of this study was to determine whether changes within peripheral neurones could account for the modality specific hyperalgesia we previously observed after blocking the C-fibre barrage during UV_A-light exposure, which prevented the development of thermal but not mechanical hyperalgesia³⁶. Although we investigated the firing properties of the fibres to a variety of noxious challenges, we did not describe the heat and mechanical thresholds, nor the full receptive field area of A δ -fibres nor C-fibres in this study. The reason for not describing these parameters was due to the instability of peripheral fibre preparations and deleterious effects of repeated noxious thermal and mechanical challenges on their receptive fields.

Dissection and recording from peripheral fibres was technically very difficult due to the fragility of fibres and their tendency to either stop firing or develop tachyphylaxis to the noxious challenges applied. We were therefore not able to measure all the possible parameters that would have been ideal, that was, their heat and mechanical thresholds, their full receptive field area and their responses to the variety of noxious challenges. We therefore prioritised our investigation to focus on those parameters that would be most helpful in either proving or disproving our hypothesis that primary mechanical hyperalgesia that was present 24 hours after UV_A-light exposure was due to A δ - and C-fibre sensitization, and that primary thermal hyperalgesia was not solely a peripherally mediated phenomenon.

Our data seems to contradict studies that have shown that UV-light causes peripheral thermal sensitization^{11,21,27,33}. Polymodal receptors or silent nociceptors were sensitized after UV-light (spectrum not stated) exposure of rabbit ears³³, and UV_B-light exposure of rat hind paw¹¹, human skin²¹ and pig skin²⁷. In this study, we used UV_A-light, not UV_B-light, to cause hyperalgesia, and UV_A-light causes hyperalgesia through different mechanisms than does UV_B-light. After UV_A-light exposure of rat tail skin a mild chronic inflammatory response was evident histologically in rats exposed to UV_A light as well as rats shielded from UV_A-light, and the inflammatory process did not parallel hyperalgesia that was present³⁵. In addition, although the pro-inflammatory mediators prostaglandins and histamine are increased after UV_A-light exposure^{15,17}, only a handful of studies have reversed hyperalgesia through the administration of suitable anti-inflammatory agents, such as a cyclooxygenase inhibitor²⁴ and bradykinin antagonists^{23,25}. Therefore, there is minimal evidence to support the hypothesis that UV_A-light causes an acute inflammatory hyperalgesia. In

contrast, the histological features after UV_B-light exposure of human skin were consistent with an acute inflammatory response ¹⁵. In addition, there are numerous studies that show that UV_B-light caused an increase in pro-inflammatory mediators such as prostaglandins ^{4,5}, nitric oxide ^{26,34}, pro-inflammatory cytokines ^{10,20,28}, and nerve growth factor ¹⁶. Furthermore, UV_B-light induced hyperalgesia can be reversed through the administration of suitable anti-inflammatory agents, such as cyclooxygenase inhibitors ^{2,3,32}, and anti-inflammatory cytokines ²⁸. Therefore, UV_B-light does cause an acute inflammatory hyperalgesia. It therefore follows that if UV_A-light causes hyperalgesia through different mechanisms than does UV_B-light comparisons cannot be made between studies of different wavelengths. In the study described by Szolcsanyi ³³ the spectrum of UV-light used was not described thereby precluding proper comparisons to other studies. In addition, no studies have described peripheral hypersensitivity to noxious thermal or mechanical challenges after UV_A-light exposure.

The only study so far that has investigated peripheral neuronal hyperexcitability after UV_A-light exposure of the rat hind paws showed an increase in spontaneous firing of polymodal receptors ¹. We have not included the spontaneous firing rates of the fibres that we investigated. An increase in spontaneous firing indicates an increase in the sensitivity of the fibres measured, but does not discriminate as to which noxious challenges the fibres become sensitized. For, example an increase in the spontaneous firing rate of the C-fibre could represent an increase in sensitivity to noxious mechanical but not noxious thermal challenge. Any change in spontaneous firing does not alter our conclusions that A δ - and C-fibres that encode for noxious punctate and

blunt challenges were sensitized while C-fibres responsible for encoding noxious thermal challenges were not sensitized by UV_A-light exposure.

In our current study, we measured behavioural and neuronal responses 24 hours after UV_A-light exposure. We have shown previously though that after such UV_A-light exposure primary mechanical hyperalgesia to noxious challenges applied to the rat's tail lasted for two weeks after the UV_A-light exposure^{35,36}. Though sensitization of peripheral neurones could account for the hyperalgesia that was present to the blunt and punctate challenges one day after UV_A-light exposure, we do not know whether that sensitization lasts long enough to account for the hyperalgesia observed two weeks after exposure to UV_A light, whether there was prolonged input from rat tail nociceptors over the weeks, or whether a subsequent increase in dorsal horn sensitivity was responsible for the sustained mechanical hyperalgesia. Urban and colleagues⁴⁰ observed continuous low-threshold mechanoreceptor input to the dorsal horn over the first three days after brief UV_A-light exposure of the rat hind-paw. Subsequently, the same group³⁷ showed enhanced summation of ventral root potentials, a measure of wind-up, in response to A- and C-fibre stimulation. In the animals in which we previously demonstrated prolonged hyperalgesia, continuous input from low-threshold mechanoreceptors after UV_A-light exposure may have led to progressive wind-up in dorsal horn neurones and subsequent central sensitization¹⁸. Determining the neuronal basis of the prolonged hyperalgesia would require a study in which primary afferent and dorsal horn neurones responses are measured in rats up to two weeks after UV_A-light exposure.

It is not known what causes the peripheral sensitization of peripheral mechanoreceptive pathways after UV_A-light exposure. It is not an inflammatory response at the exposure site, since we have shown histologically that at the site of exposure a mild chronic inflammatory cell infiltrate was present in rats whose tails were exposed to UV_A-light and those that were shielded from UV_A-light³⁵. However inflammatory mediators may be released without producing histological evidence of inflammation. Ischaemia and reperfusion of the rat tail causes a 60 minute hyperalgesia to noxious heat (49°C), reversible by anti-inflammatory drugs, without histological evidence of inflammation. Bradykinin and histamine are released during tail ischaemia and reperfusion, and contribute to the subsequent hyperalgesia^{12,13}.

The concept that a conditioning event, UV_A-light exposure in our case, can result in mechanical hyperalgesia, for which sensitization of primary afferent fibres may account, and thermal hyperalgesia, for which sensitization of primary afferent fibres does not account, is not a unique phenomenon. Intraplantar injection of bee venom in rats caused a centrally-mediated thermal hyperalgesia and a primary mechanical hyperalgesia^{6,7,9}. The thermal hyperalgesia was bilateral, but the mechanical hyperalgesia only ipsilateral^{6,7}. Contralateral hyperalgesia is a centrally-mediated phenomenon, which in the case of bee venom injection could be blocked by systemic administration of a non-competitive NMDA receptor antagonist^{6,8}.

In conclusion, we have demonstrated that primary mechanical hyperalgesia 24 hours after UV_A-light exposure is due to A δ - and C-fibre sensitization. We have shown that the C-fibres responsible for encoding noxious thermal challenges were not sensitized 24 hours after UV_A-light exposure. We have therefore demonstrated that primary

thermal hyperalgesia was not solely a peripherally mediated phenomenon and may be caused by central sensitization. Such a paradigm shift may provide the answers to our poor success in treating chronic pain and the development of novel pharmacological targets and new treatment regimes.

Acknowledgements

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Figure legends

Figure 1: Mean (SD) tail flick latencies to a noxious thermal challenge (A), mean (SD) aversive pressure thresholds to a noxious punctate mechanical challenge (B), and (C) mean (SD) aversive escape latencies to a noxious blunt mechanical challenge, before and one day after UV_A-light exposure, for rats with tails that had been exposed (black bars, n = 19) and shielded (open bars, n = 16) during UV_A light exposure. Exposure to UV_A light resulted in significant decreases in tail flick latencies to the thermal challenge (A), pressure thresholds to the punctate mechanical challenge (B), and escape latencies to the blunt mechanical challenge (C). There were no significant differences between the two groups (exposed and shielded) for any of the three measurements before exposure to UV_A light, and no significant changes in tail flick latency, pressure thresholds or escape latencies occurred in the shielded animals before and after exposure to UV_A light (* P < 0.05 exposed versus shielded, two-way ANOVA with Tukey post-hoc test).

