Characterization and re-evaluation of experimental pain models in healthy subjects

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Chapter 1

PHARMACODYNAMIC EVALUATION: PAIN METHODOLOGIES

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ABSTRACT

Despite many advances in the last decades in understanding pain, the development of new analgesic compounds has not followed at the same pace. The development of more targeted analgesic compounds with fewer side effects is therefore essential. With an increased demand to demonstrate pharmacodynamic effects of new analgesic compounds, the importance of human evoked pain models is now higher than ever.

Pharmacodynamic evaluation with human evoked pain models offers the possibility to determine the dose ranges at which new analgesics exert their pharmacological effect. Pain models may also aid in the choice of target population, determine which modality of pain a new drug is expected to be most suitable, help to differentiate between a central or more peripheral mode of action of new drugs, and help determine which other effects contribute to its mode of action *e.g.*, sedation.

Human evoked pain models are conducted in standardized laboratories where factors like stimulus intensity, frequency, duration and location can be controlled. Using pain models in healthy volunteers has important advantages over assessing the effects of new drugs in patients with pain; the pain elicited in human pain models is predictable in its intensity while clinical pain will naturally fluctuate. Analgesic properties can be investigated with pain models without the influence of accompanying symptoms that are often seen in patients with pain.

GENERAL INTRODUCTION

Pain is intended as a warning to the body that a noxious stimulus can (potentially) harm the body. The International Association for the Study of Pain (IASP) defines pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.[22] Prevalence studies show that in Western Countries 19-31% of the adult population suffer from a form of chronic pain.[110,123] Despite the availability of potent analgesics such as opioids, chronic pain remains a high unmet medical need as many effective analgesics have important side effects and chronic treatment with opioids leads to tolerance and addiction. The development of better and more specific analgesic compounds therefore remains essential. With an increased demand to demonstrate pharmacodynamic effects of new compounds as early as possible in clinical drug studies, the importance of human evoked pain models is now more than ever.

In a pure neurophysiological sense, nociceptive pain occurs when nociceptors are stimulated by noxious stimuli (*e.g.*, mechanical, thermal, electrical or chemical stimuli). After a threshold has been reached, the nociceptive nerve fibre transmits the pain signal to the spinal cord. The signal is modulated at several locations along ascending pathways through the dorsal horn and spinal cord. From the spinal cord, the pain signal is projected to supraspinal centres where the brain can modulate the excitatory activity via descending control.[137] Perception of pain is even more complex where more than one sensory system is responsible for transmission of the painful stimulus.[2]

From a more neuropsychosocial point of view, pain is a complex experience influenced by many factors such as emotion, fear, anxiety, but also cultural background, sex, genetics and educational background. Due to its complexity, it can be difficult to assess the effects of analgesic drugs on pain in patients and animal pain models demonstrate low predictability for clinical efficacy in humans. Several explanations are receptor dissimilarity between species, differences in pharmacokinetics and morphological and functional differences between the brains of animals and humans.[137] Human evoked pain models can control some of these influencing factors. Therefore, these models are an important step in the translation of animal research to pain patients.

Pharmacodynamic evaluation though human evoked pain models offers the possibility to differentiate between a centrally or peripherally acting drug, for which modality of pain a new drug will be most suitable (nociceptive, neuropathic or inflammatory), and which other effects contribute to its mode of action (*e.g.*, sedation, tolerance).[133,135,137,173] This can be done in early clinical trials with healthy volunteers, which is not only cost-reducing but also time saving. Other advantages of using human evoked pain models are (1) stimulus intensity, duration and modality are controlled and do not vary over time; (2) differentiated responses to different standardized stimulus modalities; (3) the response can be assessed quantitatively and compared over time; (4) pain sensitivity can be compared quantitatively between various normal/affected/treated regions; (5) models of pathological conditions can be studied and the effects of drugs on such mechanisms quantified; and (6) pain models can be easily performed in healthy subjects, who are easier to recruit into clinical studies.[10]

Evoked pain is mostly short-lasting, with most stimuli being applied exogenously and are generally focused on cutaneous nociceptor activation. Arguably, evoked pain models are limited in intensity due to ethical constraints related to the risk of tissue damage. In contrast to natural occurring pain which is mostly caused by endogenous factors, longer lasting and influenced by complex emotions.[122] Since clinical pain is a complex sensation involving psychological, physiological and cognitive factors, no single pain model is able to replicate all aspects of clinical pain.[135]

Several methods exist for evoking pain in humans, such as mechanical, thermal, electrical and chemical stimulation. A stimulus can be either phasic or tonic. Stimuli can be applied to different tissue types for instance skin, muscles or viscera.[10] This chapter focuses on the different pain models that are used to induce evoked pain in humans and the means to assess the evoked pain. Human evoked pain models are divided into the energy domain (*i.e.*, mechanical, thermal, electrical and chemical induction) and are further subdivided into area of stimulation (*i.e.*, skin, muscle and viscera) that is stimulated.

PAIN ASSESSMENT TECHNIQUES

An evoked pain model consists of two elements; a stimulus needs to be applied to evoke pain and the related pain response needs to be measured.[57] Possible assessment techniques for evoked pain responses can be divided into several categories: psychophysical, electrophysiological and imaging.

Psychophysical methods

Psychophysical methods aim to describe the relationship between physical stimuli (Section 3) and corresponding subjective responses in a quantitative manner.

These methods are widely used to study stimulus processing in various research fields, including pain processing. The methods to quantify the relationship between stimulus and response can be subdivided into unidimensional methods and multidimensional methods.

Unidimensional measures

PURPOSE AND RATIONALE

Unidimensional questionnaires such as the Numeric Rating Scale (NRS), the Verbal Rating Scale (VRS), or the Visual Analogue Scale (VAS) provide a single subjective measure to the intensity of a stimulus. More quantitative unidimensional measures are thresholds: the intensity where a stimulus is first perceived as painful (pain detection threshold), and the intensity where the stimulus is no longer tolerable (pain tolerance threshold).

PROCEDURE AND EVALUATION

The NRS consists of a horizontal line with evenly spaced perpendicular lines. The patient or subject is asked to rate his or her pain from 0 to 10 (11 point scale) or from 0 to 100 (101 point scale) where 0 equals 'no pain' and 10 or 100 equals 'worst possible pain'. The VRS consists of a list of descriptive terms to express the different levels of pain. It at least should include the extremes, in this case 'no pain' and 'worst possible pain'. Additional term usually used are 'very mild pain', 'mild pain', 'moderate pain', 'severe pain, and 'very severe pain'. The VAS is a horizontal line consisting of the two extremes and subjects are asked to indicate on the horizontal line indicating the perceived intensity.

For the determination of pain thresholds, the stimulus intensity increases until a certain pain threshold is reached.[57] The pain detection threshold (PDT) is reached when a change in sensation from non-painful to painful is felt by the subject. The pain tolerance threshold (PTT) is the stimulus intensity at which the pain is no longer tolerable. Depending on the method used, the stimulus is ceased before or when reaching the pain tolerance threshold.

Several stimulus modalities (such as thermal, mechanical or electrical) can be used to quantify pain perception and to assess sensory function.[12] These can then also be used as an indicator of the current state of the pain system. For example, it can be used to detect hyperalgesia, which is a condition in which an enhanced pain response to noxious stimuli is observed. Hyperalgesia is indicated by a decrease in the pain thresholds and an increase in pain to suprathreshold stimuli.

CRITICAL ASSESSMENT OF THE METHOD

The main disadvantage of NRS, VRS and VAS scales is that they measure a single qualitative aspect of pain, namely intensity or unpleasantness, while pain consists of more qualities (Section "Multidimensional measures"). Moreover, even though these (subjective) unidimensional scales can be used as a coarse measure of the level of pain a subject experiences, they cannot be used to distinguish individual contributions of nociceptive and pain related mechanisms.

MODIFICATION OF THE METHOD

To obtain a more detailed description of pain perception and qualities, multidimensional questionnaires can be used (Section "Multidimensional measures").

More advanced psychophysical procedures are being developed to relate stimulus properties (*e.g.*, pulse width, number of pulses, and inter-pulse interval) and perceptions to nociceptive processes.[39,193] Also, combining unidimensional measures with neurophysiological measures may provide more information on underlying processes.

Multidimensional measures

PURPOSE AND RATIONALE

Unidimensional questionnaires are often found to be limited in their capabilities in describing pain perceptions. Therefore, multidimensional measures provide means to describe perception using multiple sensory and affective qualities of pain.

PROCEDURE

In contrast to the unidimensional measures, subjects are asked to fill in several answers to various questions, or have to choose from a large range of adjectives to describe their pain perception. One of the most widely used multidimensional tool is the McGill Pain Questionnaire.[114] This questionnaire not only measures the pain intensity but also measures the sensory and affective qualities of pain. The McGill Pain Questionnaire has been used in a large number of studies, and has been translated and validated in multiple languages.[115,116]

CRITICAL ASSESSMENT OF THE METHOD

Multidimensional questionnaires often take more time to complete than simpler unidimensional questionnaires. Particularly in a clinical setting, multidimensional questionnaires either need to be compressed (such as the short-form

McGill Pain Questionnaire) or replaced by unidimensional questionnaires. Additionally, in the experimental setting of evoked pain models not all components of a questionnaire may be applicable, *e.g.*, items related to affective aspects of pain sensation.

