Development and validation of a human bio-marker for cutaneous inflammatory pain (the UVB-model)

- a methodological study in healthy volunteers

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TITLE:
Development and validation of a human bio-marker for cutaneous inflammatory pain (the UVB-model) - a methodological study in healthy volunteers

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Background: In the early clinical trials, human experimental pain models are important tools for defining the analgesic efficacy of drugs. The Ultraviolet-B (UVB) pain model is used for the induction of a local cutaneous inflammation in a circumscribed skin area with primary hyperalgesia (increased sensitivity to painful stimuli) and possible area of secondary hyperalgesia (sAREA).

Aim: To investigate the potential effect of the UVB-irradiation in this pain model and the reliability of this model by detecting the inter- and intra- individual variations in this model and to produce sample size estimates for a parallel and a cross over study design.

Methods: Inflammatory hyperalgesia was induced on the upper arms of 15 healthy male volunteers by irradiating a circular spot (5 cm diameter) with three times minimal erythema dose (MED) of the UVB-irradiation. The inflammatory response assessed by measures of erythema, superficial blood flow and skin temperature. The sensory tests including the brush induced allodynia, von Frey and pinprick stimuli, pressure pain threshold (PPT) and heat pain threshold (HPT), all measurements were performed at baseline, 24h, 48h and 72h after the irradiation, the whole procedure was repeated on the opposite arm with a two week's interval. sAREA was assessed to both von Frey filament and pinprick after the UVB-exposure.

Results: A significant increase in the erythema, mean blood flow (BF) and skin temperature was detected after the irradiation (P <0.001 in all three cases), but no changes in the BF in the sAREA was detected (P=0.664). Brush induced allodynia was detected 48h after the irradiation (P=0.02). No significant increase was detected in primary hyperalgesia to von Frey filament (P=0.124). A significant increase in the pinprick induced primary hyperalgesia was detected (P<0.001) and a significant difference between the pin sizes (P<0.001). Both heat pain threshold (HPT) and pressure pain threshold (PPT) significantly decreased after the irradiation (P<0.001 in both cases), but the PPT decreased differently in both arms (P<0.001).

Conclusion: The present study concluded that the UVB-pain model was successful in inducing a local cutaneous inflammation in a circumscribed skin area, as both alteration in the BF and visually erythema was noticed. Furthermore, significantly increase in both skin temperature and erythema were detected.

For detection of mechanical induced primary hyperalgesia the present study concluded that the pinprick was the most suitable test in comparison to von Frey filaments.
Detection of the heat pain threshold was found to be a highly reliable test in the UVB-pain model. PPT only seemed to be reliable on the dominant side. Area of secondary hyperalgesai was detected to both von Frey filament and pinprick stimuli and both test seemed to be reliable, the issues of which test should be preferred need to be addressed further.

Baggrund: Humane eksperimentelle smerte modeller er et vigtigt redskab i den tidlige kliniske udvikling hvor de smertestillende medikamenter efficacy bestemmes. Ultraviolet-B smerte modellen anvendes til fremkaldelse af en lokal kutan inflammation med primær hyperalgesi (øget sensibilisering overfor smertefulde stimuli)i et afgrænset område i huden. UVB- bestråling kan muligvis også fremkalde et areal i huden, omkring det bestrålede område, med sekundær hyperalgesi (sAREA).

Formål: At undersøge de potentielle effekter UVB- bestråling medfører i denne smertemodel. At undersøge UVB-modellens reliabilitet ved at finde den inter- og intra- individelle variation for denne model. At udforme en tabel med sample size beregninger for både et parallelt og et Cross over studie design.

Metoder: Inflammatoriske hyperalgesi fremkaldes på overarmen af 15 raske mandlige forsøgsdeltagere, et cirkulært areal (5 cm diameter) bestråles med tre gange den minimal erythma dose (MED). Det inflammatoriske respons bedømmes ved måling af erythema, den overfladiske blodgennemstrømning og temperaturen i huden. De sensoriske test inkluderede børste induceret allodyni, stimulering med von Frey filament og pinprick, smertetærsklen for tryk og smertetærsklen for varme. Alle målinger blev fortaget før bestrålingen (baseline), 24t, 48t og 72t efter bestrålingen, proceduren blev gentaget på den modsatte arm med et to ugers interval. Efter UVB-bestrålingen blev sAREA bestemt med von Frey filament og pinprick stimuli.

Resultater: En signifikant stigning blev fundet for erythema, den gennemsnitlige overfladiske blodgennemstrømning og hud temperaturen (P <0,001 for alle), der var ingen signifikant ændring i den overfladiske blodgennemstrømning i det sAREA (P=0,664). Børste induceret allodyni blev fundet 48t efter bestrålingen (P=0,02). Der var ingen signifikant stigning i den primære hyperalgesi for von Frey filament eller pinprick (P=0,124). En signifikant stigning blev fundet for pinprick induceret primær hyperalgesi (P=0.001) og der var en signifikant forskel mellem størrelsen på pinen (P<0,001). Smertetærsklerne for både tryk (PPT) og varme (HPT) var signifikant sænket efter UVB-bestrålingen (P<0.001 for begge), men PPT faldt ikke med samme grad på begge arme (P<0,001).

Konklusion: Dette studie konkluderede at UVB-smerte modellen var succesfuldt i at fremkalde en lokal kutan inflammatorisk reaktion i et afgrænset område i huden, da både ændringer i den overfladiske blodgennemstrømning og synligt erythema blev observeret. Ydermere blev en signifikant stigning i både temperatur og erythema også fundet. Dette studie konkluderede at pinprick stimuli var den bedste metode til fremkaldelse af primær hyperalgesi, i forholde til von Frey filamenter. Påvisningen af smertetærsklen for varmepåvirkning ved UVB stimulering blev vurderet til a at være en test med høj pålidelighed. Påvisningen af smertetærsklen for tryk påvirkning ved UVB stimulering var kun pålidelig på den dominante side. Arealet for sekundær hyperalgesi blev påvist ved både von Frey filament og pinprick stimulering, begge test syntes at være pålidelige, men problematikker om hvorvidt hvilken test bør foretrækkes kræver yderligere udredninger.

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Preface

This project is a master project generated in the period September 2010 - May 2011 by the students of “Medicine with Industrial Specialization”. The project is experiment-based, where a study was performed on the human experimental pain model “the UVB-pain model”. The project contains a study protocol, a case report form (CRF), approvals from the Regional Ethic Committee and the Danish Data Protection Agency, a scientific article base on the study and a standard operational procedure (SOP) for the UVB-pain model.

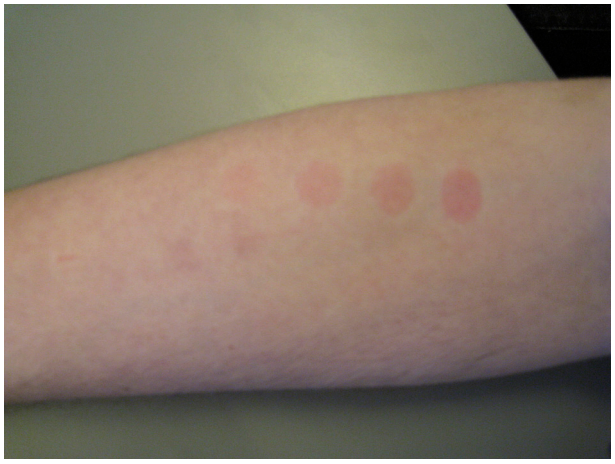
Introduction

The UVB-pain model

The Ultraviolet-B (UVB) pain model is a human experimental model. In this model UVB-irradiation is applied for induction of a local cutaneous inflammation in a circumscribed skin area with primary hyperalgesia (increased sensitivity to painful stimuli) to mechanical and thermal stimuli [1-5]. The UVB-model is a valid human inflammatory pain model of primary hyperalgesia and could serve as an early efficacy indicator in humans [2-4].

The UVB-pain model can also be applied on rats or mice. It induces thermal and mechanical hyperalgesia in both humans and rats. This cutaneous inflammatory pain model is emerging as a translational model of experimental hyperalgesia associated with a peak 24-48 h after UVB-exposure without spontaneous pain and invasive processes. This pain model is one in a few, which is characterized as a translational experimental inflammatory pain model [5-8].

1



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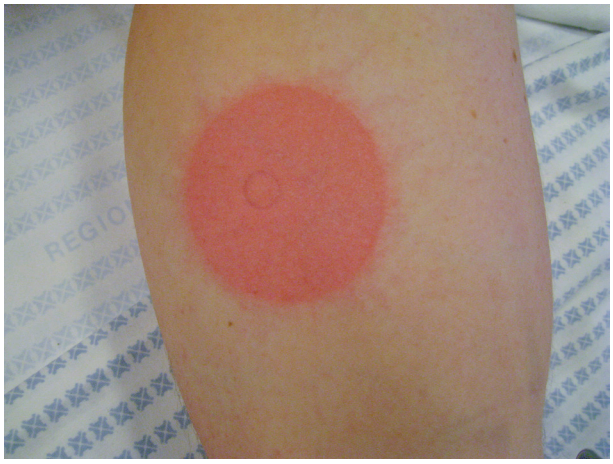


Picture 1 and 2
Picture 1 is a forearm irradiated with increasing doses of UVB for determination of MED
Picture 2 is an upper arm irradiated with 3xMED.

Pain models in general

Human experimental models of pain and hyperalgesia serve as an important tool for defining the analgesic efficacy of drugs in early clinical trials. A standardized experimental approach is an advantage in the early screening of new analgesic drugs, and pain models are required for the development of new anti-hyperalgesic drugs [2, 3]. The lack of constant conditions over a long period is an unsolved problem of most of the established pain models. Factors such as localization, intensity, frequency and duration of the pain stimuli must be controlled. An ideal pain model provides a standardized, constant and reproducible condition with high reliability of the pain assessments. The ideal pain model also has to be simple to perform and without any after-effects (i.e. no spontaneous pain), it must not cause tissue damage or psychological injury and the subject must have control of cessation during the test [2, 4].

Ultraviolet light

Ultraviolet radiation (UVR) is electromagnetic energy emitted from the sun or artificial sources. UVR (100-400 nm) is divided into three bands, long-wave ultraviolet radiation UVA (321-400), mid-wave ultraviolet radiation UVB (281-320) and short-wave ultraviolet radiation UVC (100-280) [9]. Mid range UVB is considered to be the most effective dose in inducing a cutaneous inflammatory reaction, i.e. a first degree burn [10, 11].

UVB-induced inflammation

The UVB-irradiation induces an inflammatory process which leads to exaggerated pain sensitivity within the site of irradiation (primary hyperalgesia), and in the adjacent areas (secondary hyperalgesia) [1, 3]. The primary hyperalgesia is mediated by nociceptor sensitization in the periphery nociceptive sensory neurons innervating the inflamed tissue, while secondary hyperalgesia is believed to result from activity-dependent sensitization of second and higher order neurons in the central nervous system (CNS) prominent in the spinal cord [1-3, 5, 12]. Several studies have documented a decrease in the thermal pain threshold transmitted through C-fibers, and a decrease in the mechanical pain threshold transmitted through A-β and A-δ fibers [1-3, 5].

The UVB-irradiation is used to induce a first degree sunburn reaction causing cutaneous inflammation, i.e. hyperaemia, hyperalgesia and hyperplasia. Due to inflammation, pathological conditions such as erythema, vascular hyperpermeability and edema formation is induced at the site of irradiation. The erythema in the skin is histologically characterized by vasodilatation with a mixed dermal neutrophilic and lymphocytic infiltrate [13]. Effects of UVB-irradiation also include increased pigmentation and skin thickening, i.e. hyperpigmentation and hyperplasia, respectively [10]. The UVB-irradiation is largely absorbed in the epidermis and results in apoptosis of epidermal cells, i.e. keratinocytes, melanocytes and Langerhans' cells, induced by DNA damage [6, 10, 14]. The epidermal keratinocytes are most likely to be damaged. Probably as a result of the DNA damage and damage to other chromophores[1], many cytokines and inflammatory mediators are synthesized and released into the skin [10]. A large number of inflammatory mediators such as prostaglandins (PGE2,PGF2α, PGD2), prostacyclin, leukotrienes, cytokines (IL-1β, IL-6, IL-8, IL-10 TNF-α), nitric oxide, histamine, bradykinin and nerve growth factor are believed to be involved in the sensitization of the peripheral nociceptive terminals of the sensory neurons, i.e. hyperalgesia [6, 10]. These inflammatory mediators are involved in regulation of the expression of adhesion molecules in the vascular endothelium and kreatinocytes. These substances are also involved in the recruitment and activation of mononuclear cells and neutrophils inducing vasodilatation and inflammatory process. The appearance of erythema is a clinical end-point which is the result of a series of complex biological events [10].

Erythema and determination of the individually minimal erythema dose (MED)

Erythema is an acute cutaneous inflammatory reaction induced by excessive exposure of the skin to UVB irradiation. The dose of irradiation is defined on base of the individual sensitivity of the subject and it is referred to as the minimal erythema dose (MED). The erythema response is individual and depends on the genetic and environmental factors including skin-type, hydration of the skin, age, anatomical site of the irradiation, and the wavelength, dose, altitude and latitude of the radiation [10]. The definition of the MED is not standardized, as it is varying from ill-defined

[1] Chromophores: epidermal absorbing molecules, i.e. keratin, melanin, collagen and elastin, urocanic acid, nucleic acid and DNA [10].

erythema to well-defined erythema (light pink) with definitions such as “The minimal dose in producing just perceptible erythema on the exposed skin determined 24 h after the irradiation” [14], “ The lowest dose of UVB-radiation that produces the minimum noticeable reddening of the skin 24 h after the exposure” [15], “The smallest dose causing a minimally perceptible erythema with well-defined borders at 24 h after irradiation” [10]. The MED is not a standard measure, but it does include the variable nature of individual sensitivity to the UVB irradiation. Although MED is widely used as an end-point for the assessment of erythema, it is somewhat subjective. Despite this fact the MED is a visual assessment of the erythema and it may not be the optimal reference value for dosimeter, it is an easily performed procedure, which does not require the use of any specialized equipment [10].

Applying the UVB-pain model

The UVB-pain model has been applied in several pharmacological analyses, which have shown variable results [16]. The following sections provide an overview of the studies applying the UVB-pain model. The studies can be divided into two groups, studies investigating the effects of UVB-stimulation and studies investigating the effect of a drug in the UVB-pain model. Table 1 and 2 provide an overview of the studies.

Studies investigating the UVB-pain model

To our knowledge three studies have been performed on the UVB-stimulation process.

Hoffmann and Schmelz (1999) have investigated the time course of hyperalgesia and erythema following UVB-stimulation. The source of UVB was Saalmann Multitester (Saalmann Multitester, SBB LT 400, Saalmann Medizintechnik, Herford, Germany). The study included 10 healthy volunteers (5 females and 5 males) and the MED was determined for all volunteers 24h after irradiation of ascending dosages. Five circular spots on the thigh with a diameter of 1.5 cm were irradiated with increasing dosages (10 s; 0.01–0.05J/cm2). After the MED determination volunteers were irradiated with 1xMED and 3xMED. Skin temperature, erythema, superficial blood flow (sBF), heat pain threshold (HPT), mechanical pain threshold (MPT) were assessed 1h, 6h, 12h, 24h, 48h and 96h after UVB-irradiation. UVB-irradiation resulted in a significant increase in sBF , but no difference in skin temperature was detected. Dose dependent thermal and mechanical hyperalgesia were detected with a significant decrease in both the HPT and the MPT. This study concluded that this pain model was well tolerated in all subjects and UVB-stimulation induced dose-dependent delayed hyperaemia and hyperalgesia to both thermal and mechanical stimuli. According to this study, UVB-irradiation is a suitable experimental model for long time course of stable thermal and mechanical hyperalgesia [11].