Figure 2: Example of recording from two A δ -fibres in rats whose tails had either been shielded (A) or exposed (B), to UV_A light, and the respective fibre's response to noxious punctate and blunt challenges. Arrows indicate when the challenge was applied.

Figure 3: Example of recording from two C-fibres in rats whose tails had either been shielded (A) or exposed (B) to UV_A light, and the respective fibre's response to noxious thermal, punctate and blunt challenges. Arrows indicate when the challenge was applied.

Figure 4: Mean (SD) peak firing rates of thermally-sensitive C-fibres in response to 49°C challenge in the receptive field, for rats with tails that had been shielded (open bars, n=11) and exposed (black bars, n=9) during UV_A-light exposure. There were no differences in the peak firing rates between the two groups of rats.

Figure 5: Mean (SD) area under the curve of thermally-sensitive C-fibres in response to 49°C challenge in the receptive field, for rats with tails that had been shielded (open bars, n=11) and exposed (black bars, n=9) during UV_A-light exposure. There were no differences in the areas under the curve between the two groups of rats.

Figure 6: Mean (SD) peak firing rates of mechanically-sensitive A δ -fibres in response to a noxious punctate challenge for rats with tails that had been shielded (open bars, n=8) and exposed (black bars, n=7) during UV_A-light exposure, and peak firing rates of mechanically-sensitive C-fibres in response to a noxious punctate challenge for rats with tails that had been shielded (open bars, n=8) and exposed (black bars, n=6) during UV_A-light exposure. Peak firing rate for both the A δ - and C-fibres were increased significantly after UV_A-light exposure (*P < 0.01, unpaired t-tests).

Figure 7: Mean (SD) area under the curve of mechanically-sensitive A δ -fibres in response to a noxious punctate challenge for rats with tails that had been shielded (open bars, n=8) and exposed (black bars, n=7) during UV_A-light exposure, and area under the curve of mechanically-sensitive C-fibres in response to a noxious punctate challenge for rats with tails that had been shielded (open bars, n=8) and exposed

(black bars, n=6) during UV_A-light exposure. Area under the curve for both the A δ - and C-fibres were increased significantly after UV_A-light exposure (*P < 0.01, unpaired t-tests).

Figure 8: Mean (SD) peak firing rates of mechanically-sensitive A δ -fibres in response to a noxious blunt challenge in the receptive field, for rats with tails that had been shielded (open bars, n=8) and exposed (black bars, n=7) during UV_A-light exposure, and peak firing rates of mechanically-sensitive C-fibres in response to a noxious blunt challenge for rats with tails that had been shielded (open bars, n=10) and exposed (black bars, n=6) during UV_A-light exposure. Peak firing rate for both the A δ - and C-fibres were increased significantly after UV_A-light exposure (*P < 0.01, unpaired t-tests).

Figure 9: Mean (SD) area under the curve of mechanically-sensitive A δ -fibres in response to a noxious blunt challenge in the receptive field, for rats with tails that had been shielded (open bars, n=8) and exposed (black bars, n=7) during UV_A-light exposure, and area under the curve of mechanically-sensitive C-fibres in response to a noxious blunt challenge for rats with tails that had been exposed (shielded (open bars, n=10) and exposed (black bars, n=6) during UV_A-light exposure. Area under the curve for both the A δ - and C-fibres were increased significantly after UV_A-light exposure (*P < 0.01, unpaired t-tests).