MODIFICATION OF THE METHOD

Numerous pain questionnaires exist that measure different qualities of neuropathic and non-neuropathic pain. These include the Pain Quality Assessment Scale (PQAS), Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) and PAINDETECT. Moreover, questionnaires targeting specific patients groups exist as well; the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and Knee Society Score (KSS) assign pain, stiffness, and functional scores to patients suffering osteoarthritis.

Electrophysiological and imaging methods

Electrophysiological readouts include evoked potentials via electroencephalography (EEG). Imaging readouts include functional magnetic resonance imaging (fMRI) and positron emission tomography (PET). Electrophysiological and imaging readouts provide a more objective measurement of pain. However, they have a larger variation in outcome measurements, are more expensive and are technically more difficult to perform in a large group of subjects.[10,87,188]

Functional magnetic resonance imaging

PURPOSE AND RATIONALE

Neuroimaging has identified several cortical regions in the brain that are typically active when a painful stimulus is applied to the body. With fMRI these pain responses in the brain can be observed. Structures that are active during pain perception are the primary and secondary somatosensory, the cingulate and the insular cortices which together are called the pain matrix.[81,142,149,151,181] Activation of the pain matrix due to nociceptive stimuli is the functional imaging analogue of conscious pain perception. Measuring the activities in the pain matrix during a painful stimulus can be used as an objective measure for pain perception.[23]

PROCEDURE AND EVALUATION

A subject is given a painful stimulus while being scanned in the MRI. fMRI measures brain activity by detecting changes in blood flow (hemodynamic response) associated with neuronal activation.[76] It uses the relative abundance of deoxyhemoglobin in blood that changes the proton signal from water molecules surrounding a blood vessel, producing blood oxygenation level-dependent contrasts (BOLD).[134] Via the BOLD signal an indirect index of neural activity is provided. Several fMRI methods are used in pain research, which reveal the neural correlation of pain perception and modulation by characterizing the brain response to evoked stimuli (*e.g.*, pain, allodynia), task-driven responses, or drugs (phMRI).[23]

CRITICAL ASSESSMENT OF THE METHOD

This non-invasive method of measuring pain can provide a measure of cerebral perfusion that correlates with an acute painful stimulus in healthy volunteers. Detecting chronic pain is more complex due to confounding factors like disease and treatment. Brain systems like emotion, memory and motivation are also active during measurement in these patients.[23] These systems can also be triggered by placebo analgesia, which adds to the complexity.[124] Additionally, the resolution of fMRI is inferior compared to the EEG, which means that it is not suitable to investigate the primary neuronal activity directly related to the pain stimulus and less suitable to investigate the deeper structures of the brain (*e.g.*, brainstem and thalamus).[137]

MODIFICATIONS OF THE METHOD

When including fMRI in a study, a distinction can be made in the type of pain model that will be used during the study. Mechanical,[14] thermal,[100,166] electrical[95] and chemical[14] induced pain models can be used during an fMRI scan, with the exception of models that entail the use of water and metal. Modifications can be made in the type of MRI, protocol used for scanning and analyzing protocols.

Electroencephalography and Evoked Potentials

PURPOSE AND RATIONALE

EEG is a non-invasive technique which records (spontaneous) synchronized postsynaptic neuronal activity of the human cortex. In contrast to brain imaging techniques, EEG has a high temporal resolution. This high resolution makes EEG an effective method of observing (rapid) changes in brain activity. Additionally, EEG is a suitable method for recording evoked potentials (EP) to painful stimuli (*e.g.*, thermal, mechanical, or electrical) and may provide important information on (central) pain processing.[126]

PROCEDURE AND EVALUATION

To record EEG, several electrodes are placed on the scalp, either using individual Ag/AgCl electrodes or specialized caps. The impedance should be similar among all electrodes and is advised to be held under 50 kΩ. Quantifying resting state EEG can be done by means of spectral analysis. Due to its nonstationary behavior, recorded data is divided in short epochs ranging between 2-10 seconds.[83] Each epoch is then transformed into the frequency domain and after removing or correcting epochs affected by artefacts (*e.g.*, ocular or muscular activity) averaged. The frequency range is then subdivided into bands (*i.e.*, delta, theta, alpha, beta, and gamma), and then integrated over frequency bands to obtain the total power per frequency band.

EPs are monophasic deflections of spontaneous EEG and are time and phase locked on the onset of the stimuli.[126] These waveforms are typically characterized by their polarity, latency, amplitude, and position on the scalp. As the signal-to-noise ratio (SNR) is relatively low when recording EPs, repeatedly stimulating and recording cortical responses is required. This allows improving the SNR by means of averaging and allows the characterization of the evoked response in terms of the amplitudes and latencies. The data collected generally includes the peaks and latencies of the N1, N2, P2, and P3, or the top-top amplitude between N2 and P2.[182] Moreover, the reaction time after the presentation can also be recorded. The N1 is most prominent at the contralateral temporal side (*i.e.*, T_3 or T_4) referenced to the frontal Fz. The N2 and P2 are most prominently visible at the vertex Cz referenced to the (linked) earlobes A1 and A2. The P_3, which is not thought to be nociceptive specific has a relatively long latency and is best observed at Pz referenced to the earlobes.

CRITICAL ASSESSMENT OF THE METHOD

It is important to know that EEG recordings will be contaminated with artefacts. Common sources of artefacts are ocular movements and blinks, muscle contraction and cardiac activity. Each of these artefacts have their own characteristics in both time domain and frequency domain and must be dealt with prior to analyzing EEG. Possibilities for dealing with these artefacts are either excluding parts of the recording for analysis, or correcting for them (*e.g.*, by using regression techniques, filtering, or blind source separation techniques).

EPs have been shown to be sensitive to various changes in pain pathways. EPs are sensitive to changes induced by analgesics.[161] It must be taken into account however that the amplitudes of the EPs are dependent on the attention of the subject; reduced attention results in significantly lower peak amplitudes. Moreover, the across trial variability is relatively high making comparisons between groups and trials complicating.

MODIFICATION OF THE METHOD

When several electrodes are recorded during a resting state EEG, multichannel topography allows observing the activity recorded at several locations. Time-dependent changes in power spectra can therefore also be visualized. Even though EEG has a relatively low spatial distribution in contrast to techniques such as fMRI, source localization techniques are used to find brain sources of the recorded potentials.[63] However, high-density electrode placement is required for more reliable source localization.[170]

As a result of averaging EPs, non-phase locked information is lost. Time-frequency analysis of epochs does provide the means to study non-phase-locked information.[75,126]

Nociceptive spinal flexion reflex

PURPOSE AND RATIONALE

The nociceptive spinal flexion reflex (NFR), also called the RIII reflex, is a physiological, polysynaptic reflex allowing for painful stimuli to activate an appropriate withdrawal response.[168] It is one of the available tools for objective quantification of spinal nociception in humans. The NFR reflex can be elicited in all four limbs. Here, the most standard procedure, with stimulation of the lower limb (sural nerve), is described.

PROCEDURE AND EVALUATION

Electrical constant current stimulation is delivered to the retromalleolar pathway of the sural nerve. Each stimulus consists of five pulses of 1 ms duration, separated by 4 ms, resulting in a total duration of 21 ms. Electromyographic responses are recorded from the ipsilateral biceps femoris (short head) via surface electrodes placed 4-5 cm apart over the muscle belly. The RIII reflex is identified as a polyphasic muscle response appearing with an onset latency between 90 and 130 ms after stimulation.[192] Following stimulation of the sural nerve, three responses are sequentially recorded: the tactile reflex (also known as RII), the nociceptive flexion reflex (RIII), and an involuntary movement signal.[168] For quantification of the RIII reflex response, the reflex area is obtained by integrating the rectified signal within a 50 ms analysis window starting between 90 and 120 ms after stimulation. Stimulus-response curves are recorded by increasing

stimulation intensity in 0.5 mA steps starting from 0.5 mA. Participants can rate the pain intensity of each stimulus using an NRS or VAS. The pain threshold is determined as the stimulus intensity that first evokes a painful sensation (defined as an NRS rating ≥1 or VAS > 0). The RIII threshold is defined as the stimulus intensity that first evokes a reflex response exceeding a raw area of 100 µV×ms.[157]

CRITICAL ASSESSMENT OF THE METHOD

Reduction of the NFR by a pharmaceutical compound does not necessarily imply reduction of ascending nociception, but may also indicate modulation of other components that play a role in the RIII reflex, such as deep dorsal horn interneurons[162] or motor neurons.[157]

MODIFICATIONS OF THE METHOD

NFR is affected by demographic factors. It is indicated that female subjects and children have lower NFR thresholds.[139,159] Other factor affecting the outcome include cardiac cycle, baroreceptors, stimulation site and even diurnal rhythm.[168] All should be addressed before the start of the trial.