Gustorff et al. (2004a) have investigated the within-day and between-day variability of UVB induced primary and secondary hyperalgesia over 10 h. The study was a randomized crossover design and the source of UVB was Sellasol (Sellas MedizinischeGeräte GMBH, Gevelsberg-Vogelsang, Germany; wavelength 290–320 nm). The study was performed in 8 healthy female volunteers. The MED was determined for all volunteers 24 h after irradiation of ascending dosages at the lateral side of the upper leg. A circular spot of 5 cm diameter was irradiated with 3xMED of UVB-stimulation at the ventral-medial side of an upper leg. The difference in the sBF , the heat pain tolerance threshold (HPTTT), the electrical pain tolerance threshold (EPTT), and the area of secondary hyperalgesia (sAREA) were assessed. The study consisted of two sessions, and in each session the measurements were repeated during a period of 10h with 2-h intervals between each measurement. Primary hyperalgesia was evidenced by significant decreases in both HPTT and EPTT. Large sAREA were

observed. There was no trend within one session of either primary or secondary hyperalgesia and no difference between the two sessions. The study concluded that this pain model was well tolerated in all subjects with a long time course of stable hyperalgesia with a high within-day stability and between-day repeatability for primary and secondary hyperalgesia [2].

Bishop et al. (2009) have performed three studies investigating the effects of this inflammatory hyperalgesic model on the primary and secondary area. The source of UVB in all three studies was a TL01 (Phillips, UK, max = 311 nm). The MED was determined in all volunteers, as six spots of 10 x10 mm areas of volar forearm skin were irradiated with increasing dosages. The first study investigated the time- and dose dependency of UVB irradiation. This study was performed on 12 volunteers (4 females and 8 males) and spots of 10x10 mm were irradiated by 1, 2 and 3xMED in all volunteers. The sBF, HPT and MPT were assessed 2h, 4h, 6h, 24h, 48h, 72h and 96h after irradiation. The study detected significant increase in sBF and decrease in HPT and MPT after UVB-irradiation.

In the second study, sensory changes in the primary and secondary area were investigated. The UVB-irradiation site was a 32x32 mm spot between the wrist and the elbow and 12 volunteers (4 female and 8 male) were irradiated with 3xMED. The sBF, HPT and MPT were assessed in the primary area 24h after irradiation. The study detected a significant increase in sBF and decrease in HPT and MPT in the area of primary hyperalgesia. In the secondary area measurement for erythema, brush-evoked allodynia and pinprick hyperalgesia were taken. No erythema, hyperalgesia or brush evoked allodynia was detedted in the sAREA.

In the third study the MPT was investigated in 13 volunteers (3 female and 9 male). A round annular spot (50mm and 20mm central un-irradiated) between the wrist and the elbow was irradiated with 3xMED and the MPT was measured 24h after the irradiation. The study concluded that the MPT decrease was restricted to the primary area.

Over all the study concluded that this model was well tolerated in all subjects and it produced significant primary hyperalgesia but no significant sAREA [5].

Studies investigating the effect of drugs in the UVB-pain model

To our knowledge seven studies have investigated the effect of a drug in the UVB-pain model.

Bickel et al. (1998) have investigated the anti-inflammatory and analgesic effect of ibuprofen and a peripherally acting opioid (k-agonist) in this inflammatory hyperalgesic model. The study was a randomized double-blind, cross-over study in 21 healthy male volunteers. The source of UVB was the Saalmann Multitester (SBB LT 400, Saalmann Medizintechnik, Herford,Germany). For MED determination five circular spots, 15 mm in diameter, were irradiated, at the ventral side of the upper leg, with increasing intensities of irradiation (0.02–0.06 J/cm2). In order to induce mechanical and thermal hyperalgesia the UVB-irradiation was applied on the ventral side of the upper leg 24 h before treatment. The UVB-irradiation was applied in two doses, i.e. 1xMED and 3xMED. The increase in BF, the HPT and MPT were assessed. Measurements were taken 24 h after the irradiation, before the treatment and again post-treatment. The study determined that UVB-induced mechanical and thermal hyperalgesia at dosage of 3xMED. This study concluded that this pain model was well tolerated in all subjects and the UVB-pain model is suitable for establishing antihyperalgesic effects of NSAIDs, but probably not of k-receptor agonists [17].

Koppert et al. (1999) have investigated the analgesic effects of morphine in this inflammatory hyperalgesic model. The study was performed in 12 healthy male volunteers. The source of UVB was the Saalmann Multitester (SBB LT 400, Saalmann Medizintechnik, Herford, Germany). For MED determination five circular spots with a diameter of 1.5 cm at the ventral side of the upper leg were irradiated with increasing intensities of UVB-irradiation (0.02– 0.06 J/cm2). Two spots on the ventral sides of both forearms were irradiated with 1xMED and 3xMED 24h before treatment. The HPT and MPT were assessed. Measurements were taken 24 h after the irradiation, before the treatment and again post-treatment. This study concluded that the UVB-pain model was well tolerated in all subjects and the suppression of mechanical hyperalgesia could be predominantly due to inhibition of secondary (central) mechanical hyperalgesia [18].

Sycha et al. (2003) have investigated the anti-inflammatory and analgesic effects of ibuprofen in this inflammatory hyperalgesic model. The study was a randomized, double-blind, placebo-controlled, two-way crossover design in 32 healthy volunteers (18 females and 16 males). The source of UVB was a metal halogen lamp (Sellasol; Sellas Medizinische Geräte GMBH, Gevelsberg-Vogelsang, Germany). A small skin area (diameter 5 cm) of the ventro-medial upper leg was irradiated with 3xMED. Skin temperature, HPT and HPTT were measured at the site of irradiation. Measurements were taken 24 h after the irradiation, before the treatment and again post-treatment. No alteration in skin temperature was detected. The study concluded that the effects of ibuprofen were highly significant and this pain model was well tolerated in all subjects and the model is suitable for establishing anti-hyperalgesic and anti-inflammatory effects of NSAIDs [4].

Gustorff et al. (2004b) have investigated the analgesic effect of remifentanil, gabapentin, and the combination of both drugs in this inflammatory hyperalgesic model. The study was designed as a double-blinded, active placebo-controlled, 4-way-crossover design. The study was performed in 16 healthy volunteers (8 females and 8 males) and the source of UVB was Sellasol (Sellas MedizinischeGeräte GMBH, Gevelsberg-Vogelsang, Germany; wavelength 290–320 nm). The MED was determined for all volunteers 24 h after ascending dosages at the lateral side of an upper leg. A circular spot of 5 cm diameter was irradiated with 3x MED at the ventral-medial side of an upper leg. The HPT, HPTT and the sAREA were investigated. Measurements were taken 24 h after the irradiation, before the treatment and again post-treatment. The study detected a stable decrease in the HPT and the HPTT compared to the normal skin. Large sAREA developed in all the subjects. The study concluded that this pain model was well tolerated in all subjects and this opioid analgesia was reliably demonstrated in this model [3].

Sycha et al. (2005) have investigated the anti-inflammatory and analgesic effects of Rofecoxib in this inflammatory hyperalgesic model. The study was a randomized, double blinded, placebo-controlled cross-over design. The study was performed in 42 healthy volunteers (21 females and 21 males) and the source of UVB was Sellasol (Sellas MedizinischeGeräte GMBH, Gevelsberg-Vogelsang, Germany; wavelength 290–320 nm). The MED was determined in all volunteers and a circular spot of 5 cm diameter was irradiated with 3xMED on the ventral-medial aspect of the upper leg. Increase in the BF, HPT and the sAREA were assessed. Measurements were taken 24 h after the irradiation, before the treatment and again post-treatment. This study concluded that this pain model was well tolerated in all subjects. Rofecoxib showed peripheral effects in this human inflammatory UVB-pain model and provided circumstantial evidence that even a standard clinical dose of rofecoxib reduces central hyperalgesia in inflammatory pain [1].

Sycha et al. (2006) have investigated the anti-inflammatory and analgesic effects of botulinum toxin A (BoNT/A) in this inflammatory hyperalgesic model. The study was randomized, double-blind, paired study design. The study was performed in 6 healthy voluenteers (1 female and 5 male) and the UVB source was Sellasol (Sellas MedizinischeGeräte GMBH, Gevelsberg-Vogelsang, Germany; wavelength 290–320 nm). The MED was determined in all volunteers and a circular spot of 5 cm in diameter was irradiated with 3xMED on the previously injected areas on the upper ventral aspect of both legs. BF, cold pain threshold (CPT), HPT, brush induced allodynia, MPT and the presence of a sAREA were assessed. The skin was irradiated at injection side 48 h after the injection. Measurements were performed at baseline (before the injection procedure), 48 h thereafter (in normal skin), and 24 h after the UVB irradiation (72 h after the injection procedure) when UVB-induced sensitization was stable. The study detected a significant increase in the CPT and a significant decrease in the HPT, MPT and blood flow after UVB-irradiation. Mechanical allodynia was not observed. sAREA was detected as well. The study concluded that this pain model was well tolerated in all subjects. No anti inflammatory or anti nociceptive effects of BoNT/A were found in this human inflammatory pain model [19].

Chizh et al. (2007) investigated the anti-inflammatory and analgesic effects of a TRPV1 antagonist in this inflammatory hyperalgesic model. The study was a randomized placebo-controlled single-blind cross-over design. The study was performed in 19 healthy volunteers and the Source of UVB was the Saalmann Multitester (SBB LT 400, Saalmann Medizintechnik, Herford, Germany). The MED was determined by irradiating five circular spots with a diameter of 1.5 cm with increasing intensities in the ventral side of the upper left leg. Erythema was assessed visually by using colorimetry 24h later. Subjects who did not develop erythema at the maximum dose of 130mJ/cm2 were excluded from the study. A circular spot of 2 cm diameter on the upper leg was irradiated with 3xMED. Increase in BF and HPT were assessed. Measurements were taken 24 h after the irradiation, before the treatment and again post-treatment. The study detected a significant decrease in HPT and HPTT compared to the respective values from the non-irradiated leg. The TRPV1 antagonist significantly increased both HPT and HPTT in the irradiated site compared with placebo treatment. The study concluded that this pain model was well tolerated in all subjects and TRPV1 antagonist may alleviate the pain and hyperalgesia associated with inflammation pain [20].

Author	Publication year	UVB source	Number of volunteers	MED	Area of irradiation and location	Irradiation intensity	Measurements performed	Time of measurement
Hoffmann and Schmelz	1999	Saalmann Multitester (290-320nm)	10 (5 females and 5 males	0.01-0.05J/cm² 5 spots 1.5 d	5 spots 1.5 d ventral side of the upper leg	1xMED 3xMED	Skin temp., erythema, BF, HPT, MPT	1h, 6h, 12h, 24h, 48h, 96h, (132h)
Gustorff et al.	2004	Sellasol 290-320nm)	8 (females)	Lateral side of an upper leg 60 cm distance	5 cm d. ventral-medial side of an upper leg	3xMED	BF, HPTT,EPTT, sAREA von Frey (150g)	20h, 22h, 24h, 26h, 28h, 30h
Bishop et al.	2009	a TL01 (Phillips, UK, kmax = 311 nm	12 (4 females and 8 males)	476+/- 20.6 mJ/cm²	3 spots 10x10 mm volar forearm	1xMED 2xMED 3xMED	Erythema, BF, HPT, MPT	Baseline, 2h, 4h, 6h, 24h, 48h, 72h, 96h
Bishop et al.	2009	a TL01 (Phillips, UK, kmax = 311 nm	12 (4 females and 8 males)	439+/- 28.3 mJ/cm²	32x32 mm between wrist and elbow	3xMED	sAREA to von Frey (10 g) and brush	24h
Bishop et al.	2009	a TL01 (Phillips, UK, kmax = 311 nm	12 (3 females and 9 males)	454+/- 25.7 mJ/cm²	Annulus-shaped 50mm d 20mm central un stimulated zonebetween wrist and elbow	3xMED	MPT	24h

Tabel 1 An overview of the studies investigating the UVB-pain model.

Author	Publication year	UVB source	Number of volunteers	MED	Area of irradiation and location	Irradiation intensity	Measurements performed	Design and drug
Bickel et al.	1998	Saalmann Multitester (290-320nm)	21 (male)	5 spot 15mm ventral side of the upper leg 0.02-0.06J/cm²	ventral side of the upper leg	1xMED 3xMED	BF, HPT, MPT	7days interval cross-over ibuprofen selective kappa-opioid
Koppert et al.	1999	Saalmann Multitester (290-320nm)	12 (4 female and 8 male)	5 spots 15mm ventral side of the upper leg0.02-0.06J/cm²	Ventral side of both forearms different doses	1xMED 3XMED	HPT,MPT	Morphine
Sycha et al.	2003	Sellasol (290-320nm)	32 (16 female and 16 male)		Ventro medial upper leg 5cm d	3xMED	Skin temp., HPT, HPTT	Two-way Cross-over ibuprofen
Gustorff et al.	2003	Sellasol (290-320nm)	16	Ascending dose Upper leg	5 cm d. Ventral medial side of an upper leg	3xMED	BF, HPT, HPTT, sAREA von Frey (150g)	4-way cross-over remifentalin, gabapentin and a combination of both
Sycha et al.	2005	Sellasol (290-320nm)	42 (21 female and 21 male)		5 cm d. ventral medial side of an upper leg	3xMED	BF, sAREA von Frey (150g), HPT, HPTT,	Cross-over rofecoxib
Sycha et al.	2006	Sellasol (290-320nm)	6 (1femal and 5 male)	Upper ventral aspects of both legs	5 cm d. ventral side of an upper leg	3XMED	Brush allodynia, BF, CPT,HPT, MPT, sAREA pin Prick (256mN)	Paired study Botulinum toxin A
Chizh et al.	2007	Saalmann Multitester (290-320nm)	45 (2 female and 43 male)	5 spots 15mm of the upper mex dose 130mJ/cm²	2 cm d. on the upper leg	3xMED	BF, HPT, HPTT,	Cross-over TRPV1 antagonist

Tabel 2 An overview of the studies investigating the effect of drugs in the UVB-pain model.

Aim of this project

The UVB pain model appears to be a suitable human cutaneous inflammatory pain model for testing analgesic drugs with pheripheral and central antihyperalgesic effects [1]. This pain model produces prolonged primary hyperalgesia for more than 48 h after the UVB irradiation [1, 2]. This stability of hyperalgesia over hours offers favorable conditions for pharmacological studies, which will be able to provide useful information about the mode of action and thereby providing a basis for mechanism based treatment of pain [1]. Only few data exist on the intersession variability of this pain model [2]. Besides the primary hyperalgesia, some studies have found an sAREA in this model [1-3, 19], but the literature is conflicting on this point [5, 6, 21-23]. The aim of this project was to detect the potential effect of UVB-irradiation in this pain model and to validate the UVB-pain model by investigating the inter- and intra-individual variation and thereby assessing the reliability, i.e. reproducibility, of this hyperalgesic pain model. The second purpose of this study was to produce sample size calculations based on the effect of this model, which is only an estimate.

Approvals

Approvals from the Regional Ethic Committee and the Danish Data Protection Agency were obtained prior to execution of the study. Because of technical problems with the Multitester the authorizations were extended. The approved study protocol and the documentation of the approvals can be found in Appendix A and B.

Pilot study

A pilot study was performed prior to the initiation of the principal study. Due to some technical problems the pilot study was performed with a hand-held UVB – lamp (Philips TL-01; Narrow band λ = 305-315 nm). This preliminary study included 4 healthy male volunteers. The study design was similar to the principal study with the exception that it was not repeated. The UVB-lamp was calibrated before use and the data on calibration can be found on the CD in Appendix F. The raw data collected in the pilot study can be found the Appendix E.

Case report form and requisition form

The CRF and requisition form used in the study can be found in Appendix C and D.