Figure 1

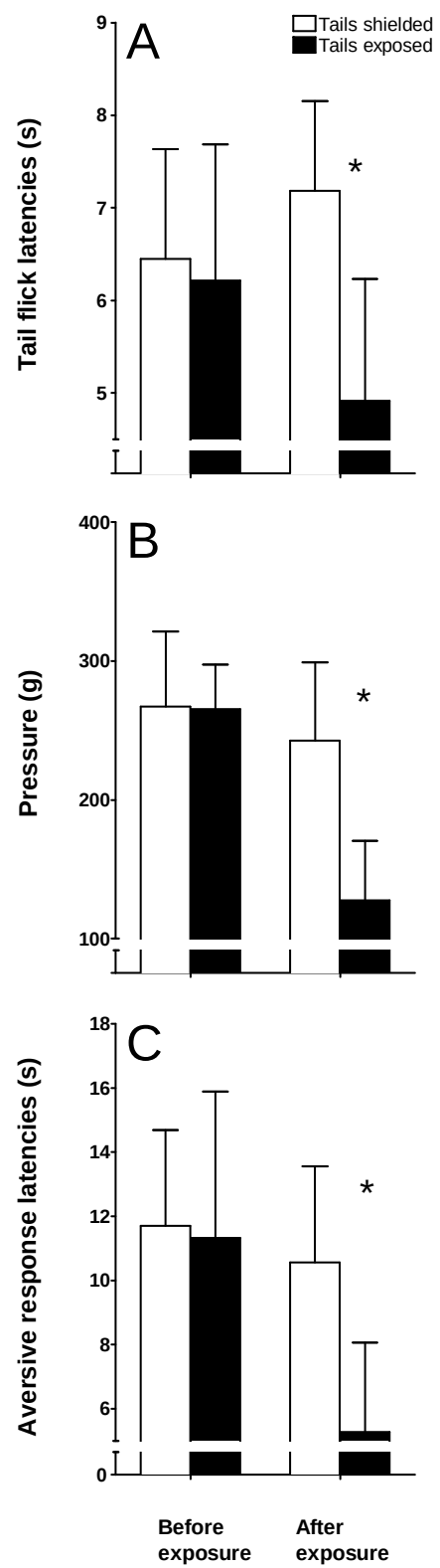


Figure 2

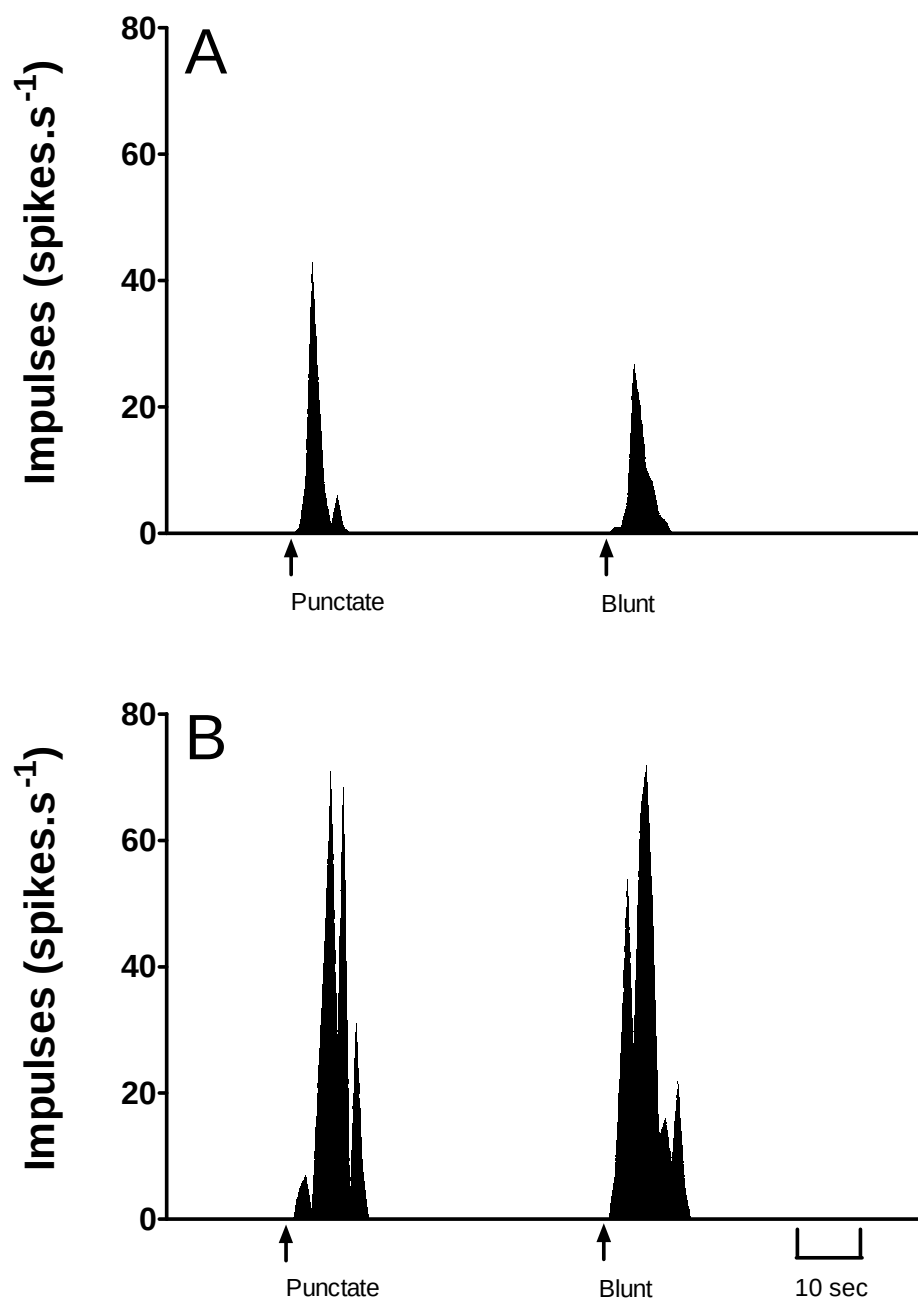


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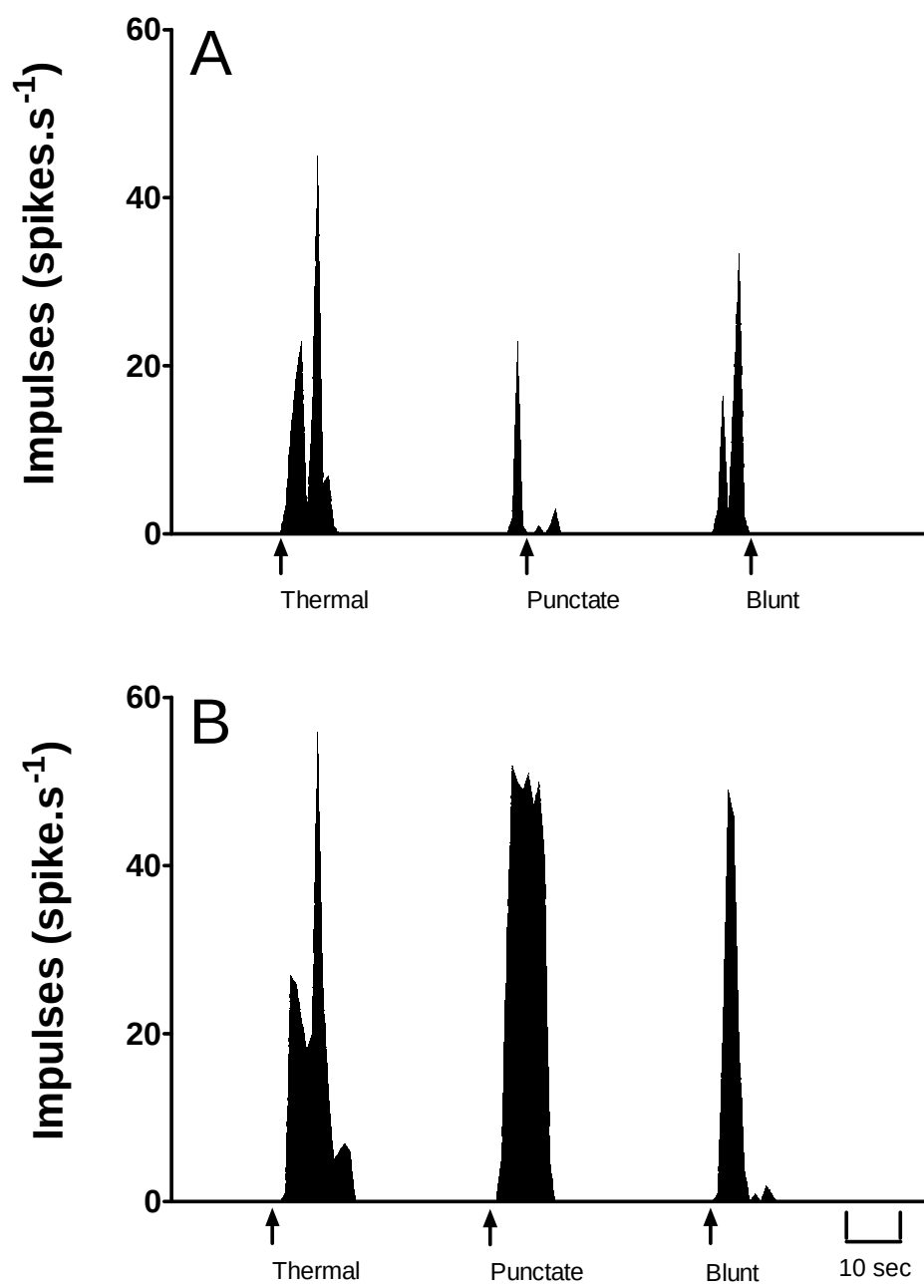