Conditioned Pain Modulation

PURPOSE AND RATIONALE

Conditioned pain modulation (CPM) (also known as descending noxious inhibitory control (DNIC) for animal studies or heterotopic noxious counter-stimulation) is a paradigm that uses a conditioning stimulus to influence a test stimulus and can be used to assess the endogenous analgesic capacity of both healthy subjects and patients.[130] The assessment of CPM provides an indication of the balance between descending facilitation and inhibition. It is hypothesized that malfunction of pain modulation network may be the cause rather than the effect, of chronic pain development.[194]

The endogenous network is mediated via descending serotonergic, noradrenergic, and dopaminergic pathways, with the conditioning stimulus activating decreasing the activity of on-cells in the rostral ventromedial medulla (descending noxious inhibitory control).[73]

PROCEDURE AND EVALUATION

CPM is assessed by using a conditioning stimulus which is generally a tonic nociceptive stimulus, however non-painful conditioning stimuli have also been reported.[24,103] Various methods can be used as the conditioning stimulus. The method most commonly used is the cold pressor test.[150] However, other stimuli have also been reported including hot water, ischemic pain, heat, chemically induced pain, electrical induced pain, physically induced muscle pain.[148]

Various methods are used as the test stimulus including nociceptive flexion reflex, electrical, heat and pressure stimulation amongst others. CPM is considered to be a systemic experience, and as such, heterotopic stimulation is used for the assessment of CPM. CPM is assessed by comparing the endpoint from the test stimulus before and after administration of the conditioning stimulus. The duration of effect from the conditioned stimuli may be assessed in parallel or soon after administration of the conditioning stimulation however the duration of effect is paradigm-dependent with reports of CPM effects lasting 5 minutes, 30 minutes and up to 60 minutes after application of the conditioning stimulus.[53,60,183]

CRITICAL ASSESSMENT OF THE METHOD

CPM paradigms are reported to have both reasonable to high intra- and inter-individual variability especially due to the endpoint being derived from subjective pain reports.[130] Furthermore, divergent terminology used in the literature for the same phenomena complicate comparisons. Factors affecting CPM are use of medication, psychological state of subjects (anxiety, depression, emotional status and attention span), and even ethnic origin.[55]

MODIFICATIONS OF THE METHOD

As highlighted above and as with other pain models, there are numerous ways to assess CPM including different methods for the conditioning and test stimulus including using different application area. Furthermore, the endpoints vary considerably between research groups, with some groups assessing change in the test stimuli endpoint before and after the conditioning stimuli while others reporting changes in the endpoint during administration of the conditioning stimulus.[38]

PAIN STIMULATION TECHNIQUES

Mechanical Stimulation

Human evoked pain models date back to the late 19th century, in which mechanical pressure was used to induce pain.[68] Over time, mechanical stimulation techniques became more accurate and are used to stimulate the skin, muscle or viscera. The skin is the most used organ, because of its practical

implementation. However, muscles can also be targeted both endogenous (post-exercise or ischemic) or exogenous (saline injection). Balloon distention in the viscera can be used to investigate new analgesic compounds in healthy volunteers but also as a diagnostic tool in patients (*e.g.*, gut disorders). Mechanical stimulation can be divided into touch/pinprick, pressure or pinching methods. This chapter focusses on the different techniques related to mechanical stimulation on the skin, muscles and viscera using touch/pinprick, pressure or pinching methods.

Mechanical skin stimulation

Touch and pinprick

PURPOSE AND RATIONALE

Mechanical stimulation via touch can be done with a cotton swab or a brushstroke (light mechanical stimulation) or pinprick. Light mechanical stimulation will not induce pain, but can be used to assess allodynia (lowered activation threshold for a non-painful stimuli) by other pain stimuli.

A common method of applying pinprick stimulation is the use of von Frey filaments. These filaments are used to quantify touch as well as the PDT and the PTT. In animal and patient experiments, it is mostly used to determine functional recovery of Aδ- or Aβ-fibers. In human pain models it is also used to determine hyperalgesia effects (*e.g.*, in the capsaicin/UVB model).

PROCEDURE AND EVALUATION

Von Frey filaments are calibrated filaments, originally made of human or animal hair, and later with acrylic or synthetic fibres or optical glass fibres. The filaments bend at a certain designated force.[52] The applied force is dependent on the stiffness, which is in turn dependent on the diameter of each filament. During stimulation, the filament is placed perpendicular to the skin and pressed down with a constant increase in force until it bends for 1 second. Subsequently, the filament is removed with a constant decrease in force and the subject is given some time to evaluate the stimulation.

CRITICAL ASSESSMENT OF THE METHOD

The von Frey test is characterized by its simplicity, thereby showing advantages in clinical settings for rough quantification of functional nerve regeneration. In addition, it can be used to classify responders in the evaluation of hyperalgesia.

Although the von Frey method is still commonly used to evaluate peripheral nerve function in patients, it has several disadvantages as a human pain model. Pinprick stimulation always co-activates non-nociceptive Aβ-fibers. This may contaminate the signal and influence the results affecting the specificity. Additionally, intra- and inter-observer variability of this method is high. The skill of the observer will have an important impact on the results. Furthermore, each filament is produced for one constant standardized level of pressure, which means that many filaments are needed to determine sensory or pain thresholds with a high precision. Environmental changes may affect the calibration of the filaments. Lastly, it is difficult to combine von Frey stimulation with quantification methods that require time-locked responses, such as evoked potentials or time-frequency analyses in EEG.

MODIFICATIONS OF THE METHOD

Von Frey filaments are inherently limited for method modification, because each filament is calibrated for one designated force application. Depending on the calibrated force, the filaments activate mainly Aβ-fibers (0.5-128 mN) or Aδ-fibers (128-512 mN).[35] Both conventional as electronic pinprick paradigms have been described.[121] To eliminate the intra- and inter-observer variation one can consider electronic pinprick devices.

Impactometers / Pinch interdigital web / joint

PURPOSE AND RATIONALE

Pressure algometers are usually applied to the muscle or bone, but other sites including or the interdigital web, skinflap, earlobe, or a finger or toe joint can be used as an area of investigation.[27,34,172] Handheld or computer controlled pressure algometers are clinically similar to palpation.[137] For methods using a pinch methodology, the pain is due to a combination of mechanical stimulation and local ischemia, while when pressure is applied to muscle the pain is related to muscle strain.[137]

PROCEDURE AND EVALUATION

A pressure algometer is applied to the area of interest. In a controlled manner the pressure increases at a constant rate until a psychophysical endpoint of interest is attained. Many commercial handheld pressure algometers provide user feedback to ensure pressure is applied at a constant rate. For repeated applications, the pressure algometer should be applied to the same area to ensure

intra-individual variability is minimized as variation in location can lead to different thresholds. PDT and PTT are the usual primary outcome measures.

CRITICAL ASSESSMENT OF THE METHOD

For handheld pressure algometers, control of the rate of onset, muscle contraction and examiner expectations are the primary limitations.[190] Differences in the size and shape of the probe limit comparisons between research groups.

MODIFICATIONS OF THE METHOD

The model can be used in combination with other methods that induce hyperalgesia or sensitization. Stimulus-response curves can be compared with non-sensitized locations.

Rather than using a punctate pressure algometer, cuff algometry can be used with the advantage that the model is generally computer-controlled thereby controlling the rate of application of the pressure) pain.[147] Pain induced by cuff algometry is primarily related to muscle pain with minimal contribution from skin nociceptors.

Mechanical muscle stimulation

Post-exercise muscle soreness

PURPOSE AND RATIONALE

Delayed onset muscle soreness (DOMS) is believed to be mediated by a combination of lactic acid, muscle spasm, connective tissue damage, muscle damage, inflammation, and endogenous substances (*e.g.*, bradykinin and prostaglandines).[129] This pain model is thought to mimic clinical pain by inducing central sensitization while having no spontaneous pain at rest compared with exogenous induced pain models.[137] Central sensitization is an increase in general excitability of the dorsal horn neurons which can be caused by nerve injury.[99] Secondary hyperalgesia is thought of as a transient state of central sensitization.[180]

PROCEDURE AND EVALUATION

Subjects perform an eccentric exercise that they are unaccustomed to with insufficient rest periods. Peak allodynia/hyperalgesia occurs 24-48 hours post-exercise. The affected muscles are assessed with a VAS to evaluate pain intensity and pressure algometry to evaluate allodynia/hyperalgesia.

CRITICAL ASSESSMENT OF THE METHOD

The method is somewhat non-specific, with an inflammatory component but these may be site specific.[171]

MODIFICATIONS OF THE METHOD

The method can be modified by using various locations/muscle groups with development of hyperalgesia dependent on the size of the muscle.[176]

Ischemic tourniquet

PURPOSE AND RATIONALE

The tourniquet model is a tonic pain model with nociceptive contributions from the muscle, skin and periosteum (the vascular connective tissue enveloping the bones). Clinically, tourniquets are used to perform intravenous regional anaesthesia or to provide a bloodless operating field. The tourniquet leads to metabolic changes, primarily acidosis, and compression which leads to the release of prostaglandins. Neuropathic pain induced by nerve compression may also contribute to the pain felt.[98] The method can also be used as a conditioning stimuli for the CPM paradigm (Section "Conditioned Pain Modulation").

The pain is thought to be mediated by the unmyelinated, slow conducting C-fibers that are usually inhibited by the Aδ-fibers.[98] The Aδ-fibers are blocked by mechanical compression after about 30 min, while the C-fibers continue to function.[31] Tourniquet compression leads to release of prostaglandins by the injured cells.[98] These prostaglandins increase pain perception by sensitizing and exciting pain receptors. Also, limb ischemia causes central sensitization.[98]

PROCEDURE AND EVALUATION

A pneumatic tourniquet is applied to an extremity, generally the thigh, following exsanguination of the leg. The cuff is inflated above the systolic blood pressure with ranges of 100-600 mmHg above the systolic pressure having been reported.[169]

CRITICAL ASSESSMENT OF THE METHOD

For healthy subjects, the tourniquet can be left for up to 2 hours, however pain is non-specific with pain being felt under the tourniquet and/or in the lower limb. The method can also lead to temporary hypoaesthesia and lower limb paralysis. Following reperfusion, the subject may experience hyperalgesia/allodynia and muscle cramps in the affected limb.