Calibration

Because of the technical problems with the Multitester a radiometer from Dr. Gröbel (Dr. Gröbel UV-Elektronik GmbH, Ettlingen, Germany) was acquired, making it possible to perform calibration of the Multitester. Several calibrations have been performed in order to document the technical problem. A description of the radiometer and the calibration technique can be found in the SOP Appendix I. Examples of the actual measurements and CD with all the measurements performed can be found in Appendix F.

Development and validation of a human bio-marker for cutaneous inflammatory pain (the UVB-model) - a methodological study in healthy volunteers

Asiah Rahi and Line Christensen 2011

Abstract

Background: In the early clinical trials, human experimental pain models are important tools for defining the analgesic efficacy of drugs. The Ultraviolet-B (UVB) pain model is used for the induction of a local cutaneous inflammation in a circumscribed skin area with primary hyperalgesia (increased sensitivity to painful stimuli) and possible area of secondary hyperalgesia (sAREA).

Aim: To investigate the potential effect of the UVB-irradiation in this pain model and the reliability of this model by detecting the inter- and intra- individual variations in this model and to produce sample size estimates for a parallel and a cross over study design.

Methods: Inflammatory hyperalgesia was induced on the upper arms of 15 healthy male volunteers by irradiating a circular spot (5 cm diameter) with three times minimal erythema dose (MED) of the UVB-irradiation. The inflammatory response assessed by measures of erythema, superficial blood flow and skin temperature. The sensory tests including the brush induced hyperalgesia, von Frey and pinprick stimuli, pressure pain threshold (PPT) and heat pain threshold (HPT), all measurements were performed at baseline, 24h, 48h and 72h after the irradiation, the whole procedure was repeated on the opposite arm with a two week's interval. sAREA was assessed to both von Frey filament and pinprick after the UVB-exposure.

Results: A significant increase in the erythema, mean blood flow (BF) and skin temperature was detected after the irradiation ($P < 0.001$ in all three cases), but no changes in the BF in the sAREA were detected ($P=0.664$). Brush induced allodynia was detected 48h after the irradiation ($P=0.02$). No significant increase was detected in primary hyperalgesia to von Frey filament ($P=0.124$). A significant increase in the pinprick induced primary hyperalgesia was detected ($P<0.001$) and a significant difference between the pin sizes ($P<0.001$). Both heat pain threshold (HPT) and pressure pain threshold (PPT) significantly decreased after the irradiation ($P<0.001$ in both cases), but the PPT decreased differently in both arms ($P<0.001$).

Conclusion: The present study concluded that the UVB-pain model was successful in inducing a local cutaneous inflammation in a circumscribed skin area, as both alteration in the BF and visually erythema was noticed. Furthermore, significant increases in both skin temperature and erythema were detected.
For detection of mechanical induced primary hyperalgesia the present study concluded that the pinprick was the most suitable test in comparison to von Frey filaments.
Detection of the HPT was found to be a highly reliable test in the UVB-pain model. PPT only seemed to be reliable on the dominant side. Area of secondary hyperalgesai was detected to both von Frey filament and pinprick stimuli and both test seemed to be reliable, the issues of which test should be preferred need to be addressed further

Introduction

Human experimental models of pain and hyperalgesia serve as an important tool for defining the analgesic efficacy of drugs in early clinical trials. A standardized experimental approach is an advantage in the early screening of new analgesic drugs, and pain models are required for the development of new antihyperalgesic drugs [2, 3]. The lack of constant conditions over a long period is an unsolved problem of most of the established pain models. An ideal pain model provides a standardized, constant, and reproducible condition with a high reliability of the pain assessments. The ideal pain model also has to be simple to perform and without any after-effects (i.e. no spontaneous pain), it must not cause tissue damage or psychological injury, and the subject must have control of cessation during the test [2, 4]. Ultraviolet-B (UVB) irradiation is applied in the UVB-pain model in order to induce a local cutaneous inflammation in a circumscribed skin area with primary hyperalgesia to mechanical and thermal stimuli [1-5]. The UVB-model is a valid human inflammatory pain model of primary hyperalgesia and could serve as an early efficacy indicator in humans [2-4].

The UVB-model induces reduction in thermal and mechanical pain thresholds (hyperalgesia) at the site of irradiation [1, 3, 5]. The inflammatory process leads to exaggerated pain sensitivity within the site of irradiation (primary hyperalgesia) and in the adjacent areas (secondary hyperalgesia) [1, 3]. The primary hyperalgesia is mediated by nociceptor sensitization in the periphery nociceptive sensory neurons innervating the inflamed tissue, while secondary hyperalgesia is believed to result from activity-dependent sensitization of second and higher order neurons in the central nervous system (CNS) prominent in the spinal cord [1-3, 5, 12].

Studies indicate that this pain model produces prolonged primary hyperalgesia for more than 48 h after the UVB-irradiation [1, 2]. This stability of hyperalgesia over hours offers favorable conditions for pharmacological studies, which will be able to provide useful information about the mode of action and thereby providing a basis for mechanism based treatment of pain [1]. However data are conflicting on the time of peak hyperalgesia between 24 and 48 h after irradiation and only few data exist on the intersession variability of this pain model [2]. Some studies have found that beside the primary hyperalgesia this model also results in an area of secondary hyperalgesia (sAREA) [1-3, 19], but the literature is also conflicting on this point [5, 6, 21-23].

Erythema is an acute cutaneous inflammatory reaction induced by excessive exposure of the skin to UVB-irradiation. The dose of irradiation is defined on the basis of each individual's sensitivity to UVB-irradiation and it is referred to as the minimal erythema dose (MED). The erythema response is individual and depends on the genetic and environmental factors including skin-type, hydration of the skin, age, anatomical site of the irradiation, and the wavelength, dose, altitude and latitude of the radiation [10]. The definition of MED is not standardized, as it is both defined as "the minimal dose in producing just perceptible erythema" and "the smallest dose causing a minimally perceptible erythema with well defined borders" [10, 14]. Although MED is widely used as an end-point for the assessment of erythema, it is somewhat subjective.

The present study investigated the development of primary hyperalgesia in experimentally induced inflammatory skin during time intervals of 24 h, 48 h and 72 h after the UVB irradiation. The study also investigated if a sAREA develops adjacent to the irradiated skin patch and the change in this effect over time. The UVB irradiated skin patch was tested with mechanical and thermal stimuli at baseline and 24, 48 and 72 h after the irradiation, in order to investigate the inter-individual variation. The intra-individual variation was also investigated as the same procedures were repeated with a

two week´s interval. The final outcome of this study was a table with sample size both for a parallel and a cross over study. The aims of the study are:

- To investigate the potential effect of the UVB-irradiation in this pain model.
- To investigate the inter-individual variation over time (24h, 48h and 72h).
- To investigate the intra-individual variation between sessions (two weeks interval).
- To produce a table of sample size estimates for parallel and cross over studies.

without giving a reason, and that they would be financially compensated for the time spent. Before performing any test procedure a full medical history was taken and a pre-study examination was performed. The exclusion criteria were as follows: BMI outside the normal range; age below 18 years and above 30 years; red-haired; any kind of infection; presence of acute pain; abuse of alcohol, hash or any other euphoric drug; participation in a clinical trial in the preceding 2 weeks or during the study; history of any neurological,

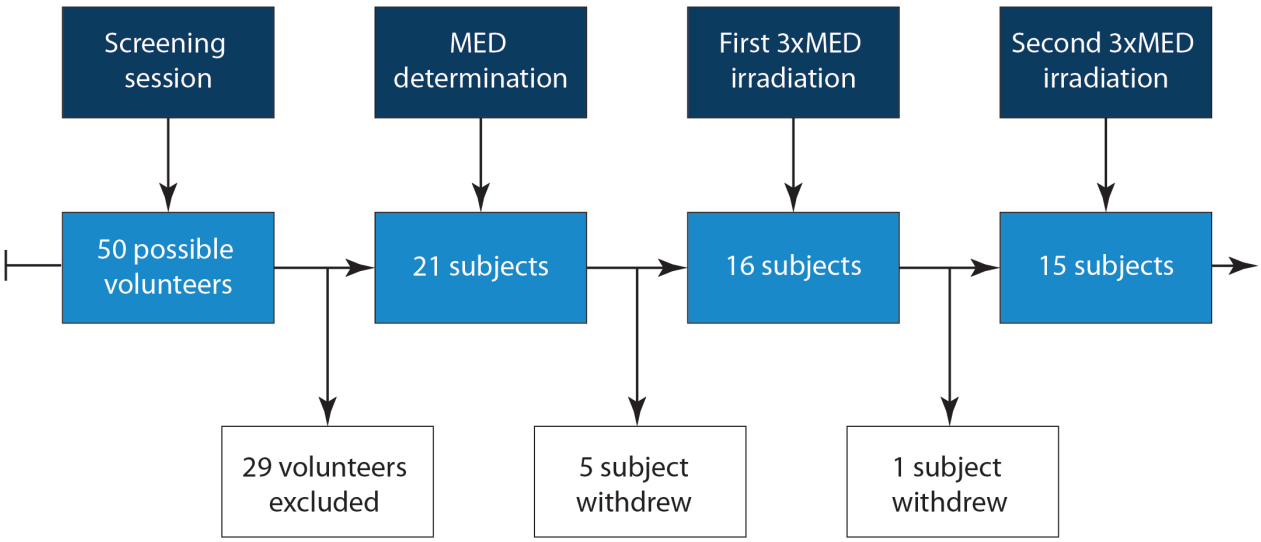


Figure 1
50 possible volunteers were screened 21 of them did not posses any of the exclusion criteria and were enrolled in the study. 5 subjects withdrew after the MED determination and 1 subject withdrew after having completed the first 3xMED irradiation and the first four study sessions. 15 subjects completed the study.

Methods

Subjects

Fifteen healthy, pain-free male volunteers participated in this study after giving their written informed consent. The volunteers were Danish speaking males aged between 18 and 27 years with a body mass index (BMI) within the normal range (18,5-24,9). The volunteers had been informed that they had the right to withdraw from the study at any time and

musculoskeletal or psychological diseases; any skin disease at the area of irradiation which can alter the response to UVB irradiation, sunbathing and intake of caffeine or any analgesic drug 24 h before the trial or between the sessions or lack of interpersonal skills. Only those volunteers who did not posses any of the exclusion criteria entered the study and were considered as study subjects. The recruiting process is illustrated in fig. 1.

Study design

The study was conducted at Aalborg University and performed in accordance with the 1996 version of the Declaration of Helsinki. The study was approved by the local ethics committee "Region Nordjylland Videnskabsetisk Komité" and by the Danish Data Protection Agency. The study consisted of a screening session, a training session with the determination of the MED and 2x4 identical study sessions with 2 weeks' interval. This means that each subject was his own control person. The measurements for each subject were taken at exactly the same time each day. The study design is illustrated in Fig. 2.

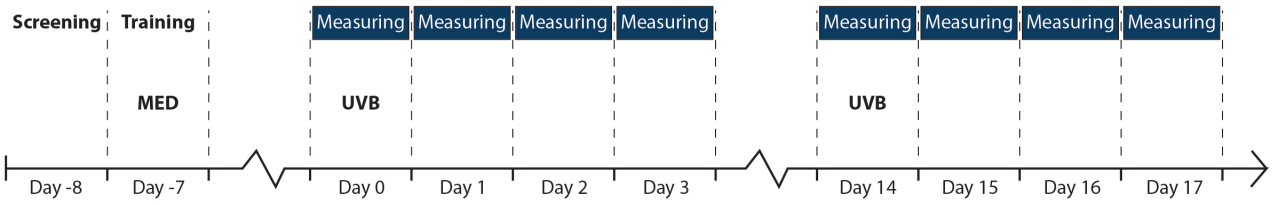


Figure 2
The figure illustrates the study design including a screening session, a training session with the determination of the MED followed by 2x4 study sessions with 14 days' interval.

Screening: After receiving both oral and written information about the study and thereafter discussing the procedure, the volunteers gave their written consent. When the consent was received, a full medical history and a pre-study examination were taken, the subjects were asked questions regarding their health status, in order to ensure that they did not posses any of the exclusion criteria.

Training session and MED determination: The aim of this session was to ensure that the subjects got familiar with the testing procedures, the sensations caused by the testing systems and the pain-rating procedures. For pain rating this study has used an electrical visual analog scale (eVAS) (Aalborg university, Aalborg, Denmark) ranging from 0-10, where 0 means "no pain" and 10 mean "maximal imagining pain". The subjects were instructed during the training session that the middle of the scale is the pain threshold, and the non-painful pricks should be rated below the midpoint and the painful pricks should be rated above the midpoint. In this regard, all the tests were applied on a non-irradiated skin area and during the procedures the subjects were asked to keep their eyes closed. The data obtained in this training session were checked for plausibility, but not included in further analysis. Subsequently, the MED was determined.

Session 1-4: Sensory tests and the assessments of the inflammation intensity were performed at baseline and post irradiation at 24h, 48h and 72 h. These sessions were repeated with a two week's interval. On session one, i.e. day 0, the assessment of inflammation and the sensory testing including the thermal and mechanical stimulation were applied on a non-irradiated skin area and thereby the baseline measurements were achieved. Subsequently, the subjects were irradiated on the ventro-medial site of the upper arm with 3xMED of UVB-irradiation. Fourteen days after session one, the same procedures were repeated and this time the UVB-irradiation was applied on the opposite upper arm.

Experimental pain model - UVB and MED

Prior to the first study session the MED for UVB-irradiation was determined with a calibrated UVB source (290-320nm) Saalmann Multitester (Saalmann, SBC LT 400 Herford, Germany),calibrated with a radiometer (RM 12, Dr.Gröbel, UV-elektronik GmbH). Site of irradiation was non-exposed skin at the anterior surface of the forearm, 5 circular spots with a diameter of 1,5 cm were irradiated with increasing intensities of UVB-irradiation (40-160 mJ/cm2). 24 h after irradiation, the MED was determined visually (well defined erythema) and by reading the erythema of the irradiated skin with a colometer (DSM II ColorMeter, Cortex Technology, Hadsund, Denmark). The visual MED reading was as followed:

0. No erythema
1. Very slight erythema (barely perceptible)
2. Well-defined erythema
3. Moderate to severe erythema
4. Severe erythema (beet) redness to eschar formation [16]

After the determination of MED, the first degree sunburn was induced with the calibrated UVB-source (Saalmann Multitester, SBC LT 400, Herford, Germany). A circular spot with a 5 cm diameter at the ventral-medial site of the upper arm was irradiated with 3xMED of UVB-irradiation and this session was repeated on the other arm with a two week's interval. The study was performed in the spring and the volunteers were asked to avoid additional UV-exposure at the sites of irradiation.

Sensory testing and inflammatory assessment

Tests for the assessments of inflammatory intensity included measurement of erythema, skin temperature and superficial blood flow (sBF). The sensory tests included measurements of the brush induced allodynia, detection of primary hyperalgesia to von Frey and pinprick, pressure pain threshold (PPT) and heat pain threshold (HPT).

Assessments of inflammation

Erythema

In this test, the responsive area, redness of the skin was measured with a colormeter (DSM II ColorMeter, Cortex Technology, Hadsund, Denmark). Increased erythema at the site of irradiation was taken as a surrogate for the inflammatory response.

Skin temperature

The skin temperature was measured with an infrared thermometer (Fluke 561 IR and contact thermometer, Fluke Corporation, Eindhoven, The Netherlands). Increased skin temperature in the irradiated skin was taken as a surrogate for the inflammatory response.