Figure 4

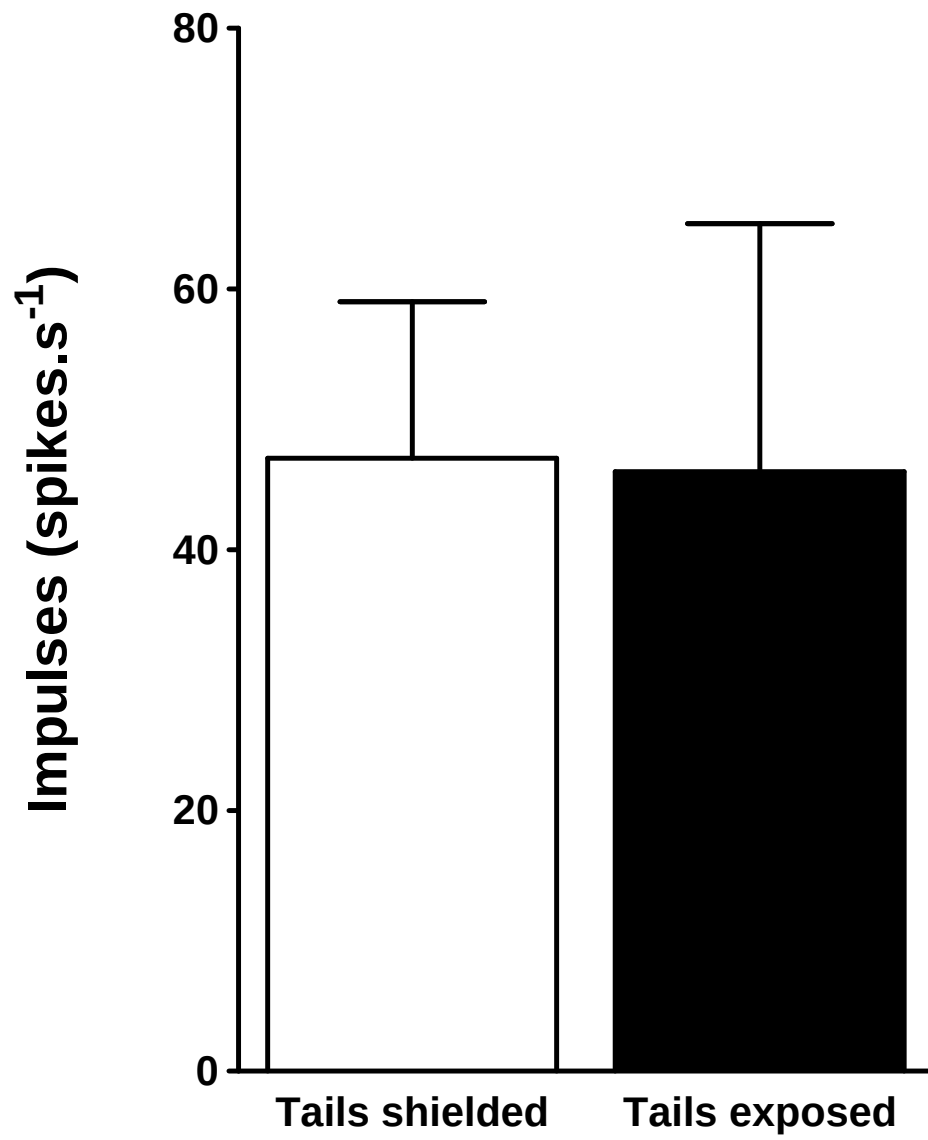


Figure 5

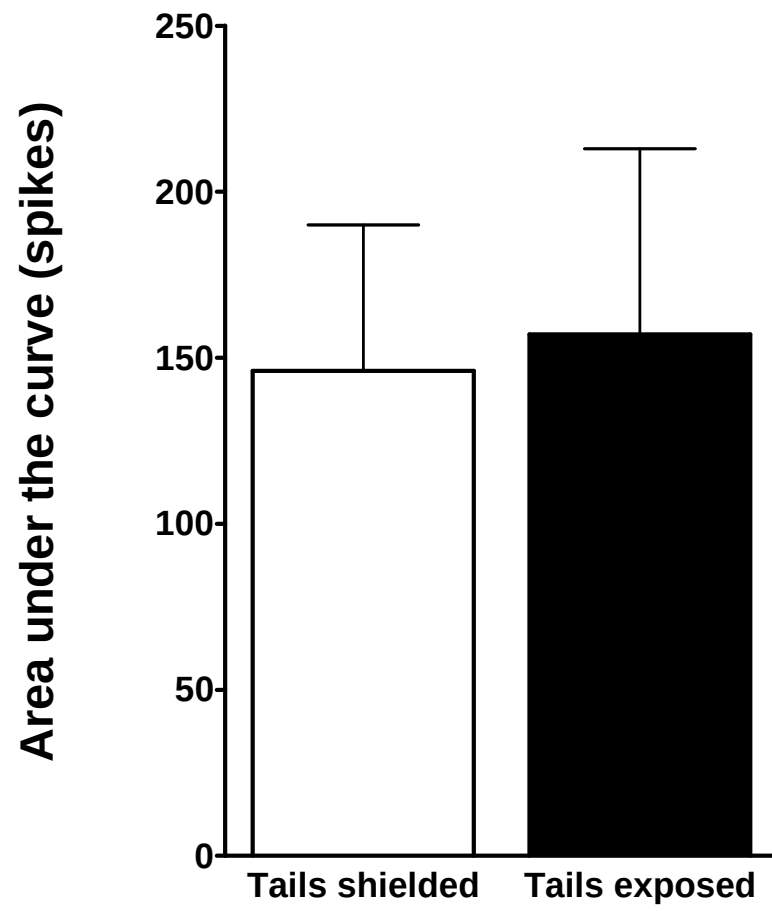


Figure 6

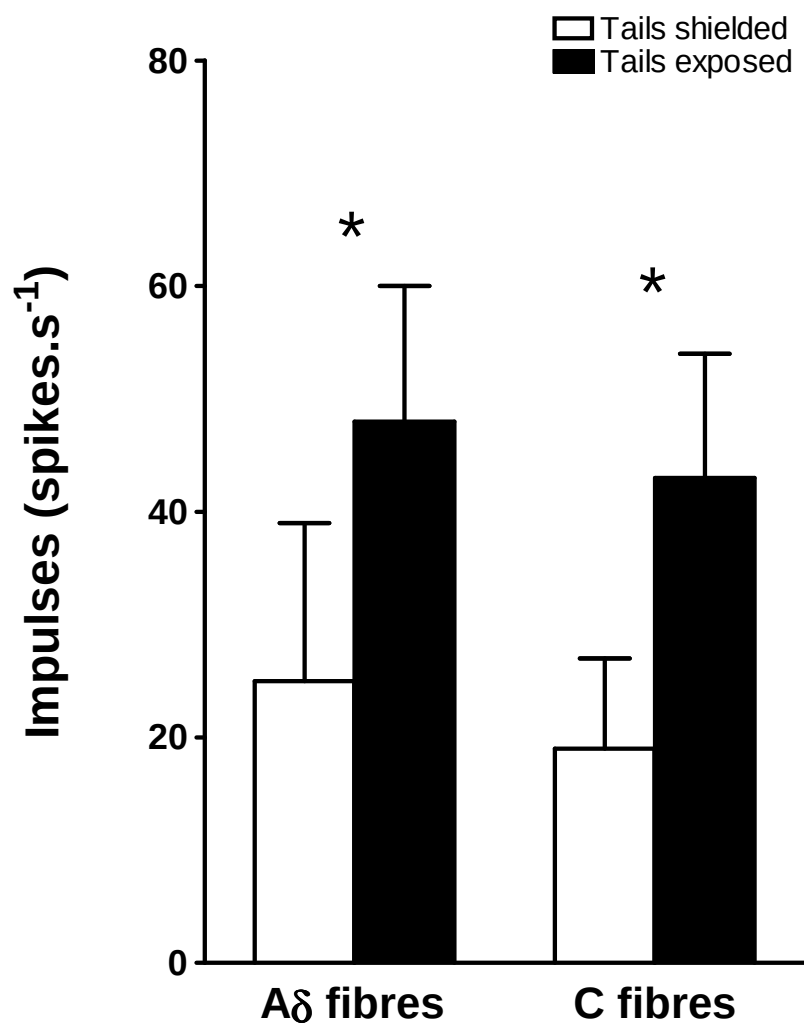


Figure 7

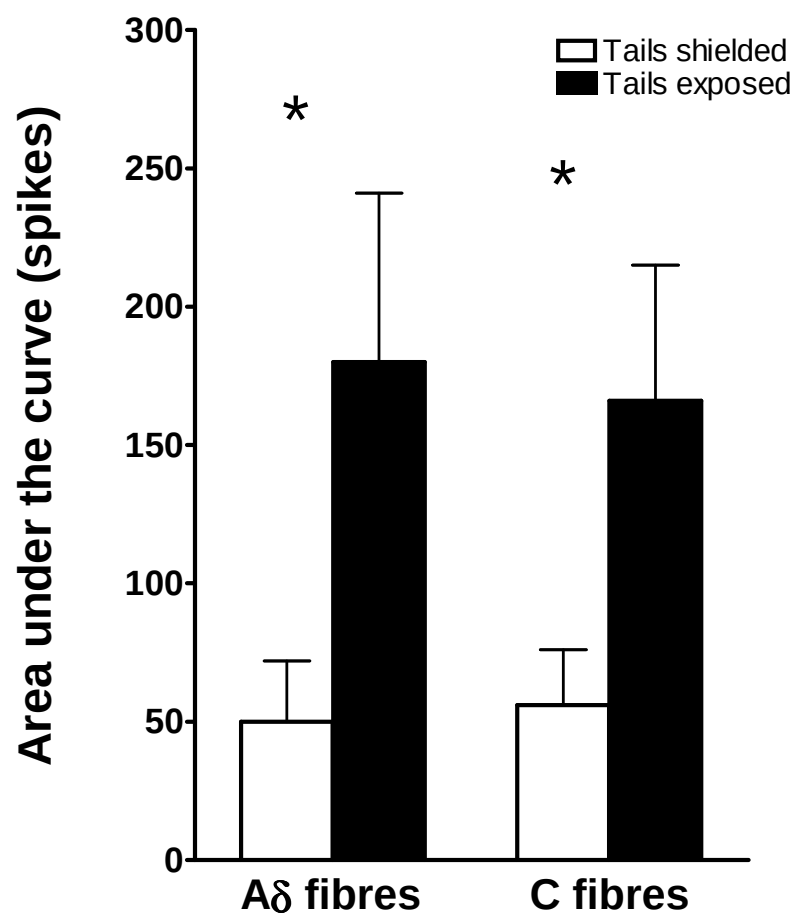


Figure 8

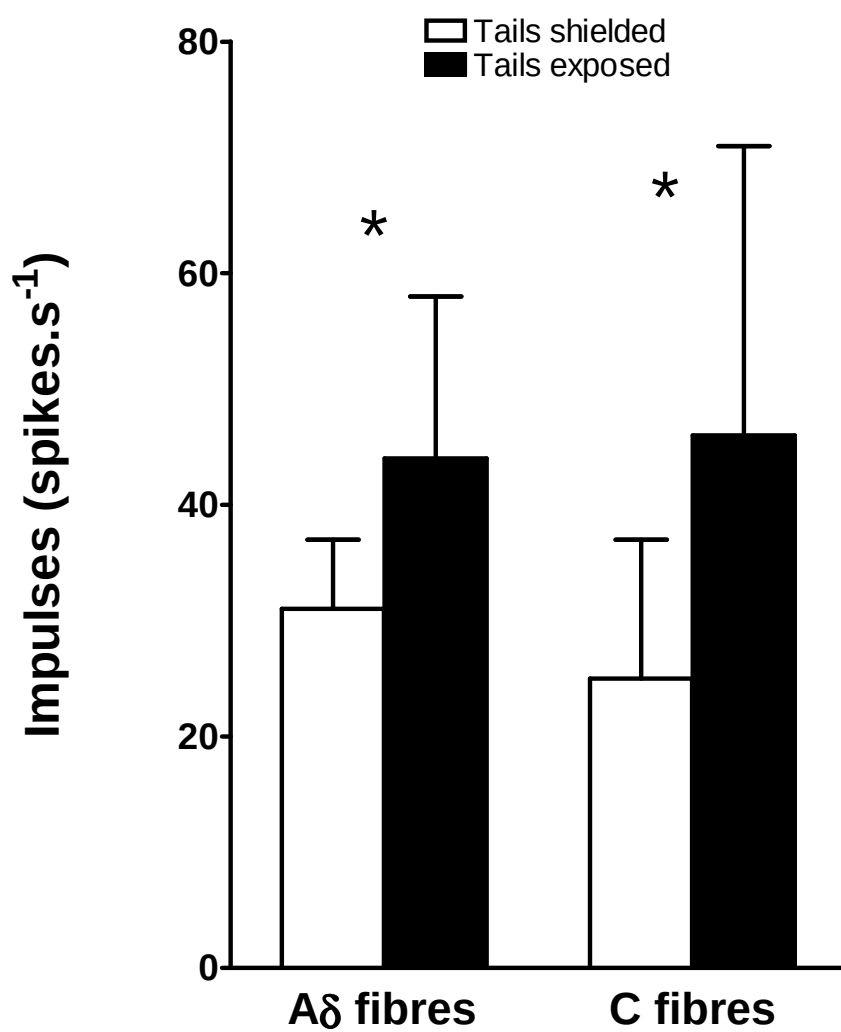
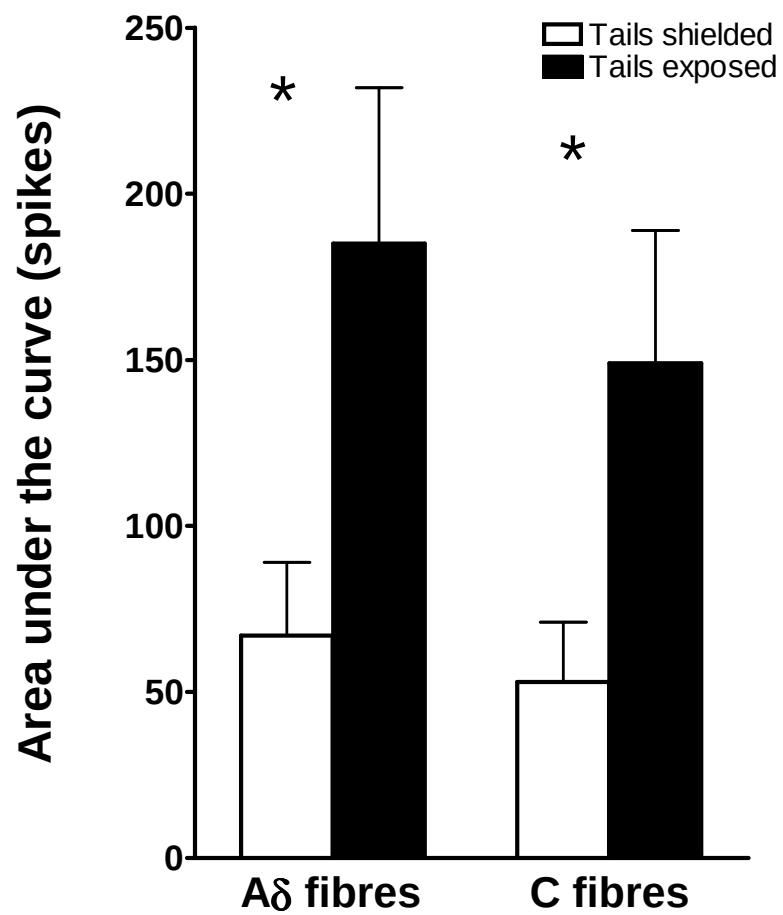


Figure 9



CHAPTER 5

Conclusions

Since it was first described in 1993, researchers have explored the physiological mechanisms through which UV_A- and UV_B-light cause sustained hyperalgesia. UV_B-light causes acute inflammation with a subsequent inflammatory hyperalgesia, and its major advantage as an experimental tool for studying hyperalgesia is that relies on the release of endogenous compounds for the induction of hyperalgesia and it can be used both in humans and animals. Alternatively, UV_A-light offers previously undescribed pathophysiological pathways to cause sustained hyperalgesia. In my thesis I completed three experiments, each concentrating on issues that have not been adequately addressed in the literature. In my first experiment I developed my own model of UV_A-light induced hyperalgesia, independently verified the spectrum of my UV_A-light and histologically determined whether inflammation was present after UV_A-light exposure. In my second and third experiments I investigated the role of the C-fibre barrage and changes within peripheral afferent neurones after UV_A-light exposure. I have summarised the results of my experiments in this chapter.

5.1 UV-light and behavioural hyperalgesia

In order to investigate the pathophysiology of UV_A-light I needed a protocol of UV_A-light exposure that would cause prolonged hyperalgesia to noxious thermal and mechanical challenges. After experimenting with multiple exposure durations and repetitions, I found that exposing the dorsal surface of rats' tails to UV_A-light eight