MODIFICATIONS OF THE METHOD

The method can also be used with combination with voluntary muscle contractions or exsanguination of the leg by gravity or esmarch bandage.[65,137] The width and type of tourniquet and the maximum inflation pressure can be used to modify the method.

Hypertonic saline injection

PURPOSE AND RATIONALE

Intramuscular injection of hypertonic sodium chloride (4-6%), results in a tonic, deep, diffuse pain. The pain usually lasts for several minutes following infection and leads to both local and referred pain. The pain is primarily mediated via direct excitation of C-fibers than caused by saline-induced tissue injury.[163,175] The procedure can also be used to induce local, cutaneous hypoesthesia.[59]

PROCEDURE AND EVALUATION

A bolus saline solution is injected intramuscularly using computer-controlled infusion pump. Earlier models used manual bolus injections.[61] Pain intensity is measured by using a VAS with peak pain or area under the curve (AUC) being the primary outcome measures. Referred pain patterns and changes in the pressure pain thresholds of the local and referred pain areas can also be assessed.[54]

CRITICAL ASSESSMENT OF THE METHOD

A limitation of the model is that hypertonic injection may elicit excitation from both non-nociceptive and nociceptive nerve fibers.[96]

MODIFICATIONS OF THE METHOD

Potassium chloride is occasionally used instead of sodium chloride. Various sites can be used for injection with the most common being the musculus trapezius and the musculus tibialis anterior.[54,163]

Mechanical visceral stimulation

Barostat / Oesophagal distention / Bladder distention

PURPOSE AND RATIONALE

Induction of pain in viscera is difficult to perform due to the location of the organs associated with visceral pain as pain originates from the internal thoracic, pelvic or abdominal organs.[84] Evoked pain models assessing mechanical visceral pain are generally limited to different accessible areas of the gastrointestinal (GI) tract, the urinary tract[111] and the uterine cervix.[42]

PROCEDURE AND EVALUATION

Mechanical stimulation of the viscera is generally performed using a balloon placed in the GI tract with the preferred locations being the oesophagus or rectum. The most common method used is the barostat method whereby changes in air volume within a balloon while maintaining constant pressure are measured.[41] Generally the balloon/bag is inflated until moderate pain is reported (up to approximately 7 on a VAS) and the corresponding balloon volume is reported.[172]

CRITICAL ASSESSMENT OF THE METHOD

One of the main limitations of organ distention is the distortion of the balloon. However, calculation of strain by impedance planimetry or calculation of balloon radius may overcome these limitations.[43,172] Assessment of visceral pain is difficult due to the diffuse, referred, vague and deep nature of the pain associated. Furthermore, autonomic reactions and the risk of perforation may limit the use of visceral pain models.[128]

MODIFICATIONS OF THE METHOD

Perfusion of the GI tract with chemical substances (*e.g.*, acid or capsaicin) can be used to sensitize the organs and nervous system and generally mimics the clinical situation where the organs are hypersensitive.[84]

Thermal Stimulation

Thermal stimuli can be used to induce pain. These can be roughly subdivided in cold and heat stimuli. Cold stimulation can be induced by emerging a body part in a cold water bath (cold pressor test), by using a cooling thermode, by inducing a freezing lesion or by applying ice directly to the skin. Heat pain tasks are among the most widely used pain models in human volunteers to investigate nociception, due to the relative ease of application and robustness. Nociceptive nerve fibers are activated by changes in temperature detected by a range of thermal receptors, of which Transient Receptor Potential ion channel subfamily vanilloid (TRPV) is most responsible. When a sharp increase in temperature (>43°) is detected, Aδ-fibers are activated, whereas C-fibers are activated by slower or more

dull temperature changes. Different clinical pain states can lead to sensitization to heat pain stimuli and consequently lower activation thresholds, which can be replicated using hyperalgesia models.

Thermal skin stimulation

Cold stimulation: Cold pressor

PURPOSE AND RATIONALE

The cold pressor test can be used to investigate nociception, but also cardiovascular responses (sympathetic functions) and can be used as to induce CPM. The cold sensation and pain induced by this method is mediated by activity of Aδ-fibers (cold sensation) and C-fibers (cold pain).[137]

PROCEDURE AND EVALUATION

One of the methods of induction of cold pressor pain is based on methods previously described by Eckhardt et al. and Jones et al..[47,85] Here, subjects place their non-dominant hand into a warm water bath for 2 minutes. At 1 minute 45 seconds a blood pressure cuff on the upper-arm is inflated to 20 mmHg below resting diastolic pressure. At 2 minutes the subject moves their hand from the warm water bath, directly placing their hand into a cold water bath. The baths are two thermostat-controlled, circulating water baths set at 35.0 ± 0.5°C and 1.0 ± 0.5°C. The subject rates their pain intensity using a rating scale (*e.g.*, VAS or NRS). When pain tolerance is reached, or when a time limit is reached, subjects are instructed to remove their arm from the water, at which point the blood pressure cuff is deflated. Typically PTT expressed in seconds after immersion in the cold water is recorded as primary outcome measure.

CRITICAL ASSESSMENT OF THE METHOD

Many different methodologies have been described which negatively influences the extent to which different studies can be compared. Small variations in water temperature can result in significant changes in pain intensity and tolerance times.[120] Therefore, it is important to use water baths that are able to circulate that water to prevent warming of the water around the hand.

MODIFICATION OF THE METHOD

Different temperature settings of the water baths can be used and different body parts can be immersed in the baths. Instead of a cold water bath, also a cool gel substance has been reported to induce pain which makes it suitable for fMRI testing.[100] Also a blood pressure cuff can be used to prevent compensatory blood flow to the hand.

Cold stimulation: Cooling thermode

PROCEDURE AND EVALUATION

The cold pressor test (Section "Cold pressor") is the most commonly used method to induce cold pain. However a cooling thermode can also be used to induce cold pain. A contact thermode is attached to a part of the human body. The method consists of administering a temperature with an intensity that gradually decreases at a constant rate, usually 1°C per second. The subject halts the stimulus when the cold pain threshold is reached or when a set lower cut off temperature is reached. Most studies using this methodology only report the cold pain detection threshold; the temperature at which the sensation has 'just become painful'.

CRITICAL ASSESSMENT OF THE METHOD

For none of these compounds this methodology provided evidence for analgesic efficacy, while other pain induction methods were able to provide this evidence.[173,174] The added value of this methodology in clinical pharmacology studies is limited.

MODIFICATION OF THE METHOD

Several adjustments can be made to this paradigm. The temperature at which the test starts, the rate at which the temperature decreases and the temperature at which the tests ends can be modified. Cold hyperalgesia can be induced by applying menthol to the skin prior to testing.[7] A mean threshold of multiple measurements can be taken to minimize subject variability.

Cold stimulation: Thermal grill

PURPOSE AND RATIONALE

The thermal grill is based on the phenomenon in which simultaneous application of innocuous cutaneous warm and cold stimuli can induce a sensation of burning pain, the so-called 'thermal grill illusion'. The illusion is thought to be caused by the central integration of ascending pain and temperature sensory channels, where the inhibition that is usually exerted by the cold afferents on the nociceptive system is reduced.[25,33]

PROCEDURE AND EVALUATION

The thermal grill consists of a number of juxtaposed bars of cold and warm non-painful temperatures (*e.g.*, 18°C and 42°C) on which the subject places a body part for a certain period of time. Possible outcome measures, during and after the test, can be cold and hot sensation, pain intensity and sensation of unpleasantness.

CRITICAL ASSESSMENT OF THE METHOD

Studies in which the thermal grill has been used applied a range of combinations of warm and cold stimuli to assess relationships between painful and non-painful sensations.[1,88,135] The occurrence of paradoxical pain elicited by the thermal grill illusion can be variable. A study by Bouhassira and colleagues reported a large subpopulation of subjects who only reported paradoxical pain when large cold-warm differentials were applied.[25] Due to the apparent necessity to tailor this method to each individual subject, it is difficult to standardize this method.

MODIFICATION OF THE METHOD

For the induction of the thermal grill illusion different temperature combinations of the cold and warm bars can be used. Also a different number and width of the bars and a different distance between the bars can be used.

Cold stimulation: Skin freezing

PURPOSE AND RATIONALE

Skin freezing is an induction method of hyperalgesia. Hyperalgesia can be experimentally induced with chemical and electrical stimulation or by injuring tissue using UVB or freeze lesions.[89,107]

PROCEDURE AND EVALUATION

A copper cylinder is cooled to -28°C and placed to a part of the skin for a brief period of time. For better thermal contact, a filter paper soaked with saline can be placed between the skin and the copper cylinder. This freezing induces cutaneous inflammation and hyperalgesia. Approximately 24 hours after induction sensory testing can be performed. After induction of the freeze lesion, pain and sensation testing can be performed via mechanical stimulation with stroking brushes (subjects have to indicate if a stroke with a certain load is painful), von Frey filaments (with increasing strength, subject have to indicate when the punctuation becomes painful) and blunt pressure using a pressure algometer (threshold in N/cm^2), or electrical stimulation using a constant current device (thresholds in mA).

CRITICAL ASSESSMENT OF THE METHOD

There are only a handful of studies reporting using freeze lesions. An advantage of this method is the extent to which this methodology can be standardized. The temperature, pressure and exposure time for induction of the lesion can be controlled. Furthermore, the lesion provides stable test conditions 1 day after induction. The lesion ceases over a period of days.[107] The freeze lesion may cause a hyperpigmentation, which can be visible for several months.[89]

MODIFICATION OF THE METHOD

The location, temperature, pressure and exposure time for induction of the lesion can be varied. Furthermore, the time window between induction of the lesion and testing can be changed.