Superficial blood flow (sBF)

Differences in the mean sBF between the irradiated and non-irradiated skin were measured with a laser Doppler flow (Moor V5.2 Instruments, Devon, UK). This device scans with a 2-mW helium laser across the skin surface and registers the shifted frequency from the backscattered light. Thereby, the velocity of moving erythrocytes is calculated and presented as a color coded picture representing a relative measure of perfusion (or flux) in 2 dimensions. The laser head was positioned 30 cm above the measurement site. The mean BF in the area of primary hyperalgesia (d 5 cm) was estimated by scanning an area of 8 x 8 cm around the irradiated skin with a resolution of 228,230 pixels. The mean sBF in the primary area and the sAREA was calculated using relative flux (arbitrary units); this served as surrogate for the inflammatory response. The images were analyzed using dedicated image-processing software (Moor V5.2 Instruments Ltd.).

Mechanical and thermal stimuli

Brush incused allodynia

The degree of brush-induced allodynia in the area of primary hyperalgesia was assessed with a brush (SENSELab Brush-05, Somedic AB, Hörby, Sweden). The brush stimulus consisted of three 2 cm longitudinal strokes with an interval of 10 sec. along the irradiated skin. The strokes were applied at an angle of 45o. After applying the strokes, the subjects were asked for an average scale on eVAS for the sensation induced by the brush. The subjects were asked to keep their eyes closed during the procedure.

(diameter 0.6 mm) with different forces: 0.8, 1.6, 3.2, 6.4, 12.8, 25.6, 51.2 and 60.0 mN were used. Each pin was applied 3 times (holding the pin for 2 sec.) in the area of primary hyperalgesia and the subjects were asked to rate the pricking on eVAS.

Pressure pain threshold (PPT)

The PPT was assessed by applying pressure on the irradiated skin with an algometer (Somedic algometer, Somedic AB, Hörby, Sweden). The pressure was applied with a velocity of 30 kPa/sec, by using a probe with an area of 0,5 cm2.

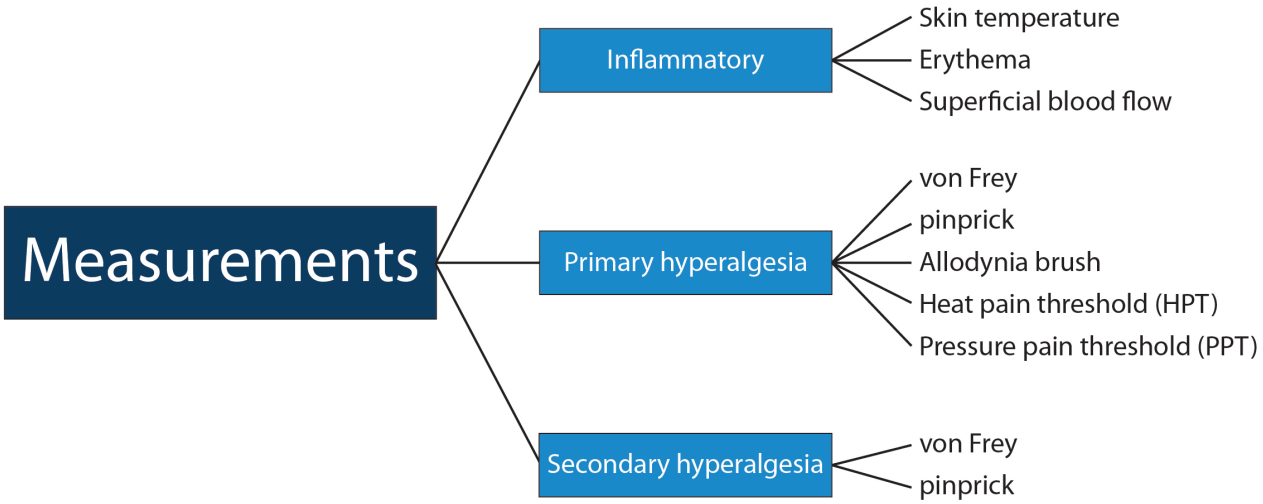


Figure 3
The figure illustrates the measurements performed in the study.

Von Frey induced primary hyperalgesia

Von Frey filaments (Aesthesiometer, Somedic AB, Hörby, Sweden) were applied on the irradiated skin, starting from the one with the thinnest filament and increasing the thickness of the filament until the subject reported the sensation of a prick. Each filament was applied three times on the skin. The subjects were asked to keep their eyes closed during the procedure and rate the pricking sensation on eVAS.

Pinprick induced primary hyperalgesia

The pinprick was applied on the irradiated area by using pinprick set (Aalborg university, Denmark). For this test, a pinprick set consisting of 8 pins

For determination of PPT the subjects were instructed to press a response button on the algometer when the pressure became painful.

Heat pain threshold (HPT)

HPT was determined by the methods of limits using a thermal sensory testing device (TSA 2001 MedocTM, Ramat Yishai, Israel). The size of the thermode was 30 x 30 mm. The thermode was attached to the skin at the site of irradiation using an elastic band. Care was taken to consider upper arm curvatures when placing the probe in order to achieve optimal contact between the probe and the surface of the upper arm. Skin adaptation temperature

was 32°C, and the temperature was gradually increased at a rate of 0,6 °C/s. When the test was stopped the temperature of the thermode returned to baseline with a return rate for 1,0 °C/s. Stimulator temperature range was 32–52 °C. Subjects were initially trained in a standardized manner to report the heat pain thresholds. Subjects were instructed to press a response button on the thermal sensory device when thermal sensation became unpleasant and aching. Pushing the button resulted in a quick cooling of the thermode until adaptation temperature was reached.

Determination of the area of secondary hyperalgesia (sAREA)

The sAREA was determined by using a 26 g von Frey filament and a pin with 6,4 mN. During the two procedures, the subjects were asked to keep their eyes closed while the skin surrounding the erythema was pricked with the von Frey filament or the pin.

The stimulation was induced 8 cm away from the area of erythema and was repeated along a pattern of 8 radial spokes. With movement along each spoke at a distance of 5 mm, the subjects were asked to report when the sensation of the pricking changed definitely, i.e. changed from a normal to "different" or "burning" or "unpleasant" sensation. The spot was marked with a pen and erased after the measurement to avoid bias during the following measurements. The two sAREA were determined from these 8 spots by calculating the area of an octagon using the software Vistamatirx (version 1.36.0m, SkillCrest LLC ©).

Statistical analysis

All statistical analysis was carried out using the statistical programs SPSS (SPSS Inc. IMB Company ©, V19 2010) and Microsoft Excel (2007). The data were analyzed by means of analysis of varaiance (ANOVA) with repeated measures ANOVA (RM ANOVA) with fixed factors time (four levels; baseline, 24h, 48h and 72h) and arms (two levels; right and left arm) and the random factor subjects. The time-by-arm interaction was also included in the model to test whether the changes detected after the irradiation were dependent on dominant or non-dominant arm. P-values for all tests were reported and the significance level was set at $P < 0.05$. To correct for sphericity the Greenhouse-Geisser estimate has been used. The post hoc test Bonferroni Correction was applied on the data to perform multiple comparisons, to assess any systemic bias over time.

For assessment of the reliability test, i.e. stability of the model over time, the intra-individual variance was assessed. For this purpose the intra-class correlation (ICC) was calculated for measurements at 24h, 48h and 72h after the irradiation. The ICC values were calculated in SPSS by the formula:

$$ICC = 1 - \frac{\sigma_{within}}{\sigma_{total}}$$

where σ_{within} is the variance within individuals and σ_{total} is the sum of the variance within individuals and between the repetitive measurements. ICC evaluates each person's ability to reproduce a response across sessions and illustrates the difference between a person's scores at repeated sessions [24, 25]. The ICC values were calculated in SPSS using two-way mixed model with absolute agreement. ICC values >0.80 was considered excellent [24, 25].

For assessment of the reliability test, the overall inter-individual variance, the coefficient of variation (CV) also was calculated. CV reflects the overall variability of the model [25] and it was calculated for measurements at 24h, 48h and 72h after the irradiation. Microsoft Excel was used for the calculation of CV values by the following formula:

$$CV = \frac{SD}{\mu} \times 100\%$$

where SD is the standard deviation and μ is the mean of the data.

For estimation of the sample sizes the following formulas were used [26, 27]. These formulas are based on the method of comparing the means of two groups. In this regard, means of the baseline (from both arms) measurements were compared with the means of the measurements at 24h (from both arms) after the irradiation. In these calculations, α was set to be 0.05 and β was set to be 20 %, which means that a power of 80 % was required.

Parallel study:

$$n = \frac{2 \times SD^2}{E^2} \times k$$

where SD is the standard deviations of means, E is the minimum clinical relevant change to be detected and k is a constant which depends on the required power (1-β) and significane levels (α). E was set to be 30 %, i.e. 30 % change from the baseline mearurements.

Cross over study:

and

$$n = \frac{s^2}{\delta_2}(t_{\alpha(2)\,v} + t_{\beta(1)\,v})^2$$

$$\delta = \sqrt{\frac{s^2}{n}(t_{\alpha(2)\,v} + t_{\beta(1)\,v})}$$

where s is the SD of means and δ is the minimal detectable, v is the degree of freedom, which is 29 in this case and (2) represents a two-tailed paired t-test. The values $t_{\alpha(2),v}$ and $t_{\beta(1),v}$ were looked up in a table. A two tailed paired- t-test was applied on the data in order to calculate the sample size for a cross over study.

The SD's were calculated by the formula:

$$SD = \sqrt{\frac{\Sigma(X - \overline{X})^2}{n-1}}$$

where X is a number from the data, $\overline{X}$ is the mean of the values in the data and n is the number of values.

Results

Fifteen subjects completed the study. None of the subjects reported pain during the UVB-irradiation and no blister or skin damage above the intended range appeared in any of the subjects. The UVB-exposed skin areas showed no alterations immediately after the expositions. Results were obtained from both right and left arm at baseline and 24h, 48h and 72h after irradiation.

Determination of MED

The MED was determined on the forearm of twenty one subjects. The MED of the fifteen subjects who completed the study were between 55-160mJ/cm2.

Inflammatory assessments

Erythema

UVB-irradiation resulted in a cutaneous inflammation in all subjects after the exposure. This was evidenced by visible erythema 24h after the irradiation. RM ANOVA test detected a significant difference (P <0.001) between the erythema at baseline and the erythema measured after 24h, 48h and 72h. The model produced the same significantly increased degree of erythema on both arms, i.e. no difference between the arms (P=0.875), which remained stable during 24-72h. The reliability test indicated stability at 24-48h since an "acceptable" ICC was obtained for both arms (right arm: ICC= 0.561, P=0.015; left arm: ICC=0.515, P=0.018). This model was also stable during 48-72h since the reliability tests had an "acceptable" ICC on for both arms (right arm: ICC 0.513, P=0.021; left arm: ICC=0.687, P=0.002). The intra-individual variance between 24-72h is too low for both arms (right arm: ICC 0,421, P=0,055, left arm: ICC=0,443, P= 0,040). The CV values supported the stability of the model during 24-48h, by low CV values for both arms (right arm: CV=5.9 %; left arm: CV=6.4 %). A similar low CV value supported the stability of the model between 48h-72h for both arms (right arm: CV=6,8%; left arm: CV= 4,7%). The

CV values for both arms between 24-72h were low as well (right arm: CV=7,3%; left arm: CV= 6,9%). The overall inter-individual variance of this model was low.

Mean BF in the area of primary hyperalgesia

The mean BF was measured in the primary hyperalgesic area. The data were analyzed as the percentage-wise increase in the mean BF 24h, 48h and 72h after irradiation compared to the baseline measurement. An increase in the mean BF after the irradiation was indicated. RM ANOVA test did not detect a significant difference between the arms (P=0.826). The model did not produce a similar increase in the mean BF , during 24h, 48h and 72 h (P=0.000). The percentage-wise increase of the mean BF was 861.5 %, 726.5% and 618.9% after 24h, 48h and 72h, respectively. The percentage-wise increase in the mean BF was not stable during 24-72h. This instability is a significant bias between the sessions, which would invalidate any reliability analysis.

Skin temperature

RM ANOVA test detected a significant increase in the skin temperature after the irradiation. There was a significant difference between the baseline temperature and the measurements from 24h, 48h and 72h after UVB exposure (P<0.001). No significant difference between the arms was detected (P=0.146). The model induced a similar increase in the temperature during 24-72h, but the reliability test only revealed “acceptable” ICC values during 24-48h (ICC=0.599, P= 0.003) and during 48-72h (ICC=0.632, P=0.003) in the right and the left arm, respectively. The CV values indicated a small overall inter-individual variation between the sessions for both arms (right arm: mean CV= 1.7 %; left arm: mean CV=2.7%).

Erythema					
Right arm	Right arm	Right arm	Left arm	Left arm	Left arm
ICC 24-48h	ICC 48-72h	ICC 24-72h	ICC 24-48h	ICC 48-72h	ICC 24-72h
0,561	0,513	0,421	0,515	0,687	0,443
P-value	P-value	P-value	P-value	P-value	P-value
0,015	0,021	0,055	0,018	0,002	0,040
Skin temperature					
Right arm	Right arm	Right arm	Left arm	Left arm	Left arm
ICC 24-48h	ICC 48-72h	ICC 24-72h	ICC 24-48h	ICC 48-72h	ICC 24-72h
0,599	0,448	0,462	0,144	0,632	0,306
P-value	P-value	P-value	P-value	P-value	P-value
0,003	0,045	0,013	0,307	0,003	0,111

Erythema					
Right arm CV 24-48h	Right arm CV 48-72h	Right arm CV 24-72h	Left arm CV 24-48h	Left arm CV 48-72h	Left arm CV 24-72h
5,9%	**6,8%**	**7,3%**	**6,4%**	**4,7%**	**6,9%**
Mean CV			Mean CV		
6,7%			**6,0%**		
Skin temperature					
Right arm CV 24-48h	Right arm CV 48-72h	Right arm CV 24-72h	Left arm CV 24-48h	Left arm CV 48-72h	Left arm CV 24-72h
1,5%	**2,0%**	**1,6%**	**2,9%**	**2,4%**	**2,9%**
Mean CV			Mean CV		
1,7%			**2,7%**		

Primary hyperalgesia

Brush induced allodynia

RM ANOVA test detected a significant increase in brush induced allodynia at 48h after the irradiation (P=0.02). The model produced the same degree of allodynia in both arms (P =0.249). The model did not produce a brush induced allodynia between 24-72h, as it only was detected at 48h. This instability is a significant bias between the sessions, which would invalidate any reliability analysis.

Von Frey induced primary hyperalgesia

RM ANOVA test did not detect a significant increase in primary hyperalgesia induced by von Frey filaments (P=0.124). The subjects did not have similar sensation in both arms (P=0.025). Primary hyperalgesia was not detected by this test during 24-72h. This instability is a significant bias between the sessions, which would invalidate any reliability analysis.

Pinprick induced primary hyperalgesia

RM ANOVA test detected a significant increase in the pinprick induced primary hyperalgesia after the irradiation (P<0.001). The intensity of primary hyperalgesia did not differ between the arms (P=0.755). RM ANOVA test detected a significant difference between the pin sizes (P<0.001). The intensity of primary hyperalgesia was proportionally increased with an increase in pin size. The model was stable during 24-72h and the reliability test also revealed stability except for the pin with 1.6 mN (see table 3). The CV values indicated a small overall inter-individual variation between the sessions for pins with a weight of 6.4, 12.8, 25.6, 50.1 and 60.0 mN (see table 4).