times for 40 seconds each, with 260 seconds between each exposure, caused hyperalgesia to a noxious thermal challenge, a 49°C water bath, and two noxious mechanical challenges, a bar algometer that generated a suprathreshold noxious static force of 3.9 N (Chapter 2), and a noxious punctate challenge, an electronic von Frey

anaesthesiometer that generated progressively increasing pressures (Chapter 3). If I exposed rats' tails to UV_A-light for longer than 40 seconds the rats' tails would become severely damaged and would be unsuitable for behavioural testing. If I used fewer than eight exposures the hyperalgesia would either not develop or would resolve too quickly. My exposure protocol did not resemble exposure protocols previously described, as a single 90-second exposure usually was sufficient to produce hyperalgesia, and I used the rat tail and not the rat hind paw, the usual substrate for pain testing (Perkins *et al.*, 1992; Perkins *et al.*, 1993; Perkins & Kelly, 1993). I felt that the rats would suffer less having their tails be hyperalgesic as opposed to the load-bearing hind paw and my laboratory has far more experience in testing for pain using the rat tail than the rat hindpaw.

Although my protocol was unusual it produced the desired results. After UV_A-light exposure, the hyperalgesia to a noxious thermal challenge lasted for eight days, to a noxious blunt mechanical challenge for at least 16 days (Chapter 2), and to a noxious punctate challenge for at least 14 days (Chapter 3). I could not test mechanical hyperalgesia for longer because the rats outgrew their restrainers. The amplitude of the hyperalgesia was substantial, with the response latency to the noxious thermal challenge shortened by nearly a third in the hyperalgesic state, and the response latency to the noxious mechanical challenge shortened by over half.