Heat stimulation: Heating thermode

PURPOSE AND RATIONALE

Heat pain thresholds can be determined by applying a peltier element to the skin, where the increase in temperature activates nociceptors via TRPV and TRPM channels.[32,106]

PROCEDURE AND EVALUATION

A contact heat thermode probe, typically with a surface of 9.0-12.5 cm^2, is placed on the skin at a standardized non-painful baseline temperature between 30-39°C. Temperature is subsequently increased in a tonic or phasic fashion at a predetermined rate up to a temperature of 50-52°C. After a subject has indicated its pain detection or tolerance threshold, the probe is rapidly cooled to the baseline temperature. To reduce variability, the test can be repeated consecutively three times, and the average of these measurements is considered the pain threshold.[21] Outcome measures consists of pain thresholds as well as subjective pain scores (NRS, VAS).

CRITICAL ASSESSMENT OF THE METHOD

Heat pain thresholds are considered to be robust and reproducible endpoints, due to their clear physiological relationship with nociceptor activation thresholds. This method is widely used and contact heat thermodes are commercially available. Limitations to using a contact heating thermode is the relatively slow heating and cooling rate of the thermode, and the fact that the thermode touches the skin compared to for example laser, making it less suitable for investigating temporal summation or specific activation of Aδ-fibers.

MODIFICATIONS OF THE METHOD

In addition to investigating pain sensation in healthy skin, this method is often used to quantify sensitization, by comparing pain sensation of normal skin to an area of sensitized (UVB, capsaicin, menthol, cinnamaldehyde) skin.[154,161] The contact heat thermode can be used in conjunction with an EEG or fMRI modality, together known as CHEPs (Contact Heat Evoked Potentials).[153,154]

Heat stimulation: UVB erythema

PURPOSE AND RATIONALE

Inflammation is the biological response to any type of bodily injury and is recognized by increased blood flow, elevated cellular metabolism, vasodilatation and the release of soluble mediators, extravasation of fluids and cellular influx. Many different neuro-active factors are released during inflammation which stimulate nociceptors itself or by lowering the depolarization threshold of afferent nerves. The UVB (or 'sunburn') model is regarded as a model for inflammatory pain and as such it is most sensitive to the effects of NSAIDs[21,184]; in this model, hyperalgesia is evoked by exposing an area of skin to an individualized dose of UVB on the skin.

PROCEDURE AND EVALUATION

Prior to the start of the study, the minimal erythema dose (MED) for a subject is determined.[160] Subsequently, a one-, two- or threefold multiple of this dose is applied to the skin. Over the course of 2-96 hours, a clearly discernible dose-related area of erythema becomes apparent, where allodynia and hyperalgesia is observed. Maximum hyperalgesia is reached at 24 hours after irradiation. Typically no background pain is observed. UVB induced hyperalgesia or allodynia can be quantified using a thermal (heat or cold) or mechanical (stroking, pinprick, pressure algometry) challenge. Pain thresholds or a subjective pain score can be used as endpoints. Mechanical allodynia to pinpricks or a pressure algometer can be expressed as a PDT, when ascending strengths of von Frey filaments are used. Moreover, the area of allodynia is measured using a fixed von Frey filament or brush.

CRITICAL ASSESSMENT OF THE METHOD

The UVB model has been proven to be valuable tool to induce hyperalgesia and allodynia associated with inflammatory pain. One caveat however, is the risk of post inflammatory hyperpigmentation (PIH).[26] PIH is a harmless condition in which areas of skin become darker in color compared to the surrounding skin. PIH can occur at any age and any skin type, however it is more common in patients with darker skin (Fitzpatrick skin type 4-6).[50]

MODIFICATIONS OF THE METHOD

In general there are three degrees of freedom to modify the UVB method: (1) the dose can be altered between estimated 1 to 3 MED[16,64,80]; (2) the location can be varied between leg, arm, back; (3) the time between UVB exposure and hyperalgesia assessment may vary between 12-36 hours.

Heat stimulation: Heat burn model

PURPOSE AND RATIONALE

A first degree burn, comparable to a slight sunburn, resulting from a heat stimulus is used to initiate a local inflammatory response which results in reduced pain sensation thresholds.[179] Additionally, the intense nociceptive excitation is thought to induce central sensitization,[140,189] rendering the burn model a model for both peripheral and central neuronal sensitization.

PROCEDURE AND EVALUATION

A superficial cutaneous burn is induced using a thermode at a fixed temperature of 45-47°C, for a period of 2-7 minutes, which is applied at a standardized pressure on the skin. The leg is predominantly selected as the location, but the arm is also used. The acceptable timeframe for detectable hyperalgesia and allodynia is typically up to 4 hours after exposure to the heat stimulus. A distinction in sensitization can be made when investigating responses in the primary (exposed) area and the secondary (adjacent, non-exposed) area. Hyperalgesia resulting from the heat burn model is most distinctly quantified using a thermal or mechanical stimulus, due to locally reduced pain sensation thresholds in the primary area.[184] The PDT is predominantly selected as an outcome measure. Furthermore, the area of secondary hyperalgesia can be quantified using mechanical (pinprick, stroking) stimuli.

CRITICAL ASSESSMENT OF THE METHOD

The heat burn model in combination with a mechanical (pinprick) assessment of sensitization is moderately sensitive to the effects of NMDA receptor antagonists.[79,119] Analgesic effects of other treatments are less conclusive. As an evoked pain model, its principle is founded in controlled tissue damage, by inducing a first degree burn, with reports of blistering in up to 20% of the studies conducted with this paradigm.[184] This may be considered to be an advantage in terms

of external validity. However, from an ethical perspective a more short-lasting model without actual tissue damage may be preferred.

MODIFICATIONS OF THE METHOD

The execution can vary from using a contact heat thermode with a short and intense stimulus (100 seconds at 50°C), to the more commonly used prolonged exposure at lower temperature (7 minutes at 47°C). Other heat sources, including laser stimulation or heat radiation can be used. The arm or leg can be used as location of exposure.

Heat stimulation: Lasers

PURPOSE AND RATIONALE

Laser stimulation (LS) uses the energy to heat up the epidermis and parts of the dermis with very brief (range of ms) and powerful (8-200 mJ/mm^2) stimuli directed at the skin.[144] This type of stimulation causes a characteristic double pain sensation, consisting of an initial sharp pinpricking-like pain (Aδ-fibers) and a second longer burning pain (C-fibers).[145,146] Most commonly used laser stimulators are based on CO2, Argon and the YAG (yttrium-aluminium-garnet).

PROCEDURE AND EVALUATION

LS can stimulate the skin in a well reproducible manner making it useful as a tool to elicit evoked potentials. Evoked potentials via EEG can easily be registered due to the brief nature of the stimulus of which the timing can be controlled as well. EEG has been used in combination with laser stimulation to distinguish between Aδ- and C-nociceptive activities.[125]

CRITICAL ASSESSMENT OF THE METHOD

Importantly, during LS no (Aβ-fibers) tactile mechanoreceptors are activated, making LS a useful tool for investigating the nociceptive system without the interference of non-nociceptive input. However, due to the fast rise in temperature of the skin, overstimulation may cause nociceptors to become fatigued over repetitive stimuli.[77] This in turn has an unwanted effect on quantification of the nociceptive system, as laser evoked potential habituation may occur.[77,182] It is therefore advised to vary the stimulation location slightly after each stimulus and use a randomized inter-stimulus interval. Additionally to habituation, precise settings are necessary to prevent damage to the skin. Hence, power, duration and surface area must be properly set up.

MODIFICATIONS OF THE METHOD

LS stimulates both Aδ- and C-fibers by thermal activation. However, adjusting the stimulation method may shift the preferential activation of either nociceptor. Preferential C-fiber stimulation is based on a characteristic difference in heat threshold (Aδ-fiber ± 46°C, C-fiber ± 40°C) and distribution density in the upper skin.[132] Shifting between Aδ- and C-fiber activation using LS is possible by choosing the right pulse width, stimulation area in combination with keeping track of the skin temperature and reaction time.

Thermal muscle stimulation

Heated saline

PURPOSE AND RATIONALE

Thermosensitive receptors located on muscle tissue afferents are thought to be involved in thermoregulation.[74] As such, these have been identified as potential targets to investigate nociception of deep muscle tissue. This is investigated by exposing muscular tissue to a high intensity thermal stimulation.[62] Only a single study was found using this method.

PROCEDURE AND EVALUATION

An intramuscular injection of sterile isotonic (1.5 mL) heated saline is injected over 20 seconds (270 mL/hr) into the musculus tibialis anterior. Hyperalgesia can quantified using a thermal and mechanical stimulus.

CRITICAL ASSESSMENT OF THE METHOD

Compared to hypertonic saline in the same study, peak pain score resulting from intramuscular injection of isotonic saline at different temperatures was significantly lower. Mechanical sensitization appeared to be largest after injection at the highest temperature (48°C). To avoid cutaneous sensations, the injection site was anaesthetized with intradermal injections of 0.2 mL lidocaine before the intramuscular injection.[62]

MODIFICATIONS OF THE METHOD

In the single study using this method, intramuscular injections of isotonic saline at different temperatures were investigated, ranging from 8°C to 48°C. Different muscles can be used for injection.