0.8 pin Prick primary hyperalgesia					
Right arm	Right arm	Right arm	Left arm	Left arm	Left arm
ICC 24-48h	ICC 48-72h	ICC 24-72h	ICC 24-48h	ICC 48-72h	ICC 24-72h
0,793	0,723	0,832	0,467	0,791	0,578
P-value	P-value	P-value	P-value	P-value	P-value
0,000	0,001	0,000	0,040	0,00	0,009

1.6 pin Prick primary hyperalgesia					
Right arm	Right arm	Right arm	Left arm	Left arm	Left arm
ICC 24-48h	ICC 48-72h	ICC 24-72h	ICC 24-48h	ICC 48-72h	ICC 24-72h
0,758	0,786	0,742	0,675	0,488	0,046
P-value	P-value	P-value	P-value	P-value	P-value
0,000	0,000	0,001	0,003	0,033	0,438

3.2 pin Prick primary hyperalgesia					
Right arm	Right arm	Right arm	Left arm	Left arm	Left arm
ICC 24-48h	ICC 48-72h	ICC 24-72h	ICC 24-48h	ICC 48-72h	ICC 24-72h
0,601	0,735	0,500	0,650	0,823	0,641
P-value	P-value	P-value	P-value	P-value	P-value
0,008	0,001	0,028	0,003	0,000	0,005

6.4 pin Prick primary hyperalgesia					
Right arm	Right arm	Right arm	Left arm	Left arm	Left arm
ICC 24-48h	ICC 48-72h	ICC 24-72h	ICC 24-48h	ICC 48-72h	ICC 24-72h
0,468	0,645	0,513	0,719	0,853	0,605
P-value	P-value	P-value	P-value	P-value	P-value
0,037	0,004	0,025	0,001	0,000	0,008

12.8 pin Prick primary hyperalgesia					
Right arm	Right arm	Right arm	Left arm	Left arm	Left arm
ICC 24-48h	ICC 48-72h	ICC 24-72h	ICC 24-48h	ICC 48-72h	ICC 24-72h
0,481	0,799	0,531	0,772	0,805	0,692
P-value	P-value	P-value	P-value	P-value	P-value
0,031	0,000	0,020	0,000	0,000	0,002

25.6 pin Prick primary hyperalgesia					
Right arm	Right arm	Right arm	Left arm	Left arm	Left arm
ICC 24-48h	ICC 48-72h	ICC 24-72h	ICC 24-48h	ICC 48-72h	ICC 24-72h
0,637	0,718	0,616	0,774	0,870	0,675
P-value	P-value	P-value	P-value	P-value	P-value
0,003	0,001	0,004	0,000	0,000	0,002

50.1 pin Prick primary hyperalgesia					
Right arm	Right arm	Right arm	Left arm	Left arm	Left arm
ICC 24-48h	ICC 48-72h	ICC 24-72h	ICC 24-48h	ICC 48-72h	ICC 24-72h
0,819	0,822	0,678	0,774	0,858	0,716
P-value	P-value	P-value	P-value	P-value	P-value
0,000	0,000	0,002	0,000	0,000	0,001

60.0 pin Prick primary hyperalgesia					
Right arm	Right arm	Right arm	Left arm	Left arm	Left arm
ICC 24-48h	ICC 48-72h	ICC 24-72h	ICC 24-48h	ICC 48-72h	ICC 24-72h
0,844	0,814	0,678	0,707	0,857	0,718
P-value	P-value	P-value	P-value	P-value	P-value
0,000	0,000	0,001	0,001	0,000	0,001

0.8 pin Prick primary hyperalgesia					
Right arm CV 24-48h	Right arm CV 48-72h	Right arm CV 24-72h	Left arm CV 24-48h	Left arm CV 48-72h	Left arm CV 24-72h
25,9%	**30,8%**	**27,6%**	**40,1%**	**27,1%**	**37,2%**
Mean CV			Mean CV		
28,1%			**34,8%**		

1.6 pin Prick primary hyperalgesia					
Right arm CV 24-48h	Right arm CV 48-72h	Right arm CV 24-72h	Left arm CV 24-48h	Left arm CV 48-72h	Left arm CV 24-72h
21,9%	**22,6%**	**27,4%**	**28,4%**	**39,7%**	**47,7%**
Mean CV			Mean CV		
24,0%			**38,6%**		

3.2 pin Prick primary hyperalgesia					
Right arm CV 24-48h	Right arm CV 48-72h	Right arm CV 24-72h	Left arm CV 24-48h	Left arm CV 48-72h	Left arm CV 24-72h
25,1%	**20,1%**	**29,1%**	**24,8%**	**17,3%**	**26,5%**
Mean CV			Mean CV		
24,8%			**22,9%**		

6.4 pin Prick primary hyperalgesia					
Right arm CV 24-48h	Right arm CV 48-72h	Right arm CV 24-72h	Left arm CV 24-48h	Left arm CV 48-72h	Left arm CV 24-72h
20,6%	**15,5%**	**21,5%**	**18,8%**	**13,9%**	**23,4%**
Mean CV			Mean CV		
19,2%			**18,7%**		

12.8 pin Prick primary hyperalgesia					
Right arm CV 24-48h	Right arm CV 48-72h	Right arm CV 24-72h	Left arm CV 24-48h	Left arm CV 48-72h	Left arm CV 24-72h
18,5%	**10,4%**	**19,7%**	**14,8%**	**13,3%**	**17,4%**
Mean CV			Mean CV		
16,2%			**15,2%**		

25.6 pin Prick primary hyperalgesia					
Right arm CV 24-48h	Right arm CV 48-72h	Right arm CV 24-72h	Left arm CV 24-48h	Left arm CV 48-72h	Left arm CV 24-72h
13,3%	**10,4%**	**14,4%**	**13,2%**	**9,5%**	**16,8%**
Mean CV			Mean CV		
12,7%			**13,2%**		

50.1 pin Prick primary hyperalgesia					
Right arm CV 24-48h	Right arm CV 48-72h	Right arm CV 24-72h	Left arm CV 24-48h	Left arm CV 48-72h	Left arm CV 24-72h
7,6%	**7,3%**	**10,1%**	**11,7%**	**8,4%**	**13,0%**
Mean CV			Mean CV		
8,3%			**11,0%**		

60.0 pin Prick primary hyperalgesia					
Right arm CV 24-48h	Right arm CV 48-72h	Right arm CV 24-72h	Left arm CV 24-48h	Left arm CV 48-72h	Left arm CV 24-72h
6,4%	**6,6%**	**8,1%**	**11,1%**	**6,9%**	**10,8%**
Mean CV			Mean CV		
7,0%			**9,6%**		

Pressure pain threshold (PPT)

RM ANOVA test detected a significant decrease in the PPT after the irradiation (P<0.001). The PPT decreased in both arms but with significantly different degrees (P=0.02). Similar degree of PPT was detected during 24-72h, but the reliability test only revealed "excellent" ICC values for the right arm (ICC=0.892, P<0.001; ICC =0.885, P<0.001; ICC=0.808, P<0.001). The significant difference between the two arms was also supported by the low ICC values for the left arm (see table 3). The CV values indicated a larger overall inter-individual variation between the session of the left arm compared to that of the right arm (right arm: mean CV=19.5%, left arm: mean CV=29.8).

Heat pain threshold (HPT)

RM ANOVA test detected a significant decrease in the HPT after the irradiation (P<0.001). A similar degree of decrease in the HPT was detected in both arms (0.741). The model was stable during 24-48h and the reliability test revealed "excellent" ICC values for both arms (right arm: ICC=0.926, P<0.001, left arm: ICC =0.938, P<0.001). The CV values indicated a small overall inter-individual variation between the sessions 24-48h of both arms (right arm: CV=1.6%; left arm: CV=1.8%).

PPT					
Right arm	Right arm	Right arm	Left arm	Left arm	Left arm
ICC 24-48h	ICC 48-72h	ICC 24-72h	ICC 24-48h	ICC 48-72h	ICC 24-72h
0,892	0,885	0,808	0,377	0,888	0,258
P-value	P-value	P-value	P-value	P-value	P-value
0,000	0,000	0,000	0,067	0,000	0,164
HPT					
Right arm	Right arm	Right arm	Left arm	Left arm	Left arm
ICC 24-48h	ICC 48-72h	ICC 24-72h	ICC 24-48h	ICC 48-72h	ICC 24-72h
0,926	0,962	0,894	0,938	0,963	0,947
P-value	P-value	P-value	P-value	P-value	P-value
0,000	0,000	0,000	0,000	0,000	0,000

PPT					
Right arm CV 24-48h	Right arm CV 48-72h	Right arm CV 24-72h	Left arm CV 24-48h	Left arm CV 48-72h	Left arm CV 24-72h
17,5%	**19,3%**	**21,7%**	**33,3%**	**17,4%**	**38,6%**
Mean CV			Mean CV		
19,5%			**29,8%**		
HPT					
Right arm CV 24-48h	Right arm CV 48-72h	Right arm CV 24-72h	Left arm CV 24-48h	Left arm CV 48-72h	Left arm CV 24-72h
1,6%	**1,2%**	**2,0%**	**1,8%**	**1,2%**	**1,4%**
Mean CV			Mean CV		
1,6%			**1,4%**		

Area of secondary hyperalgesia (sAREA)

Mean blood flow (BF) in the area of secondary hyperalgesia

The mean BF was also measured in the sAREA. ANOVA test did not detect a significant increase in the mean BF after the irradiation (P=0.664). No significant difference was found between the two arms (0.514).

Area of secondary hyperalgesia (sAREA)

A sAREA was observed to both von Frey filament and pinprick stimulation and sAREA remained stable during 24-72h.

sAREA to von Frey filament

sAREA to von Frey filament was detected in ten subjects. RM ANOVA test did not detected a significant difference between the measurements at 24-72h (P=0.070) but a significant difference between the arms was detected (P=0.003). Interaction between time and arm was detected (p=0.037). The model was stable during 24-72h and the reliability tests revealed "excellent" ICC values for both arms (right arm: ICC= 0.889, P<0.001; ICC=0.790, P<0.001; ICC=0.631, P= 0.002 and left arm: ICC= 0.936, P<0.001; ICC=0.917, P<0.001; ICC=0.913, P<0.001) for 24h, 48h and 72h, respectively. The CV values indicated a small overall inter-individual variation (right arm: mean CV= 31.7%; let arm: mean CV=29.9%).

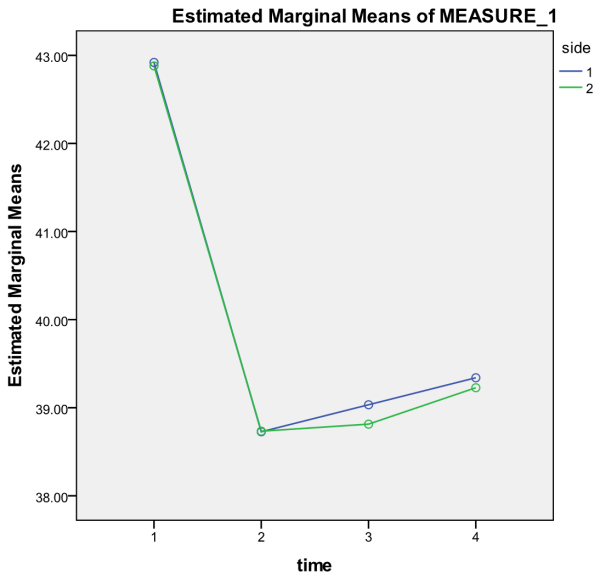
sAREA to pinprick

aAREA to pinprick was detected in eleven subjects. RM ANOVA test did not detect any significant difference between the measurements at 24-72h (P=0.223) but a significant difference between arms was detected (P=0.009). Interaction between time and arm was detected (p=0.035). The model was stable during 24-72h and the reliability tests revealed "excellent" ICC values for both arms (right arm: ICC= 0.889, P<0.001; ICC=0.790, P<0.001; ICC=0.631, P= 0.002; and left arm: ICC= 0.936, P<0.001; ICC=0.917, P<0.001; ICC=0.913, P<0.001) for 24h, 48h and 72h, respectively. The CV values indicated a small overall inter-individual variation (right arm: mean CV= 33.2%; let arm: mean CV=28.4%).

von Frey secondary hyperalgesia					
Right arm	Right arm	Right arm	Left arm	Left arm	Left arm
ICC 24-48h	ICC 48-72h	ICC 24-72h	ICC 24-48h	ICC 48-72h	ICC 24-72h
0,889	0,790	0,631	0,936	0,917	0,917
P-value	P-value	P-value	P-value	P-value	P-value
0,000	0,000	0,002	0,000	0,000	0,000
Pin prick secondary hyperalgesia					
Right arm	Right arm	Right arm	Left arm	Left arm	Left arm
ICC 24-48h	ICC 48-72h	ICC 24-72h	ICC 24-48h	ICC 48-72h	ICC 24-72h
0,896	0,922	0,874	0,957	0,932	0,874
P-value	P-value	P-value	P-value	P-value	P-value
0,000	0,000	0,000	0,000	0,000	0,000

Von Frey secondary hyperalgesia					
Right arm CV 24-48h	Right arm CV 48-72h	Right arm CV 24-72h	Left arm CV 24-48h	Left arm CV 48-72h	Left arm CV 24-72h
23,8%	**30,5%**	**40,6%**	**30,6%**	**25,7%**	**33,3%**
Mean CV			Mean CV		
31,7%			**29,9%**		
Pin Prick secondary hyperalgesia					
Right arm CV 24-48h	Right arm CV 48-72h	Right arm CV 24-72h	Left arm CV 24-48h	Left arm CV 48-72h	Left arm CV 24-72h
23,6%	**20,9%**	**27,1%**	**26,5%**	**34,2%**	**24,6%**
Mean CV			Mean CV		
23,9%			**28,4%**		

A



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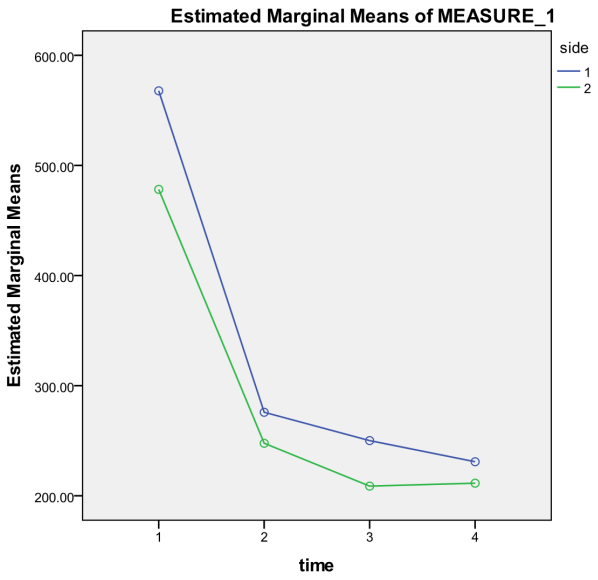
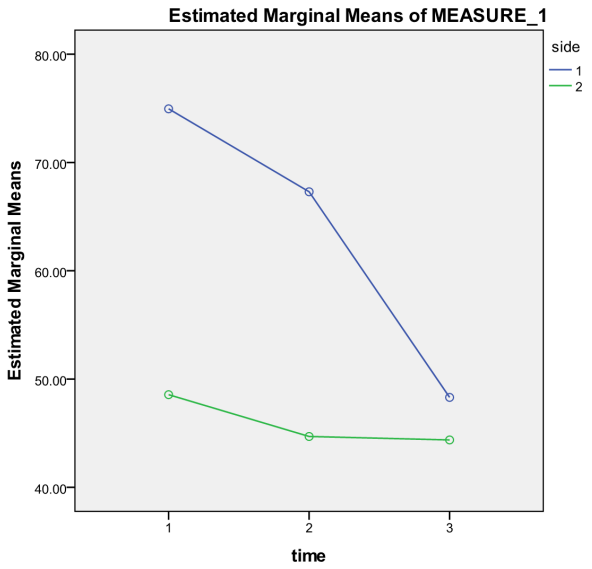


C



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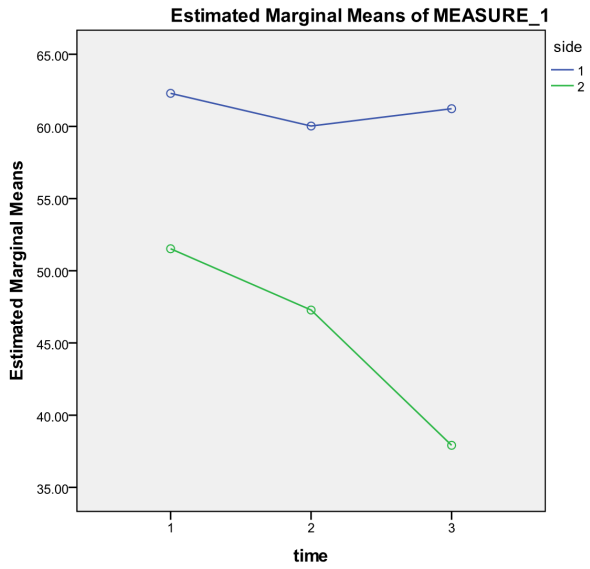

Figure A-D illustrate the dose-response curves for HPT, PPT, von Frey sAREA and pinprick sAREA, respectively.