Despite the UV_A-light causing a severe hyperalgesia, the rats were hyperalgesic for over two weeks, they did not exhibit signs of chronic distress, such as abnormal gait, reduced mobility, or reduced grooming when handled, and they gained body mass over the duration of the various experiments at the same rate as those rats shielded

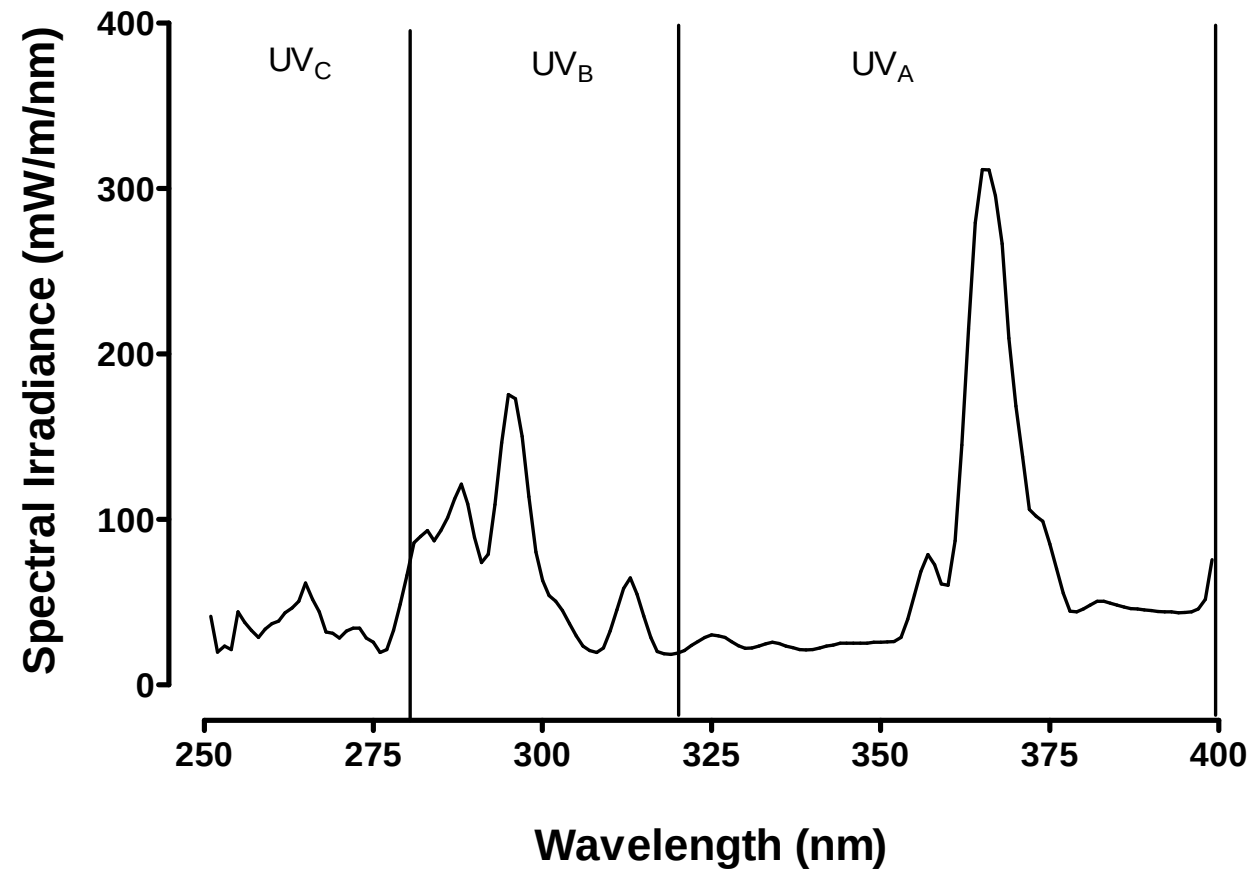
from the UV_A-light (Chapter 2). There are animal models that cause hyperalgesia that lasts for weeks to months (Chapter 2, Table 1) that aim to mimic clinical conditions such as rheumatoid arthritis, osteo-arthritis, peripheral neuropathy and fibromyalgia. However, the conditioning events necessary to induce long-lasting hyperalgesia leave the experimental animals in persistent pain that not only confounds responses to evoked pain, but also compromises the health of the animals, which characteristically lose mass, show an abnormal gait, reduced mobility, reduced grooming and disturbed sleep (Blackburn-Munro, 2004). My model displays the longest-lasting experimental hyperalgesia reported so far during which the experimental animals gain weight and appear to be healthy and pain-free. Not only were they free of impaired mobility associated with long-lasting hyperalgesia, but, because the hyperalgesia was produced in the tail, not the hind paw, they also were free of the impaired mobility associated with any lesions of the foot.

Although the rat tail offers many advantages, a significant disadvantage of using the tail as opposed to the hindpaw was that I was unable to assess for central neuronal changes by measuring behavioural changes within the contralateral appendage. However, measuring mechanical allodynia may offer an alternative assessment of central neuronal changes and is worthy of further investigation.

5.2 UV-light lamp

I used a double-ended high-pressure metal halide UV_A lamp as the source of UV_A-light in all my studies. For the purposes of my first study (Chapter 2), I commissioned experts at the Council for Scientific and Industrial Research, Tshwane, South Africa, to measure the spectrum of UV-light emitted by my lamp. They independently verified that my lamp emitted light predominantly in the UV_A-light spectrum. It had a spectral irradiance of 37 mW.cm⁻², with 60 % of the energy emitted in the UV_A range, 30% in the UV_B range and 10% in the UV_C range, in the steady state (Figure 1).

Figure 1: Spectral irradiance of UV-light lamp



Although the lamp emitted light predominantly in the UV_A-light spectrum, it began to emit a significant portion of light in the UV_B-light spectrum 30 seconds after the lamp was activated (Chapter 2). In my studies the lamp was activated only for 40-second pulses, and since my lamp began to emit UV_B-light only 30 seconds after the lamp was switched on, UV_B-light was unlikely to have contaminated my studies. I am therefore confident that my findings were as a result of the biological effects of UV_A-light, and not UV_B-light.

However, in none of the reports from the laboratories that have studied UV-light induced hyperalgesia states that the nature of the lamp used to generate UV-light had been independently verified. In previous studies where UV_A-light was the conditioning challenge, the lamps were activated for 90 seconds (Perkins *et al.*, 1992; Perkins *et al.*, 1993; Perkins & Kelly, 1993), and if their lamps were similar to the one I used in my studies, the rat tissues would have been exposed to UV_B-light for at least 60 seconds. Since the lamps were not independently verified, it was entirely possible that UV_B-light contaminated those studies. Contamination by UV_B-light has significant implications, as UV_B-light can produce inflammation and hyperalgesia at substantially lower energy levels and through different mechanisms than does UV_A-light, as explained in my Introduction. Therefore, in studies where UV_A-light was the proposed conditioning event, it was possible that UV_B-light was causing the hyperalgesia and their findings are as a result of inflammation caused by UV_B-light.

5.3 UV-light induced inflammation

A long-held and plausible assumption has been that UV-light, including UV_A- and UV_B-light, caused an inflammatory hyperalgesia (Davies *et al.*, 2005). Exposure of skin, rodent and human, to UV-light causes erythema, increased tissue temperature, oedema, pain and pruritus, characteristic clinical features of inflammation. Histological sections of human tissue after UV_B-light irradiation revealed a typical acute inflammatory cascade within the epidermis and dermis, an initial vasodilatation of blood vessels followed by a inflammatory cell infiltrate consisting primarily of neutrophils, T lymphocytes and mononuclear cells (Gilchrest *et al.*, 1981). Therefore, the histological features of acute inflammation after UV_B-light exposure are consistent with the clinical picture of the inflammatory process that follows UV-light exposure. However, the histological changes that occur after UV_A-light exposure do not parallel the features of acute inflammation. Histological sections of UV_A-light irradiated human tissues typically reveal significant dermal injury with a mixed perivascular cellular infiltrate, consisting of predominantly monocytes and T lymphocytes with few neutrophils, encroached on the deeper dermal vessels (Kumakiri *et al.*, 1977; Gilchrest *et al.*, 1983). There is no epidermal injury and a complete absence of inflammatory cells within the epidermis (Soter, 1990). Therefore, UV_A-light causes a pattern of injury completely different to that of UV_B-light.

It therefore was of vital importance to examine the rat tail tissue histologically after UV_A-light exposure to confirm the presence, or absence, of inflammation after UV_A-light irradiation, and to determine whether the tissue response to UV_A-light correlated with the hyperalgesia that was present. Since animal tissue never has been examined

histologically after UV-light, neither UV_A- nor UV_B-light, exposure as far as I could establish, there were a number of possibilities to consider. It may be that animal tissue responds differently to UV_A-light than human tissue and UV_A-light may cause an acute inflammatory response in rats that corresponds with the hyperalgesia, as UV_B-light does in human tissue. Or UV_A-light may cause a similar response in rat tissue as in human tissue and an acute inflammatory response may not be responsible for UV_A-light induced hyperalgesia. In my first study (Chapter 2) I sought to answer the above questions.