Thermal visceral stimulation

Oesophageal

PURPOSE AND RATIONALE

Thermal stimulation of the GI tract activates specific nociceptive afferents selectively through TRPV1. This in contrast to mechanical and electrical stimulation, which activate afferents both superficial and deeper in the layers of the viscera.[164] This makes thermal stimulation of the GI tract a useful technique for specific activation of non-myelinated afferents in the mucosa.

PROCEDURE AND EVALUATION

In several studies, a model was used to thermally stimulate the oesophagus.[13,41,43,97] In these experiments thermal stimuli were performed by changing the temperature (5-60°C) of re-circulating water in a bag that was placed in the lower part of the oesophagus. Temperatures were continuously measured inside the bag to control the thermal stimulation in the oesophagus. Both for cold as for heat pain, a linear stimulus-response (°C-VAS) can be observed.

CRITICAL ASSESSMENT OF THE METHOD

The upper GI tract (oesophagus) is able to differentiate between thermal stimuli in the temperature range that can be used without chronic damage. Quantification of the visceral pain is more difficult to distinguish.

MODIFICATIONS OF THE METHOD

It has been demonstrated that fast increases in temperature (1.5°C/min) affect the precision of the response.[136] Therefore, the experimental oesophageal model can be modified by using slower temperature increases to ascertain better results in pain assessments.

Electrical Stimulation

Electrical stimulation is used extensively for testing the sensitivity of the pain system in studies activating cutaneous structures, muscular structures, and in visceral structures.[4,8,102] Electrical stimulation initiates activity in nerve fibers directly without activating receptors. The stimulus intensity determines the size of the current field in the tissue and thereby the number of fibers activated.[5] In case a rectangular pulse is applied to the skin, thick fibers mediating mechanoreceptive input are activated at the lowest stimulus intensities. Increasing. Increasing the stimulus intensity leads to concurrent activation of thin myelinated fibers (Aδ-fibers), and eventually C-fibers.

Electrical skin stimulation

Stimulation can be done cutaneous or intracutaneous with various stimulation paradigms with diverse waveforms, frequencies, and durations are used to selectively activate different afferents and nervous structures and thereby evoke various pain sensations. In addition, summated neural activity, as a result of the stimuli, can activate central mechanisms,[94] which is described further in paragraph “Electrical Single Stimulation”.

Electrical single stimulation

PURPOSE AND RATIONALE

This electrical stimulation paradigm leads to a nociceptive, Aδ- and C-fiber mediated type of pain, which is well controllable. The electrical current stimulates nerve fibers directly because the intensity is far below that required to stimulate the actual receptors in the skin.[40]

PROCEDURE AND EVALUATION

For cutaneous electrical pain, two electrodes (Ag/AgCl) are placed on clean (scrubbed) skin, *e.g.*, the skin overlying the tibial bone. Electrical resistance between electrodes should be less than 2 kΩ. Each stimulus (10 Hz tetanic pulse with a duration of 0.2 ms) is controlled by a computer-controlled constant current stimulator. Current intensity increases from 0 mA in steps of 0.5 mA/sec (cutoff 50 mA). The pain intensity after each stimulation is measured using an (electronic) VAS, until pain tolerance level is reached or a maximum of 50 mA is reached.[138]

CRITICAL ASSESSMENT OF THE METHOD

Electrical stimulation is easily controlled. Electrical stimulation of the skin to induce pain has several shortcomings: (1) they excite the afferent pathways in an unnatural synchronized manner; (2) they excite the full spectrum of peripheral nerve fibers (Aβ-, Aδ- and C-fibers); and (3) stimulation bypasses the receptors on the sensory nerve endings, and therefore, all information on specific activation and transduction processes is lost.[67]

MODIFICATIONS OF THE METHOD

Aδ- and C-fibers are activated at different stimulus intensities where C-fibers have a higher activation threshold. Modeling approach can be useful for certain drug trials.[67,105] The non-specificity towards nociceptive specific stimulation is thought to be overcome by using small specialized needle-like electrodes. These electrodes slightly protrude through the epidermis and can preferentially stimulate nociceptive Aδ-fibers.[28,82,127] Intracutaneous stimulation can be chosen to mimic more a stinging/burning sensation and less throbbing.[29]

Electrical burst (temporal summation)

PURPOSE AND RATIONALE

Increasing pain in response to a series of stimuli (temporal summation) reflects the first phase of "wind-up" in animal studies. Temporal summation can be induced with mechanical, thermal and electrical stimulation.[9,58,112,129] Temporal and spatial summation evoked in the skin reflects a central nervous system modulation of the response and it is believed to mimic neuropathic pain conditions because a likely contribution of central sensitization to neuropathic pain has been demonstrated.[191] Application of transcutaneous electrical stimuli, with variation in electrical burst frequency, has been shown to be a reliable model to induce temporal summation in human subjects.[9]

PROCEDURE AND EVALUATION

For burst stimulus, each single stimulus is repeated 5 times with a frequency of 2 Hz. Pain threshold is taken as the value (mA) whereby a subject indicates either: all 5 stimuli are painful, or the train of 5 stimuli started feeling non-painful but ends feeling painful (VAS > 0).[9,71]

CRITICAL ASSESSMENT OF THE METHOD

As facilitated temporal summation is a feature in neuropathic pain patients, it has been hypothesized that induction of temporal summation using electrical stimulation can be used as a biomarker of drug effects on neuropathic pain.[11] In a recent study, drug effects of analgesic compounds, including several used in the treatment of neuropathic pain, could not be established using this evoked pain paradigm, while other evoked pain paradigms manage to demonstrate pharmacological effects convincingly. This appeared to be related to a higher intra-subject variability that may necessitate larger subject groups.[135]

High-frequency electrical stimulation

PURPOSE AND RATIONALE

High-frequency electrical stimulation (HFS) of the human skin induces increased pain sensitivity in the surrounding unconditioned skin.[185] It has been shown that sustained nociceptive input can induce activity-dependent changes in synaptic strength within nociceptive pathways, leading to an amplification of nociceptive signals.[78] This is thought to play a key role in the development and maintenance of chronic pain and in particular some forms of hyperalgesia.[101,158] HFS-induced hyperalgesia within the surrounding unconditioned skin mimics the phenomenon of secondary hyperalgesia.[118] As such, it constitutes a suitable model to study the mechanisms underlying central sensitization of nociceptive pathways.[92]

PROCEDURE AND EVALUATION

HFS is delivered to the test site, *e.g.*, the volar forearm, and consists of 5 trains of 100 Hz pulses lasting 1 second, (10-s interstimulus interval; 2-ms single pulse duration) at 10 times the detection threshold.[143] The electrical stimulation is generated by a constant-current electrical stimulator and delivered to the skin using a specifically designed electrode that has been demonstrated to activate peptidergic nociceptive afferents in the skin.[91] The heterotopical effect of HFS is usually characterized using mechanical punctate stimuli. The test stimuli are applied to the skin surrounding the area onto which HFS is applied as well as to the same skin area on the contralateral arm, which serves as control to take into account a possible time-dependent habituation.[185]

The intensity of perception elicited by the three types of test stimuli is assessed using a numerical rating scale (NRS). After approximately 1 hour, the level of heterotopical hyperalgesia starts to diminish, however is still measurable and significant from baseline up to 8 hours after HFS.[143]

CRITICAL ASSESSMENT OF THE METHOD

HFS offers an Alternative to other models that lead to secondary hyperalgesia, such as the capsaicin model or the UVB model with some important advantages. The major advantage versus the UVB model is that the mechanism underlying the secondary hyperalgesia is thought to involve heterosynaptic facilitation and, hence, to constitute a suitable model of central sensitization of nociceptive pathways,[92] while the secondary hyperalgesia in the UVB model is thought to be due to a more peripheral sensitization of nociceptors, induced by inflammation.[21] The

interval during which the secondary hyperalgesia is measurable can be carefully regulated and is relatively stable over the first hour after application of HFS.[143] The major disadvantage of this method is that is has not been used to demonstrate pharmacodynamic effects of analgesic drugs, Although one recent study did show that the model can be combined with the assessment of drug effects.[187]

MODIFICATIONS OF THE METHOD

Modifications of the method are primarily related to the type of sensory stimulus to determine the heterotopical hyperalgesic effect and to the quantification. Heterotopical hyperalgesia can be demonstrated for mechanical punctate stimuli, but also for thermonociceptive stimuli induced by heat probes or laser stimulation. Van den Broeke et al. used the model in conjunction to event related potentials to objectively demonstrate the hyperalgesic phenomena.[185]

Electrical muscle stimulation

PURPOSE AND RATIONALE

Electrical stimulation of muscle tissue can be used to elicit both local and referred muscle pain. It possesses the ability to generate referred muscle pain in an 'on and off' manner, and it is capable of maintaining referred pain for at least 10 minutes.[102] Intramuscular electrical stimulation appears to be used more often to study the nature of muscle pain than as a model to determine the pharmacodynamic effects of new analgesic compounds.