Sample size

Sample size estimations were calculated for a parallel and a cross over study design, which are provided in below.

Test	SD = standart deviation	δ= minimum detectable difference	**Cross over study n = sample size**
Erythema	2.20027	1.35	30
Skin temp.	1.20990	0.74	30
Brush	0.72907	0.45	30
Von Frey	1.35443	0.83	30
PPT	197.00091	120.71	30
HPT	2.76470	1.69	30
pinPrick 0.8	1.86213	1.14	30
pinPrick 1.6	1.95096	1.20	30
pin Prick 3.2	2.03050	1.24	30
pin Prick 6.4	1.87612	1.15	30
pin Prick 12.8	1.65641	1.01	30
pin Prick 25.6	1.72355	1.06	30
pin Prick 50.1	1.62618	0.996	30
pin Prick 60.0	1.57841	0.97	30

Test	Mean	Change to be detected	SD	**Parallel study n = sample size**
Erythema	9,14	2,742	6,649686	184
Skin temp.	32,69666667	9,809	1,228085	1
Brush	0,57	0,171	0,867173	409
Von Frey	6,533333333	1,96	1,253695	7
PPT	523,0333333	156,91	204,9947	27
HPT	42,9	12,87	3,517261	2
pin Prick 0.8	1,137931034	0,34137931	1,965151	524
pin Prick 1.6	1,283333333	0,385	2,095728	451
pin Prick 3.2	1,575333333	0,4726	2,241331	355
pin Prick 6.4	1,93	0,579	2,382739	263
pin Prick 12.8	2,23	0,669	2,459546	211
pin Prick 25.6	2,736666667	0,821	2,52978	149
pin Prick 50.1	3,56	1,068	2,592904	92
pin Prick 60.0	3,786666667	1,136	2,625371	83

Discussion

Erythema

In this study, the UVB-induced inflammation was established by a significant level of erythema after the irradiation. A similar degree of erythema was observed within 24-72h after the irradiation. To our knowledge no other study has measured the degree of erythema, but all studies have assessed erythema by visual inspection [1-5, 11, 17-20].

The overall intra-individual variance in the data was "acceptable" and the overall inter-individual variance was low. These findings indicate that the erythema was fairly reproducible in this model and a fairly reliable indicator for the UVB-induced inflammation.

Mean BF in the area of primary hyperalgesia

An increase in the mean BF was indicated after the irradiation. The data showed a decrease in the mean BF between 24h-72h after the irradiation. This indicates that the mean BF had a peak 24h after the irradiation. Several studies on UVB-model have measured the mean BF in the primary hyperalgesic area. These studies found a significant increase in the mean BF and two of them, i.e. Bickel et al. and Sycha et al., detected a significant increase at 24h [1, 2, 5, 11, 17, 19, 20]. One study, i.e. Hoffmann and Schmelz found a peak in erythema 12h after the irradiation [11]. In the present study the detected increase in the mean BF was an indicator for the UVB-induced inflammation to a lesser extent.

Skin temperature

In the present study a significant increase in the skin temperature was detected after the irradiation. No significant difference was detected between 24-27h. Two other studies have investigated the effect of UVB irradiation on the skin temperature [4, 11], but only one of them, i.e. Sycha et al., detected a significant difference between the non-irradiated and irradiated skin. Sycha et al. did not investigate the reliability of the tests. The overall intra-individual variance in the data of the present study was "acceptable" and the overall inter-individual variance was low. These findings indicate that the skin temperature was fairly reproducible in this model and a reliable indicator for the UVB-induced inflammation.

Brush induced allodynia

The present study detected a significant increase in brush induced allodynia 48h after the irradiation. The model did not induce alloynia between 24-72h. To our knowledge only one other study, i.e. Sycha et al., has investigated the presence of brush induced allodynia 24h after irradiation, but the study did not detect the presence of allodynia [19]. These results suggest that brush evoked allodynia might be present 48h after irradiation, but this issue need to be addressed further before any conclusion can be made. Allodynia is thought to be mediated by Aβ-fibres, while hyperalgesai is thought to be mediated by Aδ-fibres [5]. The results obtained from the presence study indicate that the inflammatory process mainly induce an activation of Aδ-fibres, but not Aβ-fibres, in the primary area.

Von Frey induced primary hyperalgesia

The present study was not able to detect von Frey induced primary hyperalgesia. To our knowledge no other study has yet performed a similar test. The results imply that the von Frey filaments might not be the most suitable test of detection of primary hyperalgesia in this model. Another study, Bishop et al., have assessed the present of primary hyperalgesia with an electronic von Frey system 6h, 24h, 48h, 72h and 96h after irradiation. This study detected a significant decrease in the mechanical pain threshold for all time points with a peak 24h after irradiation, but the study did not asses the reliability of the test [5]. These results suggest that an electronic von Frey system might be

a more suitable method for detecting the presence of primary hyperalgesia in this model, but this issue needs to be further addressed before any conclusion can be made. The von Frey filaments used in the present study were (4-8; 0.0034-0.320g) applying a pressure of 8-14g/mm2, and it seemed like these filaments were not able to activate the Aδ-fibres. An increase in the size, i.e. pressure applied, of the von Frey filaments can be a solution, but this issue also needs to be further addressed before any conclusion can be made.

Pinprick induced primary hyperalgesia

The present study detected a significant increase in prinprick induced primary hyperalgesia. The intensity of hyperalgesia was proportionally increased with an increase in pin size. To our knowledge only one other study, i.e. Sycha et al., has investigated the present of pinprick induced primary hyperalgesia 24h after the irradiation. This study also found that the intensity of hyperalgesia was proportionally increased with an increase in pin size [19]. Based on the results obtained from the the present study the pinprick test seems to be an overall reliable method for the detection of primary hyperalgesia in this model, i.e. a reliable indicator for the inflammatory activation of Aδ-fibres in the area of primary hyperalgesai.

Pressure pain threshold (PPT)

The present study detected a significant decrease in the PPT, but the degree of decrease differs between arms. The PPT was in general lower on the left arm in comparisons to the right arm. The issue might be a matter of dominance, as according to Wolf and Jarvik the non-dominant side is more sensitive to pain than the dominant side and thirteen of the fifteen subjects were right-handed [28]. The pain responses of the dominant side produce smaller scatter than those from the non-dominant side, and therefore are more reliable, this is in agreement the ICC and CV obtained for this test. To our knowledge no other studies have investigated the PPT in this model. Additional studies on the application of this test in the model should be performed before any conclusion can be made.

Heat pain threshold (HPT)

The present study has detected a significant decrease in the HPT with a peak at 24h. The decrease was stable between 24h and 48h, a small raise was seen at 72h, but the decrease was still significantly different from the baseline measurement. Several studies have investigated the HPT in this model and have detected a significant decrease in the HPT 24h after irradiation [3, 5, 11, 17-19]. Two other studies, Hoffmann et al. and Bishop et al. , have also investigated the time-course of the HPT with similar result as seen in the present study [5, 11], but these studies did not investigate the reliability of this test. The test for HPT seems to be a reliable based on the results from the present study, i.e. the test of HPT is a reliable indicator for the inflammatory activation of C-fibres in the primary area of hyperalgesai.

Mean BF in the area of secondary hyperalgesia

In this study the mean BF in the sAREA was investigated. No change in the mean BF was detected after the irradiation. According to previous studies, the sAREA is believed to be a result of sensitization in the neurons of the CNS and our data supports this theory [1, 2, 5]. No change in the mean BF in the sAREA indicates the fact that the detected sAREA is not a result of inflammation, but it is due to a change in the CNS. To our knowledge, no other study has investigated the mean BF in the sAREA.

Area of secondary hyperalgesia (sAREA)

In this study sAREA was observed to both cutaneous mechanical hyperalgesia induced by von Frey filament and pinprick stimuli and the area remained stable during 24-72h. Although

both methods were able to detect a sAREA, pinprick seemed to be a more sensitive test, as a higher number of volunteers showed sAREA to pinprick as compared to that of von Frey filaments. This could also be a coincidence, but since no other studies have compared the two tests, this issue needs to be further addressed before any conclusion can be made. Results from the present study indicated an "excellent" reliability for both tests, which was supported by low inter-individual variance. Based on these results of the present study, both tests seem to be reliable for the detection of the sAREA.

The UVB-induced sAREA has been investigated in several other studies [1-3, 5, 19], but different sites of irradiation were chosen and not all of the studies succeeded in the detection of the sAREA. In three studies [1-3] the site of UVB-irradiation was the ventral-medial aspect of the upper leg and a sAREA was detected by a von Frey filament (150g). One study, i.e. Sycha et al. [19], irradiated the upper ventral aspect of the leg and detected a sAREA with a custom-made weighted pinprick probe (force,256 mN). In another study, i.e. Bishop et al. [5], an irradiated area of the forearm was stimulated with von Frey filament (10g), but the study did not detect any sAREA. This finding was similar to the results obtained in a preliminary pilot study performed before the conduction of the present study. In this pilot study the forearms of four volunteers were irradiated, but sAREA was not detected in any of the volunteers. The results from the preliminary pilot study and the study performed by Bishop et al. can lead to the assumption that the presence sAREA might depend on the site of irradiation. The possible relation between the development of a sAREA and the site of the irradiation needs to be further addressed before any conclusion can be made.

Another noticeable factor is the weight of the von Frey filament used for the detection of the sAREA. The studies applying 150g von Frey [1-3] were able to detect the presence of sAREA, while the study applying the 10g, i.e. Bishop et al., von Frey filament did not detect sAREA [5]. A 10g von Frey was probably not a suitable pressure for the detection any sAREA. The present study was able to detect the sAREA with a 24g von Frey filament. Based on these results of the present study and the study by Bishop et al. the conclusion would be that the von Frey filament must weigh more than 10g. But the results from the present study indicate that the weight of 24g can be used, instead of 150 g, in order to detect sAREA.

Conclusion

The present study concluded that the UVB-pain model was successful in inducing a local cutaneous inflammation in a circumscribed skin area, as both alteration in the BF and visually erythema was noticed. Furthermore, significant increase in both skin temperature and erythema were detected.

For detection of mechanical induced primary hyperalgesia the present study concluded that the pinprick was the most suitable test in comparison to von Frey filaments.
Detection of the HPT was found to be a highly reliable test in the UVB-pain model. PPT only seemed to be reliable on the dominant side. Area of secondary hyperalgesai was detected to both von Frey filament and pinprick stimuli and both test seemed to be reliable, the issues of which test should be preferred need to be addressed further.

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List of Abbreviations

ANOVA – Analysis of Variance
BF – Blood Flow
BMI – Body Mass Index
CNS – Central Nervous System
CPT – Cold Pain Threshold
CRF – Case Report Form
CV – Coefficient of Variance
EPTT – Electrical Pain Tolerance Threshold
HPT – Heat Pain Threshold
HPTT – Heat Pain Tolerance Threshold
ICC – Intra-Class Correlation
MED – Minimal Erythema Dose
MPT – Mechanical Pain Threshold
RM ANOVA – Repeated Measures ANOVA
sBF – Superficial Blood Flow
sAREA – Area of secondary hyperalgesia
SD – Standard Deviation
SOP – Standard Operational Procedure
UVR – Ultraviolet Radiation
UVB – Ultraviolet-B

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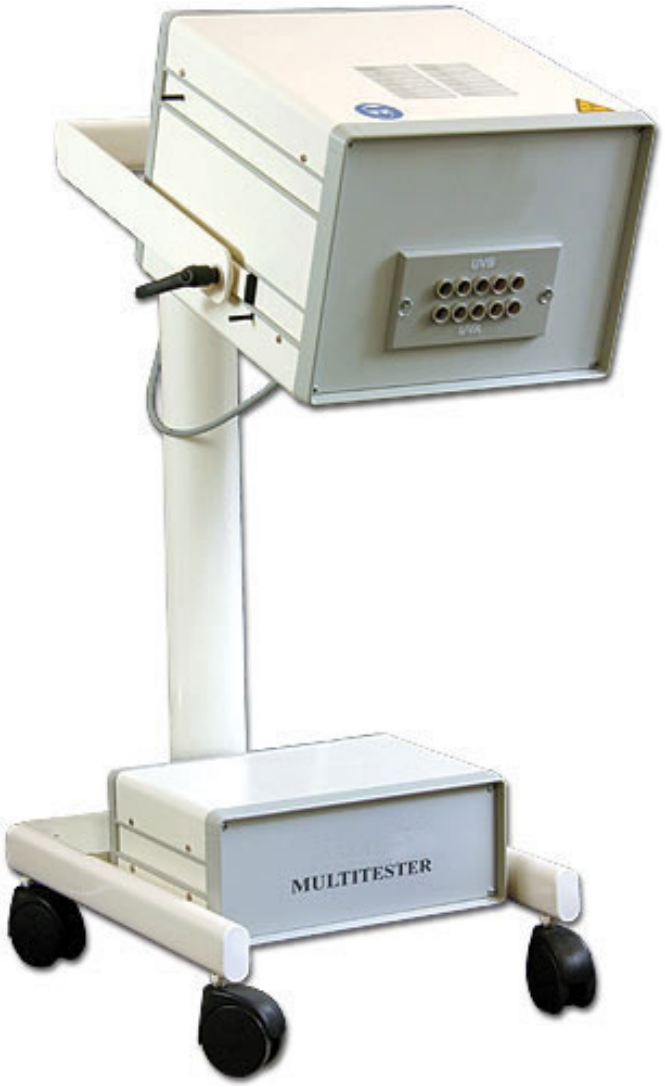
Appendix A - Protocol UVB-model

Protocol UVB-model



Videreudvikling og validering af en human biomarkør til kutan inflammatorisk smerte (UVB-model) – et metodisk studie i raske forsøgspersoner

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Forsøget udføres i overensstemmelse med nedenstående protokol og gældende myndighedskrav.

Baggrund

Smerter er et meget hyppigt forekommende symptom, der har stor betydning for den enkelte patient. Specielt har vedvarende smerter store omkostninger for den enkelte patient i form af nedsat livskvalitet som følge af nedsat mobilitet, øget risiko for depression, påvirkning af familieliv, risiko for social isolation og/eller økonomiske omkostninger ved tabt arbejdsevne mm. Ligeledes påfører patienter med vedvarende smerter samfundet store økonomiske omkostninger som følge af hyppigere kontakt til sundhedssystemet, længere hospitalsophold, flere sygedage mm.

Smerter er et subjektivt fænomen, og sundhedspersonalet oplever dagligt store udfordringer med at give den enkelte smertepatient den mest optimale smertebehandling. Tidligere blev smertesystemet opfattet som et statisk system, men de senere års forskning i nervesystemet har vist, at nervesystemet, både perifert og centralt, kan undergå væsentlige neuroplastiske forandringer, som blandt andet manifesterer sig som forøget reaktion på smertepåvirkning.