In my first study, I examined rat tail tissue histologically at the site of UV_A-light irradiation for inflammation (Themistocleous *et al.*, 2006). There was no evidence of acute inflammation, but I discovered mild chronic inflammation at the target site of irradiation both in those rats irradiated with UV_A-light and in those rats shielded from the UV_A-light. There were scattered lymphocytes and plasma cells within the dermis, and there was no change in the severity of inflammation one to nine after days after UV_A-light exposure. The rats shielded from the UV_A-light never were hyperalgesic, so the sustained hyperalgesia in irradiated rats I observed was unlikely to have been caused or maintained by the mild inflammation. The cause of the mild chronic inflammation was not clear, but I think it was caused by tail hyperthermia, which in turn was caused by an increase in temperature within the UV_A-light rig. The air in the UV_A light rig reached a peak temperature of about 45°C during activation of the UV_A-light lamp, probably because the UV_A-light rig was poorly ventilated. Even if hyperthermia was the cause of the mild chronic inflammation it did not cause hyperalgesia, as the rats shielded from the UV_A-light did not develop hyperalgesia, and so did not confound my main results. Whatever the events were which led to the

hyperalgesia, they were not visible under light microscopy in the irradiated tissue. It is not to say that components of the inflammatory soup may not still be involved, as in reperfusion hyperalgesia, where anti-inflammatory agents are anti-hyperalgesic in the absence of clinical and histological features of inflammation (Gelgor *et al.*, 1986a; Gelgor *et al.*, 1992a; Gelgor *et al.*, 1992b; Vidulich & Mitchell, 2000).

There is evidence that some components of the inflammatory soup are involved in at least initiating UV_A-light induced hyperalgesia. Prostaglandins and histamine are increased after UV_A-light exposure of skin (Gilchrest *et al.*, 1981; Hawk *et al.*, 1983). Furthermore, bradykinin receptor antagonists and prostaglandin synthesis inhibitors, such as ibuprofen, a non-selective COX inhibitor, can attenuate UV_A-light hyperalgesia when administered at the onset of hyperalgesia (Perkins *et al.*, 1992; Perkins *et al.*, 1993; Perkins & Kelly, 1993). A proviso to the studies that demonstrated an increase in pro-inflammatory mediators or the efficacy of anti-inflammatory agents in relieving hyperalgesia is that since the UV-light lamps were not independently verified and their UV-light spectrum characterised it is possible that UV_B-light contaminated their studies. If this occurred the increase in inflammatory mediators, and subsequent efficacy of anti-inflammatory agents, may reflect the effects of UV_B-light and not UV_A-light. Ultimately more studies have to be done to determine the exact role of pro-inflammatory mediators in UV_A-light induced hyperalgesia. Pro-inflammatory antagonists would have to be administered, and pro-inflammatory mediators measured, at different points in the time course of the hyperalgesia, such as at the onset of UV_A-light induced hyperalgesia, and at intervals after the onset of UV_A-light induced hyperalgesia. In addition, the various pro-inflammatory antagonists would be administered at various locations within the

neuraxis, such as the spinal cord or cerebral ventricles, to determine their point of action.

Irrespective of the role of pro-inflammatory mediators, UV_A-light induced hyperalgesia provides an alternative to almost all current animal models of hyperalgesia that rely on initiating inflammation to cause hyperalgesia. In almost all models of hyperalgesia, inflammation parallels the duration and severity of hyperalgesia (Vinegar *et al.*, 1987; Diehl *et al.*, 1988; Weber *et al.*, 2005). For example, carrageenan injected into the paw of rats and cats (Vinegar *et al.*, 1987; Diehl *et al.*, 1988) or incision of the rat tail (Weber *et al.*, 2005) causes hyperalgesia to either a noxious thermal or mechanical challenge that parallels the onset and resolution of the acute inflammatory response. The major advantage is that experimental hyperalgesia without inflammation provides a template for the testing of putative antihyperalgesic agents, because agents that relieve such hyperalgesia must act through antinociceptive rather than anti-inflammatory mechanisms.

5.4 UV-light and altered neuronal firing

My investigations of UV_A-light induced hyperalgesia began by exploring the first step in central sensitization, the C fibre barrage. The C-fibre barrage, activation of C-fibre neurones during a conditioning challenge, alter WDR neurones such that the WDR are sensitized for a duration that exceed both the conditioning challenge and the C-fibre barrage (Dickenson & Sullivan, 1987; Woolf, 1989; Thompson *et al.*, 1993b; Vattne *et al.*, 1998; Ji *et al.*, 2003). Consequently, blocking the C-fibre barrage with local anaesthetics decreases the severity and duration of hyperalgesia in certain

animal models of chronic pain (Gonzalez-Darder *et al.*, 1986; Seltzer *et al.*, 1991; Duarte *et al.*, 2005), as well as post-incisional allodynia in humans (Gordon *et al.*, 1997). Since UV_A-light irradiation of the rat hindpaw caused sensitization of WDR neurones (Urban *et al.*, 1993), I injected bupivacaine, a long-lasting local anaesthetic, into the base of rats' tails before UV_A-light irradiation in order to block the C-fibre barrage (Chapter 3).

Bupivacaine injection into the base of the rat tail before UV_A-light exposure prevented the development of hyperalgesia to a subsequent noxious thermal challenge, but did not affect the development of primary hyperalgesia to a noxious punctate mechanical challenge (Themistocleous *et al.*, 2007). Adequate afferent neuronal activity during UV_A-light exposure was therefore critical for the development of thermal, but not mechanical hyperalgesia, and my study suggested that thermal hyperalgesia after UV_A-light exposure was caused solely by central sensitization. If sensitization of peripheral thermal nociceptors occurred then thermal hyperalgesia would have developed once the bupivacaine had worn off, on the second or later days. Centrally mediated thermal hyperalgesia is not without precedent. Subcutaneous bee venom injection also caused a centrally mediated thermal hyperalgesia (Chen *et al.*, 1999; Chen & Chen, 2000; Chen *et al.*, 2000). Intraplantar injection of bee venom caused a contralateral thermal hyperalgesia, and the contralateral thermal hyperalgesia was a centrally mediated phenomenon that was blocked through the systemic administration of a NMDA receptor antagonist (Chen & Chen, 2000; Chen *et al.*, 2003). Despite preventing the development of thermal hyperalgesia, interfering with the C-fibre barrage did not prevent the development of primary hyperalgesia to a noxious punctate mechanical challenge.

It may be that the mechanical hyperalgesia depended on sensitization processes activated by myelinated afferent pathways (Meyer & Treede, 2004), which were not disabled by the local anaesthesia. Prolonged peripheral neuronal sensitization was probably responsible for the mechanical hyperalgesia; however, it still was possible that central sensitization occurred independent of an initial C-fibre. Indeed, Urban and colleagues (1993) observed continuous low-threshold mechanoreceptor input to the dorsal horn over the first three days after UV_A-light exposure of the rat hind-paw. Subsequently, the same group (Thompson *et al.*, 1994) showed enhanced summation of ventral root potentials, a measure of wind-up, in response to C- and A-fibre stimulation. Therefore in our animals, continuous input from low-threshold mechanoreceptor after UV_A-light exposure may have led to progressive wind-up in dorsal horn neurones (Herrero *et al.*, 2000) with subsequent central sensitization independent of an initial C-fibre barrage during UV_A-light exposure. To resolve the mechanisms contributing to the development and maintenance of the mechanical hyperalgesia I measured responses from the primary afferent neurones of the rat tail after UV_A-light exposure (Chapter 4).