PROCEDURE AND EVALUATION

In the intramuscular electrical stimulation paradigm two needle electrodes with uninsulated tips are inserted into a muscle (*e.g.*, the musculus tibialis anterior). A computer-controlled constant current stimulator is used to induce referred pain in the ventral part of the ankle by stimulating the muscle.[102] Each stimulation consists of five constant current rectangular pulses (1 ms) delivered at 200 Hz. The referred pain threshold is defined as the lowest stimulus intensity required for the subject to notice a 'just barely painful' sensation in the referred pain area. Referred pain thresholds are determined by a staircase regime consisting of five ascending and four descending series of stimuli.[56,102]

CRITICAL ASSESSMENT OF THE METHOD

Electrical muscle and skin stimulation can use the same modalities which makes it possible to compare both models. A disadvantage of the model is that referred pain due to intramuscular electrical stimulation does not occur in all subjects; approximately three quarters of patients experience it.[102] The referred pain typically arises approximately 40 seconds after the onset of electrical stimulation, which may mean that temporal summation is involved.[102]

MODIFICATIONS OF THE METHOD

Modifications can be made with the stimulation settings. Pulse range of 100-200 Hz have been described, as well as a pulse width of 1-2 seconds.[102,185]

Electrical visceral stimulation

In the viscera it is difficult to determine the pain threshold to a single stimulus, whereas the pain threshold is easily determined if a train of stimuli is used. Furthermore, the referred pain area gradually expands if stimulation is continued for 120 seconds.[8]

Chemical stimulation

Administration of algonenic substances to the skin, muscle or viscera is believed to be a close resemblance of clinical inflammation. Various substances have been used to induce cutaneous hyperalgesia. The most commonly used are capsaicin, Nerve Growth Factor (NGF), glutamate, mustard oil and menthol, but other chemical stimulation models exist as well. Intramuscular injection of chemical substances is less common and harder to control in a clinical trial. The oesophagus is the target organ when it comes to chemical viscera stimulation because of its easy access.

Chemical skin stimulation

Capsaicin

PURPOSE AND RATIONALE

Capsaicin is a highly selective agonist for TRPV1, notorious for its pungent property in red chili peppers. TRPV1 channels are major transducers of physically and chemically evoked sensations.[70] The vanilloid 1 subtype is activated by noxious heat (≥ 43°C)[51] and is expressed on C-fibers, and on a subset of Aδ-fibers.[104] The direct effects of applying topical capsaicin are burning sensations, hyperalgesia, allodynia and erythema. In addition, it triggers the release of pro-inflammatory agents at peripheral terminals, such as substance P and calcitonin gene-related peptide (CGRP).[86,194]

PROCEDURE AND EVALUATION

Capsaicin can be administered topically and intradermal. Intradermal injection with capsaicin 0.1 mg can cause hyperalgesia, but a dose of 100 mg or higher is needed to produce hyperalgesia for an hour.[167] A dose of 100 mg is most frequently used.[14,165,180]

Topical administration of capsaicin in low concentrations (up to 3%) can cause temporary mechanical and heat hyperalgesia. Sensitization can be induced by preheating the skin to 45°C for 5 minutes with a thermode directly before capsaicin application. Sensitization can be rekindled throughout a study by reheating the skin up to 40°C for 5 minutes. Application of the capsaicin is most commonly done on the forearm or the back, but can be done on any area of the skin. Topical application of capsaicin can induce peripheral and central sensitization shown respectively by primary mechanical/thermal hyperalgesia and by secondary mechanical hyperalgesia/allodynia. This pain model can therefore be used to study novel analgesic compounds targeting these typical symptoms of neuropathic pain. Peripheral sensitization is caused by modulation of peripheral afferents and is therefore restricted to the site of injury, *i.e.*, primary hyperalgesia. Central sensitization is caused by modulation of the nociceptive processing in the central nervous system. To quantify the effects of this pain model, laser stimulation can be used in combination with electro-encephalogram.

CRITICAL ASSESSMENT OF THE METHOD

Peripheral sensitization is closely linked to primary hyperalgesia and central sensitization is partly explained by hyperalgesia in the surrounding area, *i.e.*, secondary hyperalgesia. Moreover, nociceptive integration at spinal cord level may include non-nociceptive mechanoreceptors. Therefore, central sensitization may also cause Aβ-fiber mediated pain (allodynia). Higher concentrations (capsaicin 8%) initially causes increased sensitivity but is then followed by a decrease in sensitivity due to a reduced TRPV1 expression.[117,184] High concentration capsaicin is indicated in postherpetic neuralgia. Besides, capsaicin may also have a neurolytic property, where it (partly) eliminates epidermal nerve fibers (ENFs) in treated areas over time.[45] Re-innervation occurs over time.[77]

MODIFICATIONS OF THE METHOD

There are several variations that need to be addressed when designing a study utilizing capsaicin, *e.g.*, concentration of the capsaicin, dose administration (intradermal or topical), vehicle of the capsaicin (alcohol or cream), duration of the application, location of administration and pre-/rekindling.

Nerve growth factor injection

PURPOSE AND RATIONALE

NGF is a member of the neurotrophin family, which also includes brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT3) and neurotrophin-4/5 (NT4/5). NGF binds to both a high affinity tyrosine kinase receptor TrkA and a low affinity receptor p75. NGF can sensitize nociceptors so that they show an increased response to thermal and chemical stimuli.[17] Administration of NGF to human skin evokes mechanical sensitization and profound hyperalgesia to thermal stimuli that develops within 3 hours post injection and peaks between day 1 and 7.[46] Sensitization to heat and hyperalgesia to cold develops within days after injection and lasts up to 21 days, while hypersensitivity to mechanical impact stimuli develops over a longer period and persists for at least 49 days.[156] Intradermal NGF administration provokes a pattern of sensitization that can be used as experimental model for neuropathic pain.[156]

PROCEDURE AND EVALUATION

One microgram of human recombinant lyophilized NGF is dissolved in 50 µL saline and injected intradermally into the central volar forearm. The same volume of saline is administered into the contra-lateral site as vehicle control.[156] Vasodilatation upon NGF- and saline-injection can be recorded by laser Doppler imaging. Nociceptor sensitization can be explored to mechanical (touch, pinprick, pressure), thermal (cold, heat), and electrical (current pulses) stimuli. Stimuli for investigating static and dynamic allodynia and pinprick hyperalgesia are administered 5-7 cm distal from the injection site and continued in steps of 1 cm until the subject reports a definite increase of pinprick pain or switch from touch to an aversive sensation.[156] The point where this starts is marked on the skin and the distance to the injection site measured. Pain thresholds and subjective scores with NRS/VAS can be used to evaluate the mechanical, thermal or electrical stimulation.

CRITICAL ASSESSMENT OF THE METHOD

Increased levels of NGF have been reported in human painful disorders including arthritis.[177] Injection of NGF therefore appears to mimic processes found in clinical disease.[137] Even though NGF may also be upregulated in the UVB burn, anti-NGF has been shown to only partially reduce UVB induced hyperalgesia.[20] Apparently, the NGF induced mechanism of mechanical sensitization is different to UVB evoked primary hyperalgesia. NGF induces a particularly long lasting

mechanical sensitization including static allodynia and cold hyperalgesia without any visible signs of inflammation and therefore adds to the spectrum of human evoked pain models.[156] The long lasting local allodynia and hyperalgesia after subcutaneous or intradermal injection, up to 49 days after injection, form the most important disadvantage of the model. Even though considered a model for neuropathic pain, it is unlikely that central sensitization plays a role.

MODIFICATIONS OF THE METHOD

Systemic administration of NGF 1µg/kg I.V. has been shown to lead to mild to moderate muscle pain mainly in the bulbar and truncal musculature that lasted 2-8 days.[141]

NGF has been injected into the musculus masseter to induce allodynia and hyperalgesia and as a model of myofascial temporomandibular disorder pain.[177]

Other chemical mediated models

MUSTARD OIL

Mustard oil is a plant-derived irritant. The noxious effects of mustard oil are currently ascribed to specific activation of the cation channel transient receptor potential, subfamily A, member 1 (TRPA1) in nociceptive neurons.[137] Topical administration leads to a burning pain in the area exposed to mustard oil as well as secondary allodynia and hyperalgesia in the surrounding unaffected area, similar to the topical capsaicin model.[93]

MENTHOL

Menthol acts as an agonist on the transient receptor potential cation channel subfamily M member 8 (TRPM8) receptor. The topical application of high concentration (40%) menthol, is thought to activate and sensitize cold-sensitive TRPM8-expressing C-nociceptors and activates cold-specific Aδ-fibers.[19] Topical application has been used as an evoked pain model of cold hyperalgesia, which is a clinical symptom that occurs frequently in patients with peripheral or central nervous system lesions.[69] In addition to cold hyperalgesia, the model elicits primary and secondary mechanical (pinprick) hyperalgesia combined with the sensation of burning.[19] The menthol model has been shown to be sensitive to a range of analgesics.[3]

Chemical muscle stimulation

Nerve growth factor

Intramuscular injection with NGF is most commonly done in the musculus tibialis anterior or musculus masseter.[6,178] It induced a long-lasting hyperalgesia and lower pressure pain threshold can be observed, lasting up to 4 days in the musculus tibialis anterior and up to 14 days in the musculus masseter.[6,178] An advantage of the intramuscular NGF paradigm is the long-lasting hyperalgesia which can simulate clinical pain more than most other paradigms, but this is also the disadvantage where ethical consideration may play a role. The paradigm is difficult to control where hyperalgesia is dependent on the dose and the size of the muscle.[6]

Chemical visceral stimulation

Oesophagal (gut) perfusion with acid, alcohol, glycerol, capsaicin and hypertonic saline

PURPOSE AND RATIONALE

Chemical stimulation of the GI tract may be used to stimulate C-fibers selectively via TRPV1 receptors and modulate the visceral pain system due to their sensitization effects. Having a model of central sensitization of the viscera can be helpful in the development of new analgesics, as this is thought to be an important element of chronic visceral pain.