Perifere nociceptorer er receptorer, der findes i hud, muskler, sener, ledkapsler inkl. ledvæske, knoglehinden og selve knoglevævet. Det perifere nervesystem består af myeliniserede (Aδ) og umyeliniserede (C) afferente nervefibre med nociceptorer, der kan aktiveres af mekaniske, kemiske, termiske og elektriske stimuli. Nociceptorerne er et effektivt og livsnødvendigt advarselssystem, og ved skade på vævet vil centralnervesystemet blive informeret via nociceptorerne. I raskt væv vil omkring 30 % af nociceptorerne være inaktive og først blive aktiveret ved mere ekstreme forhold eller påvirkninger. Hvis vævet udsættes for langvarig eller gentagen inflammation, sker der direkte eller indirekte forandringer, som øger nociceptorernes følsomhed. Denne øgede sensibilisering bevirker, at der afgives flere impulser end normalt ved forholdsvis beskedne stimuli. Impulserne vil opleves som smerte og denne tilstand kaldes hyperalgesi. Fænomenet hyperalgesi er velkendt i inflammeret væv. Humane neurofysiologiske studier har vist, at primær hyperalgesi er forårsaget delvist af sensibilisering af de afferente nociceptorer, mens sekundær hyperalgesi formodentlig skyldes sensibilisering af centrale smertetransmissionsneuroner.

Der er udviklet humane eksperimentelle smertemodeller, som simulerer akut nociception og neural sensibilisering, og af etiske årsager må sådanne smertemodeller ikke indebære faktisk nerveskade. Neurale sensibiliseringsmodeller bruger længerevarende eller intens fokal smertestimulation til at inducere reversibel kutan allodyni (lavere smertetærskel) og hyperalgesi, som simulerer aspekter af klinisk nociceptiv og neuropatisk smerte. De underliggende mekanismer bag termal kutan sensibilisering synes at have karakteristiske egenskaber i lighed med de mekanismer, der ligger til grund for akutte og kroniske smertelidelser i klinisk praksis. Således menes kronisk sensibilisering af centrale smertetransmissions-neuroner at spille en rolle i udviklingen af kroniske smertesyndromer. Humane eksperimentelle smertemodeller formodes derfor at have potentiale til at udforske smertemekanismer og teste virkningen af nye smertestillende midler samt kombinationer af disse, idet de reversibelt forårsager øget sensibilisering. Eksperimentel anvendelse af humane smertemodeller har også til formål at udgøre et bindeled mellem smertemodeller i dyr og smertelidelser i klinisk praksis.

En ideel human eksperimental smertemodel skal reversibelt kunne inducere stabile og langvarige sensoriske forandringer uden at forårsage vævsskade. Da varighed og omfang af den sekundære hyperalgesi er afhængig af den initiale stimulus i primærzonen, er de oftest anvendte smertemodeller enten invasive (*Intradermal Capsaicin* Model; capsaicin injekteres under huden) eller forbundne med potentiel vævsskade (*Burn Injury* Model; varme appliceres på et kutant område).

I UVB- modellen anvendes ultraviolet B (UVB) bestråling til at fremkalde en kutan inflammation med en termisk og mekanisk sensibilisering af nociceptorerne i huden. . Denne model er specielt god til at opnå vedvarende primær hyperalgesi og muligvis sekundær hyperalgesi.

Denne aktivering medfører, at nociceptorerne sensibiliseres (bliver ekstra smertefølsomme, perifer hyperalgesi) og samtidig medfører det centrale ændringer (central hyperalgesi). Disse ændringer er vigtige at studere, da det er mekanismer, som ofte opstår hos patienter med kroniske smerter (specielt nervesmerter).

Den humane eksperimentelle smertemodel, UVB-sensibilisering, menes at kunne inducere stabil, vedvarende og reproducerbar primær og muligvis sekundær hyperalgesi i et omfang, der gør det muligt at teste smertestillende midler. Modellen har således været anvendt i flere studier med smertelindrende midlers virkning og effekt (Chizh et al 2007; Sycha et al 2006; Sycha et al 2005; Gustoff et al 2004; Sycha et al 2003; Koppert et al 1999; Bickel et al 1998).

Samtidig kan en sådan eksperimentel smertemodel anvendes til at undersøge effekten af lægemidler, som er udviklet til netop til at behandle denne typer smerter. En effektiv, sikker og anvendelig human smertemodel vil således bidrage til den medicinske smerteforskning, da det muliggør human klinisk afprøvning af ny smertestillende medicin i simulerede smertepatienter. Dette kan medvirke til en øget virkning af smertemedicin i faktiske smertepatienter. Projektet har til formål at videreudvikle og validere UVB- modellen, en sensibiliseringsmetode, for at kunne anvende modellen i fremtidig smerteforskning samt i udviklingen og afprøvningen af nye smertestillende medikamenter.

Formål

Projektets formål er:

1. At undersøge UVB-modellens intra-individuelle variation over tid.
2. At undersøge den intraindividuelle variation mellem gentagne sessioner med to uges interval.
3. At undersøge UVB-modellens interindividuelle variation.
4. At udforme en tabel med styrkeberegninger. (Hvor mange personer skal inkluderes for at detektere en given effekt).

Materiale og metoder

Forsøgspersoner

15 raske mandlige forsøgspersoner rekrutteres blandt de studerende på Aalborg Universitet via e-mail og opslag på universitet. Personer, som er egnet til deltagelse i forsøget, vil få tilsendt en deltagerinformation og blive bedt om at melde tilbage, om de er interesseret i at deltage. Deltagerinformationen fremsendes, hvilket giver de potentielle deltagere mulighed for at læse informationen grundigt igennem og evt. diskutere denne med deres pårørende. Ønsker en person at deltage, vil han blive bedt om at møde op på Laboratoriet for Eksperimentel Smerteforskning på Aalborg Universitet, Center for Sanse-Motorisk Interaktion, hvor en mundtlig gennemgang af forsøget foretages og evt. spørgsmål vil blive besvaret og diskuteret. De mulige deltagere får besked på, at det er tilladt at have en pårørende med til samtalen. De mulige forsøgsdeltagere har 24 timers betænkningstid efter have modtaget den skriftlige og mundtlige information. Når forsøgsdeltagerne har givet deres informerede samtykke, skal de gennemgå en screeningssession.

Inklusionskriterier:

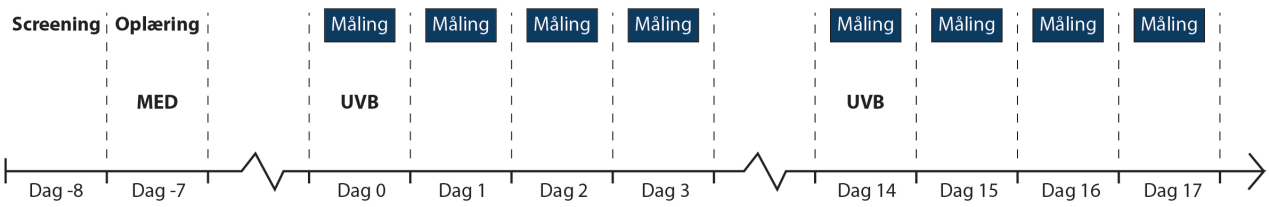
- Raske mænd (18-30 år).
- Dansk-talende forsøgspersoner.
- Normalt BMI (18,5-24,9).

Eksklusionskriterier:

- Addiktiv eller tidligere addiktiv adfærd defineret som misbrug af hash, opioder eller andre euforiserende stoffer.
- Bærer af smitsomme sygdomme.
- Akut smerte-tilstand.
- Tidligere neurologiske, muskuloskeletale eller psykiske sygdomme.
- Hudsygdomme på det bestrålede areal, som kan påvirke responset af UVB-bestrålingen.
- Manglende samarbejdsevne.
- Indtagelse af alkohol, koffein eller smertestillende medicin 24 timer inden og på undersøgelsesdagene samt imellem disse.
- Udsættelse af det bestrålede område for UV- bestråling under forsøgets forløb.
- Deltagelse i andre forsøg 2 uger før og under selve forsøget.

Forsøgsdesign

Studiet består af en screeningssession, en oplæringssession med bestemmelse af Minimal Erythema Dose (MED), dvs. den mængde UVB-lys, der skal til, for at den enkelte person får et rødt irriteret område på huden (svarende til en førstegradsforbrænding), efterfulgt af 2 x 4 målingssessioner med to ugers interval, se figur 1 for en illustration af forsøgsforløbet.

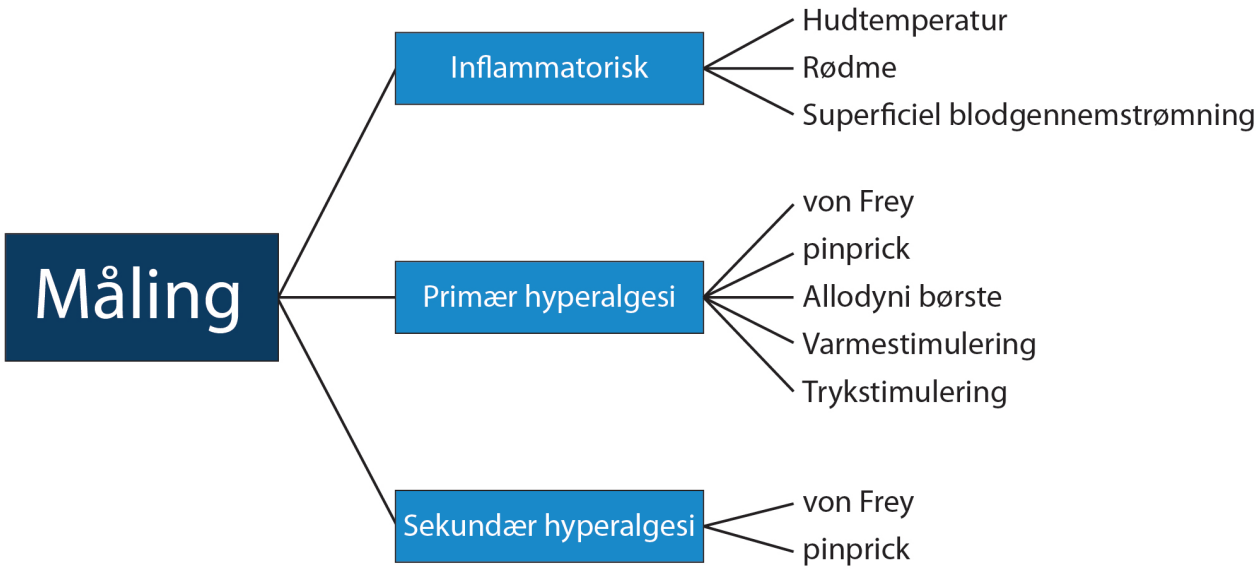


Figur 1.
Illustrerer forløbet af forsøget, som starter med en screeningssession efterfulgt af en oplæringssession. UVB-bestråling forgår på dag 0 og 14, hvor de forskellige målinger bliver taget før UVB-stimuleringen. Målingerne bliver gentaget i de efterfølgende tre dage. Alle procedurerne bliver gentaget fra dag 14 og forsøget slutter på dag 17.

Screening: Når de mulige forsøgsdeltagere har modtaget deltagerinformationen skriftligt og mundtligt, læst, diskuteret og accepteret den, kan de give deres informerede samtykke. Herefter betragtes de som forsøgsdeltagere og indgår i forsøgets sessioner. Den første session er den grundlæggende screening.

Oplæringssessionen og bestemmelse af MED: De 15 forsøgsdeltagere gøres bekendt med metoderne og lærer at skelne mellem normal perception og hyperalgesi, hvilket er kritisk for undersøgelsen. Evnen til at kunne skelne mellem normal perception og hyperalgesi er vigtig, fordi kvantificeringen af den sekundære hyperalgesi udføres på baggrund af forsøgsdeltagernes subjektive oplevelse af den mekaniske stimulering. Denne session vil maksimalt tage en time. Graden af bestråling afhænger af hudtypen, derfor bestemmes den individuelle Minimal Erythema Dose (MED), dvs. den mængde UVB-lys, der skal til, for at den enkelte person får et rødt, irriteret område.

Session 1-4: Udgør selve undersøgelsesdagene (procedure 3-12), hver af disse sessioner varer maksimalt 2 timer og udføres før bestrålingen samt 24, 48, 72 timer efter. Figur 2 illustrerer metoderne, som udføres under ét sessionsforløb.



Figur 2.
Viser de forskellige metoder, som bliver foretaget på dage 0-3 og igen på dag 14-17. Metoderne kan opdeles i tre hovedtyper. Inflammatoriske målinger, målinger for primær hyperalgasi og målinger for sekundær hyperalgesi.

Projektet er et metodisk studie, der tilstræber at validere og videreudvikle UVB-sensibiliseringsmodellen. Den samme undersøger vil stå for samtlige sessioner med en given forsøgsperson for at minimere inter-observatørvariationer.

Videnskabelige metoder

Procedure 1: Screening (60 minutter)
Under screeningssessionen vil hver forsøgsdeltagerene ved ankomst blive vist til rette i et afsides rum, hvor de vil gennemgå en grundlæggende screening, som indeholder følgende:

- Inklusions- og eksklusions-kriterier
- Anamnese
  - Tidligere og nuværende brug af medicin
- Fysisk eksamination
- Højde, vægt og alder

Procedure 2: Oplæringssession og bestemmelse af MED (60 minutter)
Alle procedurer for UVB-sensibilisering gennemgås. Alle procedure for de gentangende måle-metoderne gennemgås, så forsøgsdeltagerne får en forståelse for hver eneklt metode. Graden af bestråling afhænger af hudtypen, derfor bestemmes den individuelle Minimal Erythema Dose (MED), dvs. den mængde UVB-bestråling, der skal til, for den enkelte person får et rødt, irriteret område. MED bestemmes ved bestråling på indersiden af underarmen. Fem cirkler med en diameter på 1,5 cm bestråles med stigende doser UVB-lys.

Procedure 3: UBV-modelen, UVB-lys stimulering (5 minutter)
Indersiden af overarm (medial siden) stimuleres med 3XMED UVB-lys (290-320nm) i et cirkulært område med en diameter på 5 cm. Målinger foretages førsamt 24, 48 og 72 timer efter stimulering med UVB-lys. Ved stimuleringen opnås en førstegradsforbrænding af huden, som efterfølgende vil blive rød og irriteret.

Procedure 4: Rødme (5 minutter)
Hudens rødme måles med et ColorMeter (DSMII).

Procedure 5: Måling af den superficielle blodgennemstrømning (5 minutter)
Den superficielle blodgennemstrømning måles ved brug af et laser Doppler-system (Moor Instruments, Devon, Storbritannien). Apparatet producerer et outputsignal, som er proportionalt med blodcelleperfusionen (eller flux). Laserhovedet anbringes 30 cm over målestedet. Scanningsarealet er 8 × 8 cm. Blodgennemstrømningen måles før UVB-strålingen og efter bestrålingen. Middelblodgennemstrømningen beregnes ved brug af relativ flux (vilkårlige enheder).

Procedure 6: Von Frey stik - Kvantificering af sekundær hyperalgesi (5 minutter)
Det primære område defineres som det bestrålede område. Det sekundære område kvantificeres med en let mekanisk stimulering med et kalibreret nylonfilament (von Frey 26g). Områder, som føles smertefulde (ændring i opfattelsen af stimuleringerne), optegnes og defineres som det sekundære hyperalgesi-område.
Der stimuleres langs 8 liniære forløb arrangeret vertikalt, horisontalt og diagonalt omkring stimulationszonen ud fra fire vectorer tegnet på deltagerens overarm. Armen holdes horisontal under forløbet, og den støttes af en pude. Stimuleringen initieres udenfor det primære hyperalgesiske område og fortsætter ind mod den behandlede zone med 5-mm mellemrum med intervaller af 4 sekunder. Der fortsættes, indtil deltagerne rapporterer om en klar ændring i perceptionen af stimuli (brændende fornemmelse, ømhed, intense stik). Dette gentages for alle vectorer og de enkelte punkter, og hvor perceptionen ændrer karakter, markeres med en filtpen. Det definerede område overføres herefter til en klar transparant film (på forhånd mærket med deltagernummer og tidspunkt), og overfladearealet af den sekundære hyperalgesi kan derefter beregnes ved digitalisering. Det er ikke tilladt for deltagerne at se det testede område under proceduren.