In my third study (Chapter 4), I investigated the effect UV_A-light exposure of the rat tail on primary afferent fibres that innervate the rat tail, to determine whether changes within peripheral neurones could have accounted for the modality specific hyperalgesia observed after I blocked the C-fibre barrage and prevented the development of thermal but not mechanical hyperalgesia. I focused on nociceptive afferents and did not investigate those afferents that would respond to challenges both in the noxious and non-noxious ranges. The major finding of my third study was that I

discovered a population of peripheral afferent fibres with electrophysiological properties consistent with the behavioural hyperalgesia that developed after UV_A-light exposure. The peak firing rates and areas under the curve of post-challenge time histograms, a measure of firing rate over time, of both the A δ - and C-fibres were increased when noxious blunt and punctate challenges were applied to the rat tail. I therefore had shown that the A δ - and C-fibres that encode for noxious punctate and blunt mechanical challenges were sensitized after UV_A-light exposure. However, the peak firing rate and areas under the curve of post-challenge time histograms, a measure of firing rate over time, of C-fibres that were responsive to noxious thermal challenges were not increased after UV_A-light exposure. Therefore, C-fibres responsible for encoding noxious thermal challenges were not sensitized by UV_A-light exposure. I therefore had discovered alterations in peripheral afferent fibres that could account for the modality-specific hyperalgesia. However, since I did not investigate the firing properties of all peripheral afferent neurones, for example neurones that would respond to challenges both in the noxious and non-noxious ranges, there may be other mechanisms that contribute to UV_A-light hyperalgesia.

Hyperalgesia to blunt and punctate mechanical challenges after UV_A-light exposure of the rat tail lasted for at least two weeks. I measured neuronal responses one day after UV_A-light exposure. Therefore, sensitization of peripheral neurones could account for the hyperalgesia that was present to the blunt and punctate challenges one day after UV_A-light exposure, but I do not know whether the peripheral sensitization lasted long enough to account for the prolonged behavioural hyperalgesia. It may be that increased input from sensitized peripheral afferent neurones altered WDR neurones independently of a C-fibre barrage and thus was responsible for the sustained

mechanical hyperalgesia, or sensitized WDR neurones and peripheral afferent neurones together account for the prolonged behavioural hyperalgesia. If peripheral sensitization does account for prolonged behavioural hyperalgesia it may be that UV_A-light acting through chromophores permanently altered peripheral afferent neurones. These changes may have caused the change in the sensitivity of peripheral nociceptors specific for mechanotransduction in our rats in the absence of overt histological signs of inflammation, and thereby account for undetected peripheral mechanisms for the sustained hyperalgesia we observed. Ultimately, determining the neuronal basis of the prolonged hyperalgesia would require a study in which primary afferent and dorsal horn neurones responses are measured in rats up to two weeks after UV_A-light exposure.

In contrast to their sensitization to noxious mechanical challenges, peripheral afferent neurones were not sensitized to noxious thermal challenge. Such a finding was in keeping with the data from my second study whereby abolishing the C-fibre barrage and subsequent central sensitization prevented the development of sustained thermal hyperalgesia. Therefore, it is possible that thermal hyperalgesia after UV_A-light exposure of the rat tail was caused by central sensitization. It is possible that after UV_A-light exposure of the rat tail a sub-population of neurones within the spinal cord became sensitized to noxious thermal challenges, as was seen after intraplantar injection of bee venom, which was a central NMDA receptor mediated phenomenon (Chen & Chen, 2000; Chen *et al.*, 2003). Determining the neuronal basis of the thermal hyperalgesia would require a study in which WDR dorsal horn neurones responses are measured after UV_A-light exposure, as well as determining whether

WDR dorsal horn neurones responses are altered with the addition of suitable antagonists, such as NMDA receptor antagonists.

In my thesis I have developed my own model of UV_A-light hyperalgesia, which causes sustained behavioural hyperalgesia to noxious mechanical and thermal challenges. I independently verified that my UV-light lamp emitted light predominantly in the UV_A spectrum, and confirmed histologically that UVA-light does not cause inflammation. The sustained mechanical hyperalgesia did not depend on the activation of the C-fibre barrage, but thermal hyperalgesia did depend on the activation of a C-fibre barrage during the conditioning event of UV_A-light exposure. Furthermore, I demonstrated that primary mechanical hyperalgesia was in part due to A δ and C-fibre sensitization, and C-fibres responsible for encoding noxious thermal challenges were not sensitized after UV_A-light exposure. I have therefore demonstrated that primary thermal hyperalgesia is not solely a peripherally mediated phenomenon. I believe that the pathophysiological mechanisms that cause hyperalgesia differ according to the conditioning event. Thermal hyperalgesia after peripheral inflammation and nerve injury is caused by primary afferent sensitization and mechanical hyperalgesia is caused by central sensitization. After bee venom injection and UV_A-light exposure the reverse is true, that is, thermal hyperalgesia is caused by central sensitisation and mechanical hyperalgesia is caused by primary afferent sensitisation. Such a paradigm shift may provide the answers to our poor success in treating chronic pain and the development of novel pharmacological targets and new treatment regimes.

APPENDIX

AESC 3

STRICTLY CONFIDENTIAL**UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG****ANIMAL ETHICS SCREENING COMMITTEE****CLEARANCE CERTIFICATE NO:**

2002	89	3
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APPLICANT: Andreas Themistocleous**DEPARTMENT:** School of Physiology**PROJECT TITLE:** The Effect of Ultraviolet Light Exposure on Nociception of the Rat's Tail

Species	Number	Expiry Date
Sprague Dawley	35	2004

- i) Approval is hereby given for the experiment described in the above application.

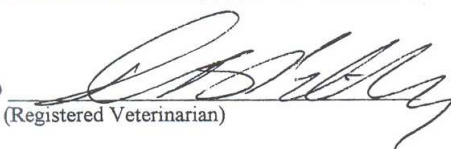
The use of these animals is subject to AESC Guidelines for the use and care of animals, is limited to the procedures specified in the application form, and to:

APPROVED Subject to to clarification of what exactly the MED is as stated in point 1 page 4 of AESC application form

SIGNED 
(Chairman: Animal Ethics Screening Committee)

DATE: 26 November 2002

- ii) I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23(1)(c) of the Veterinary and Para-veterinary Professions Act (19 of 1982)

SIGNED 
(Registered Veterinarian)

DATE: 26 November 2002

NOTE:

First-time users of the CAS should contact the Director of the CAS in order to familiarise themselves with the facilities available, and the procedures required by the CAS for the carrying out of experiments.

AESC 3

AESC 3

STRICTLY CONFIDENTIALUNIVERSITY OF THE WITWATERSRAND, JOHANNESBURGANIMAL ETHICS SCREENING COMMITTEECLEARANCE CERTIFICATE NO.:

2003	63	3
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APPLICANT: Andreas ThemistocleousDEPARTMENT: School of PhysiologyPROJECT TITLE: The effects of local anaesthetics on UV-induced hyperalgesia in rats

Species	Number	Expiry Date
Sprague Dawley	20	2005

- i) Approval is hereby given for the experiment described in the above application.

The use of these animals is subject to AESC Guidelines for the use and care of animals, is limited to the procedures specified in the application form, and to:

APPROVED

SIGNED D. A. Gray
(Chairman: Animal Ethics Screening Committee)

DATE: 27 August 2003

- ii) I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23(1)(c) of the Veterinary and Para-veterinary Professions Act (19 of 1982)

SIGNED [Signature]
(Registered Veterinarian)

DATE: 27 August 2003

NOTE:

First-time users of the CAS should contact the Director of the CAS in order to familiarise themselves with the facilities available, and the procedures required by the CAS for the carrying out of experiments.

AESC 3

STRICTLY CONFIDENTIALUNIVERSITY OF THE WITWATERSRAND, JOHANNESBURGANIMAL ETHICS SCREENING COMMITTEECLEARANCE CERTIFICATE NO.:

2005	7	3
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APPLICANT: Dr A ThermistocleousDEPARTMENT: PhysiologyPROJECT TITLE: Discharge patterns of nociceptive primary afferent fibres in the rat coccygeal nerve after ultraviolet A-light exposure

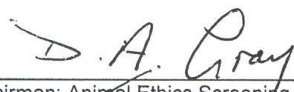
Species	Number	Expiry Date
Rat	60	January 2007

- i) Approval is hereby given for the experiment described in the above application.

The use of these animals is subject to AESC Guidelines for the use and care of animals, is limited to the procedures specified in the application form, and to:

- demonstrating competency with nerve dissection to Dr Meyer (CAS vet)

SIGNED


 (Chairman: Animal Ethics Screening Committee)

DATE: 25/01/2005

- ii) I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23(1)(c) of the Veterinary and Para-veterinary Professions Act (19 of 1982)

SIGNED


 (Registered Veterinarian)

DATE: 25/01/2005

NOTE:

CHAPTER 6

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