PROCEDURE AND EVALUATION

Using acid to stimulate the oesophagus is the most used method to sensitize the gut[18,37,44,152] However, other chemicals such as alcohol, glycerol, capsaicin and hypertonic saline are used to stimulate the gut as well.[42,43,109] The chemical compound is usually infused into a container/bag residing in the oesophagus with a small perfusion hole to release the compound into the oesophagus. Chemical stimulation is able to modulate the visceral pain system by selectively activating non-myelinated C-fibers for a longer amount of time. This tonic activation may result in central sensitization effects, which can be quantified by subsequent thermal, electrical or mechanical stimulation.

CRITICAL ASSESSMENT OF THE METHOD

A high variation in the outcome measures is seen with this model. The reproducibility is challenging because several factors are hard to control, like exposure time to the chemical stimulus, size of the treated area and latency time to onset of effects. Furthermore, tissue injury results in the release of multiple molecules working together, and to mimic this situation it may be necessary to use a mixture of chemical substances.[109] Blinding this procedure is difficult, since subjects are able to taste the compound. Therefore, the experimental setup requires that both subject and assessor are ignorant of the possible influence of the compound on the pain threshold.[43]

MODIFICATIONS OF THE METHOD

Each chemical substance will have an impact on the results. For example, the motility may interfere with the results when glycerol is used. Other stimuli, such as injection of hypertonic saline and application of capsaicin, the pain is elicited shortly after the chemical comes into contact with the mucosa, and the motility has minor impact on the results.

DISCUSSION

Healthy subjects versus patients

Despite many advances in the last decades in understanding pain, the development of new analgesic compounds lacked behind. In almost 60 years, only 59 compounds were registered for the treatment of pain, of which two-thirds were specifically developed as analgesics.[90] Historically, pain states have been classified and investigated on the basis of a disease state. Based on preclinical animal models, target patient populations were selected. In patient studies, efficacy is then reported as change in the patient's response to pain.[113] Unfortunately, several promising compounds have failed in this late-stage development where pharmacotherapy only provides meaningful pain relief in less than 50% of patients with neuropathic pain.[48,49] But a negative outcome does not automatically mean inefficacy of the compound. Pathophysiological mechanisms of pain vary between individuals with the disease state. Selecting and clustering the patients in groups of pathophysiology rather than disease might be necessary to obtain meaningful results. The use of human evoked pain models can provide more information.

Multi-modal testing in healthy volunteers can provide information about the analgesic activity of the compound and possibly find the active dose level range. In a way, by using different pain modalities, the results will create a certain pain profile of the compounds.[135] These results may reflect effects of analgesic drugs on mechanisms involved in clinical pain. Thus, multi-modal pain testing may aid in determining the optimal target population for new analgesic compounds based on their profile of effects on a diversity of pain mechanisms and depending on the contribution of each of these mechanisms in clinical pain phenotypes. In several chronic pain populations, such as chronic whiplash, rheumatoid arthritis, vulvodynia and fibromyalgia, changes in pain tolerance levels, pain modulation and augmented brain responses and altered responses to analgesics have been found.[36,66,186] Using evoked pain in these patients can provide insight into the analgesic mechanisms -or lack thereof- in these altered pain states.[137] In patients with chronic (neuropathic) pain, different sensory profiles exist. These profiles possibly match with different neurobiological mechanism of pain.[15]

Predictive value of models for drug development

Human evoked pain models in healthy volunteers can be conducted in standardized laboratories. Factors like stimulus intensity, frequency, duration and location can be controlled and when a model is stable and reproducible, it can be regarded as suitable for pharmacodynamic evaluation of new analgesic drugs. Using pain models in healthy volunteers has important advantages over assessing the effects of new drugs in patients with pain; the pain elicited in human pain models is predictable in its intensity while clinical pain will naturally fluctuate and in pain models analgesic properties can be investigated without the influence of accompanying symptoms that are often seen in patients with pain. However, it should always be asked whether a pain model at all resembles naturally occurring pain. Clinical pain is a subjective perception, influenced by cognitive processes, by emotions, social context and even cultural background, while pain models are solely based on the infliction of a noxious stimulus and its response. An important question is whether or not a positive result in a certain evoked model is also predictive of clinical efficacy.

Two approaches have been used to investigate this. Moore and colleagues investigated which naturally occurring pain was physiologically most in agreement with evoking a pain response causing the same type of pain. For instance, they concluded that intramuscular electrical stimulation closely matched clinical acute musculoskeletal pain.[122] Oertel and Lötsch evaluated the differences between human pain models and clinical efficacy. First they looked at which drugs were effective in different pain conditions (*e.g.*, NSAIDs were effective for inflammatory

arthritis), then they investigated which drugs were effective in which pain model (*e.g.*, NSAIDs influence pain response in laser evoked pain). If a certain drug was effective both in the model and in the particular clinical setting, the model was concluded to possibly be predictive for the type of clinical pain. Some level of agreement could be observed for a large number of pain models with many different clinical forms of pain.[133] In another review, the mutual agreement between pain models and clinical efficacy was statistically assessed. It was observed that a small set of pain models seemed predictive for efficacy in the clinic, for example capsaicin induced hyperalgesia with mechanical stimulation is associated with trigeminal neuralgia and renal colic, and UVB induced hyperalgesia in combination with heat stimulation can be linked to burn injuries or postoperative pain.[108]

Several reviews investigated which evoked pain models were sensitive to the analgesic effects of different classes of analgesics in healthy subjects.[133,135,173,174] With the aid of these studies, well-considered decisions can be made on which evoked pain models to include in studies investigating potentially analgesic compounds.

Multi-model assessment of pain

Pain comes in various types and can originate in many different tissues. It is obvious that different analgesics will influence different types of pain according to their respective mechanism of action. If an analgesic drug with a novel mechanism of action is studied, it can occur that a single pain model, thought to relate to a specific clinical pain syndrome, demonstrates lack of efficacy of the new compound. In these cases, a combination of human evoked pain models can be used to screen for possible analgesic effects of these compounds. For instance, a combination of a mechanical, thermal and electrical pain models: pressure stimulation assesses the nociception generated from within the muscle,[147] cold pain induced by the cold pressor test mainly activates C-fibers in the skin,[137] heat stimulation initially activates A-fibers in the skin, followed by C-fiber activation, induction of inflammation via sunburn or UVB, induces the production of cytokines that lead to sensitisation of cutaneous nociceptors,[21] and electrical stimulation directly stimulates sensory nerve endings of both Ad and C-fibers in the skin.[67] This multimodal testing with a battery of different pain models has been performed by multiple study groups.[135,136,172] The batteries have in common that they induce pain via different modalities and in different tissues and mimics clinical pain better than a single pain model can. The multimodal batteries can be used to profile the analgesic effects of new drugs, to obtain the optimal dose of new analgesics and to benchmark new drugs against profiles of well-known analgesics.[135]

This thesis is composed of two sections. The first section focusses on the efficacy of different (novel) analgesics using a battery of human evoked pain models (PainCart). Validation or improvement of human evoked pain models are discussed in section 2.

Section 1

THE EFFICACY OF DIFFERENT (NOVEL) ANALGESICS BY USING THE PAINCART

Chapter 2
An overview of the performance of two dose levels of a novel α2/α3/α5 $GABA_A$ subunit selective partial positive allosteric modulator, PF-06372865, was summarized after utilizing the PainCart. PF-06372865 has the potential to provide analgesia but with less sedation or cognitive effects than non-selective benzodiazepines.

Chapter 3
PF-06273340 is a small molecule inhibitor of Trks A, B and C that reduced chronic pain in nonclinical models where there has been some sensitization, such as ultraviolet B. The trial aimed to investigate the pharmacodynamics of this first-in-class molecule in healthy subjects to translate nonclinical effects in to men. Pharmacodynamic assessment was done with the PainCart.

Chapter 4
PF-05089771 is a potent selective $Na_V1.7$ sodium channel blocker, developed for the treatment of acute and chronic pain. The study was performed to demonstrate analgesic properties of PF-05089771, alone and in conjunction with pregabalin, using the PainCart on healthy subjects.

Section 2

VALIDATION AND IMPROVEMENT OF HUMAN EVOKED PAIN MODELS

Chapter 5
The reproducibility of the PainCart was measured by demonstrating the variability of the analgesic effects of ibuprofen and pregabalin. Results were extracted from three (ibuprofen) or four (pregabalin) studies previously completed with the PainCart.

Chapter 6

A better understanding of the analgesic profile of existing analgesics by using the pain models of the PainCart can help in a more detailed prediction of nociceptive activity of a new analgesic compound. Chapter 6 describes the pharmacodynamic results of a classic (paracetamol) and a non-classic (Δ9-THC) analgesic compound to expand knowledge of different classes being tested with the PainCart.

Chapter 7

Unexpected long-term side effects of the 3x minimal erythema dose (MED) UVB pain model were the base for a retrospective evaluation of this model. Postinflammatory hyperpigmentation (PIH) is a usually harmless condition but has impact on the subject's self-consciousness, social well-being and personal relationships. Based on finding from this study a second study was conducted to improve the UVB model to a 2x MED model with minimal risk to develop PIH.

Chapter 8

Chapter 8 describes the results of the capsaicin induced hyperalgesia model. Laser evoked potentials were included to obtain an objective outcome parameter. The first part of the study was to demonstrate the validity of the model. Analgesic properties of tramadol and duloxetine were evaluated in the second part. This was done in a multimodal test setting where the capsaicin induced hyperalgesia model was incorporated in to the PainCart.

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