Procedure 7: Pinprick stimulus-respons funktion (5 minutter)
Pinprick-testen anvendes til bestemmelse af intensiteten af gradueret pinprick hyperalgesi (stimulus-responsfunktion) i det primære og sekundære hyperalgesi-område. Indenfor området med primær hyperalgesi, ca. 5 cm, bestemmes intensiteten af graduerede pinprick-stimuli ved brug af graduerede metalprober (diameter 0,6 mm, Aalborg Universitet) med 8 forskellige fikserede vægte: 8, 16, 32, 64, 128, 256,501 og 600 mN. Hver vægt appliceres 3 gange i 2 sek. med 10 sek.

interval på omtrent samme sted. Forsøgsdeltagerne afgiver en gennemsnitlig score på en elektrisk visuel analog skala (eVAS), hvor ”0 cm” indikerer ”ingen smerter” og ”10 cm” indikerer ”maksimal smerte”. Forsøgsdeltagerne instrueres i, at midten af skalaen er smertetærsklen, styrken af de ikke-smertefulde prik-scorer placeres på den nederste halvdel, og styrken af de smertefulde prik-scorer placeres på den øverste halvdel. Scoren omsættes til en numerisk værdi, aflæses og registreres i CRF’en (Case Report Form), dvs. et struktureret skema til opsamling af patientdata.
Det er ikke tilladt for forsøgsdeltagerne at se denne værdi. Herefter kan en stimulus-responsfunktion etableres.

Procedure 8: Bestemmelse af intensiteten af UVB-induceret allodyni (5 minutter)
Bestemmelse af allodyni i området med primær hyperalgesi foretages ved at stryge en standardiseret børste (Somedic, Sverige) i en vinkel på 45° 2 cm longitudinelt mod huden svarende til en kraft på 200-400 mN. Der stryges 6 gange med 10 sek. interval, hvorefter forsøgsdeltagerne afgiver en gennemsnitlig score på eVAS, som omsættes til en numerisk værdi. Denne værdi aflæses og registreres i CRF’en. Det er ikke tilladt for forsøgsdeltagerne at se værdien.

Procedure 9: Trykalgometer til måling af smertetærsklen ved tryk (5 minutter)
Til bestemmelse af smertetærsklen ved tryk (pressure pain threshold (PPT) anvendes et trykalgometer (Somedic, Hörby, Sverige). Apparatet presses mod det primære hyperalgesi område på huden. Trykket påføres med en hastighed på 30 kPa/s ved hjælp af en sonde på et areal på 0,5 cm2. Forsøgsdeltagerne skal reagere, når de første gang føler, at trykket er smertefuldt. Alle forsøgsdeltagere får besked på at trykke på en knap, når de føler, at trykket lige netop er smertefuldt, dette tryk angives som PPT’er i CRF’en. Det er ikke tilladt for forsøgsdeltageren at se værdien.

Procedure 10: Hudtemperatur (5 minutter)
Hudtemperaturen bliver målt med et infrarødt termometer.

Procedure 11: Varmestimulering til bestemmelse af den termiske smertetolerance (HPT)
(5 minutter)
Til bestemmelse af den termiske smertetolerancetærskel anvendes en termostat (TSA II Medoc neuro sensory analyser). Det bestrålede hud område varmes op med termostaten, som er computerstyret. Den maksimale temperatur vil af sikkerhedsmæssige årsager være 52 °C. Når forsøgsdeltagerne opfatter varmestimuleringen som værende smertefuld, stoppes stimuleringen via en knap på en mus. Herved forsvinder varmen øjeblikkelig. Varmestimuleringen ophører automatisk, hvis en forsøgsdeltager ikke har trykket stop inden 52 °C. Termostaten indstiller temperaturen af huden til ca. 32 °C, inden selve stimuleringen startes. Det betyder, at udgangstemperaturen er den samme for alle forsøgsdeltagerne. Inden forsøget påbegyndes, bestemmes temperaturen af huden for hver enkelt forsøgsdeltager. Varmestimuleringen udføres ved samme temperatur på ca. 32 °C. Det vil sige, at hvis en af forsøgsdeltagerne har en lavere temperatur end den ønskede, bliver deltageren dækket med en trøje eller lignende, indtil temperaturen opnås og omvendt i tilfælde af en forhøjet temperatur. Huden bliver langsomt opvarmet, hvilket medfører en aktivering af C-fibrene.

Procedure 12: Sikkerhed
Procedurene forventes at fremkalde smerte hos forsøgsdeltagerne, men ydligere bivirkninger forventes ikke. Forsøgsdeltagerne vil være under observation igennem forsøget og vil være placeret i sengeleje under alle målinger.
Varigheden af hver undersøgelsesdag (Session1,2,3,4) vil være på maksimalt 2 timer. Forsøgsdeltagerne vil udgå af forsøget, hvis betydelige bivirkninger indtræffer. Frafaldne forsøgsdeltagere vil blive erstattet af nye.

Økonomisk godtgørelse
Betaling for deltagelse til raske forsøgspersoner udgør 150 kr./time. Beløbet er skattepligtigt, og det vil derfor blive oplyst til Skat som B-indkomst. Projektet er støttet af fondsbevillinger givet til Center for Sanse-Motorisk Interaktion.

Risici og ulemper
De anvendte metoder er alle undersøgt og udført i henhold til normale kliniske procedurer. Den klinisk ansvarlige er speciallæge. Der er ingen rapporter fra andre institutioner om langtidsbivirkninger ved metoderne. De anvendte stimulationer kan afbrydes på et hvert tidspunkt. Ulemperne ved forsøget er, at forsøgsdeltagerne vil føle mild til moderat smerte under stimulationerne, samt at der vil opstå rødme i det bestrålede område på indersiden af overarmen.

Statistik
ANOVA for gentagende målinger testen anvendes til at sammenligne resultaterne fra målingerne før og efter bestrålingen, i tilfældet af at det observerede data er normalt fordelt. Hvis data ikke er normalt fordelt, vil Freidman's test blive anvendt.
Fraser and Harris 1989 test vil blive anvendt til at analysere inter- og intravariation.

Styrkeberegning
Baseret på tidligere studier og den varians, der er rapporteret vurderes 15frivillige at være tilstrækkelig til at vurdere metodens validitet (Sycha et al 2006; Gustorff et al 2004; Koppert et al 1999; Bickel et al 1998).

Etiske overvejelser
Undersøgelsen overholder kravene i Helsinki-deklarationen og forelægges Den Videnskabsetiske Komité for Region Nordjylland. De anvendte metoder er blevet testet og udført i henhold til normale kliniske procedurer. Der er ikke rapporteret om langtidsbivirkninger fra vores eller andre institutioners forsøg.

Der er ikke nogle umiddelbare fordele ved deltagelse i forsøget for forsøgspersonerne, men forsøget vil bidrage til en videreudvikling og validering af en ikke-invasiv og sikker human eksperimentel smertemodel, der kan anvendes inden for smerteforskning samt til udvikling og afprøvning af smertestillende medikamenter. På længere sigt kan det muligvis lede til udvikling af bedre behandlingsregimer og dermed en mere effektiv behandling af patienter med kroniske smerter. Den anvendte smertestimulation kan afbrydes på ethvert tidspunkt. Ulempen ved disse forsøg er minimal sammenlignet med bidraget fra den opnåede viden til den medicinske verden.

Alle alvorlige bivirkninger vil blive rapporteret til Den Videnskabsetiske Komité af den klinisk ansvarlige læge. Forsøgspersonerne er dækket af institutionernes forsikring.

Personfølsomme data

Efter forsøget gemmes data i anonymiseret form i henhold til gældende regler. Disse data er reelt alene anvendelige for fortolkningen af nærværende forsøg, og de vil derfor være uinteressante for tredjepart. For hver person udarbejdes et registreringsskema, hvori data for personen noteres. Følgende registreres: alder, højde og vægt. Alle data vil blive anonymiseret og behandlet fortroligt. Data vil blive opbevaret på Center for Sanse-Motorisk Interaktion, Aalborg Universitet. Forsøget anmeldes til Datatilsynet.

Økonomi

Center for Sanse-Motorisk Interaktion, Aalborg Universitet igangsætter dette projekt, der er støttet økonomisk af Højteknologifonden med kr. 7 mio. kr. Forskerne bag protokollen har ingen personlig økonomisk interesse i projektet. På sigt kan metoden blive en metode, som CCBR kan tilbyde lægemiddelindustrien som en undersøgelsesmetode.

Tidsplan

Projektet forventes færdiggjort d. 30/6-11. ~~i løbet af ca. 4 måneder fra godkendelsesdato.~~

Publicering af resultater

Projektets resultater vil blive søgt offentliggjort i anerkendte tidsskrifter uanset udfaldet af forsøget. Projektdeltagerne er medforfattere til den videnskabelige artikel.

Referencer

Chizh B A, O'Donnell M B, et al. The effects of the antagonist SB-705498 on TRPV1 receptor-mediated activity and inflammatory hyperalgesia in humans. International Association for the Study of Pain 2007;132:132-141.

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Gustorff B, Hoechtl K, et al. The effects of Remifentianil and Gabapentin on Hyperalgesia in a New Extended Inflammatory Skin Pain Model in Healthy Volunteers. The International anesthesia Research Society 2004; 98:401-7

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Koppert W, Likar R, et al. Peripheral Antihyperalgesic Effect of Morphine to Heat, but Not Mechanical, Stimulation in Healthy Volunteers after Ultraviolet-B Irradiation. The International anesthesia Research Society 1999;88:117-22

Bickel A, Dorfs S, et al. Effects of antihyperalgesic drugs on experimentally induced hyperalgesia in man. International Association for the Study of Pain 1998;76:317-325.

Retningslinjer mundtlige information og informeret samtykke

Indkaldelse af mulige forsøgspersoner

Indkaldelse af mulige forsøgsdeltagere:
Når mulige forsøgsdeltagere kontaktes via e-mail eller opslag med henblik på deltagelse i forsøget, skal følgende oplyses:

- At der er tale om en anmodning om deltagelse i et videnskabeligt forsøg.
- Formålet med forsøget.
- At forsøgsdeltageren har ret til betænkningstid, før samtykket afgives, og at forsøgsdeltageren ligeledes har ret til at medbringe en bisidder, når den mundtlige information gives. Forsøgsdeltageren vil få udleveret skriftet "Forsøgspersonens rettigheder i et biomedicinsk forskningsprojekt", som indeholder oplysninger omkring tavshedspligt, aktindsigt og klageadgang.
- At det er frivilligt at deltage, og at forsøgsdeltageren når som helst kan trække sit tilsagn om deltagelse tilbage, uden at dette vil påvirke forsøgsdeltagerens nuværende eller fremtidige behandling.
- Tidspunkt og sted for informationssamtalen.

Informationssamtalen

Til informationssamtalen reserveres et egnet lokale, f.eks. et mødelokale, hvor samtalen kan gennemføres uforstyrret. Der kan evt. serveres kaffe, te og/eller sodavand. Selve informationssamtalen skal afholdes af den projektansvarlige eller en seniorforsker, der har fået bemyndigelse til dette.

Samtalen skal indeholde følgende oplysninger/spørgsmål:

- Det er frivilligt at deltage, og forsøgsdeltageren kan når som helst trække sit tilsagn om deltagelse tilbage, uden at dette vil påvirke hans nuværende eller fremtidige behandling.
- Forsøgsdeltageren har ret til betænkningstid før samtykket afgives, og forsøgsdeltageren har ligeledes ret til at medbringe en bisidder, når han modtager den mundtlige information.
- Forsøgsdeltageren spørges, om han ønsker, at der er en bisidder tilstede.
- Formålet med forsøget oplyses, og det forklares, hvordan forsøget skal udføres. Der tages udgangspunkt i "Deltagerinformation".
- Forsøgsdeltageren spørges, om han er sund og rask, og om han er bærer af en smitsom sygdom.
- Forsøgsdeltageren spørges, om vedkommende er dansk statsborger. Hvis svaret er nej, spørges vedkommende, om han har en gyldig arbejdstilladelse.
- "Deltagerinformation" samt skriftet "Forsøgspersonens rettigheder i et biomedicinsk forskningsprojekt" udleveres.
- Det forklares, at det udleverede skrift "Forsøgspersonens rettigheder i et biomedicinsk forskningsprojekt" indeholder oplysninger omkring tavshedspligt, aktindsigt og klageadgang.

- Forsøgsdeltageren bedes om, at gennemlæse ”Deltagerinformation”.
- Når forsøgsdeltageren har gennemlæst deltagerinformationen, spørges forsøgsdeltageren, om han har spørgsmål til forsøget.
- Herefter gives en demonstration i laboratoriet af div. måleudstyr, og deres anvendelse i forsøget fremvises til forsøgsdeltageren.
- Forsøgsdeltageren gøres opmærksom på, at han har ret til betænkningstid, før samtykket afgives. (Den Centrale Videnskabsetiske Komité anbefaler 24 timers betænkningstid).
- Forsøgsdeltageren gøres igen opmærksom på, at det er frivilligt at deltage, og at han når som helst kan trække sit tilsagn om deltagelse tilbage, uden at dette vil påvirke den nuværende eller fremtidige behandling.
- Såfremt forsøgsdeltageren ikke ønsker at gøre brug af betænkningstiden, kan samtykket afgives herefter.
- Tidspunkt og sted for forsøgets afholdelse aftales.
- Forsøgsdeltageren informeres om, hvem der er kontaktpersoner for projektet (det vises, at disse navne fremgår af ”Deltagerinformation”), og at disse til hver en tid kan kontaktes, hvis der er yderligere spørgsmål.

Lægmandsinformation

Knap 20 %, hver femte voksne dansker, har kroniske smerter, og antallet af smertepatienter vokser med 6.000- 7.000 patienter om året. Smerter har store samfundsøkonomiske konsekvenser. Smertepatienterne har således et dobbelt så højt sygefravær sammenlignet med normalbefolkningen, og en tredjedel af smertepatienterne er ophørt med at arbejde på grund af deres sygdom. Derudover har smertepatienterne væsentlig flere kontakter til sundhedsvæsenet end normalbefolkningen, ligesom de har et særdeles stort medicinforbrug.

Indsatsen i sundhedsvæsenet er imidlertid mangelfuld grundet manglende viden, ligesom medicineringen ofte ikke er optimal. Der er derfor behov for en mere målrettet indsats, som kan forbedre livskvaliteten for patienter med kroniske smerter.

Udviklingen af humane smertemodeller, der kortvarigt simulerer en smertereaktion, kan bidrage til større forståelse samt udvikling og afprøvning af nye smertelindrende midler. Ved hjælp af smertemodeller, kan virkningen og effekten af smertemedicin testes under fiktive smerteforhold i raske forsøgspersoner, så medicinering af smertepatienter kan optimeres.

Formål:
Dette projekt har til formål at videreudvikle og validere en smertemodel i raske mandlige forsøgsdeltagere. UVB-modellen (bestråling med Ultra Violet B-lys) anvendes til at fremkalde en overfladisk inflammatorisk smertereaktion, som simulerer de mekanismer der indgår i den kroniske smerte.

Formålet er at undersøge, om smertemodellens simulering af smerter kan genskabes over tid; forekommer der variation 1) over 24, 48, 72 timer i samme forsøgsdeltager, 2) over dage (to uges interval) i samme forsøgsdeltager, 3) og forskellige imellem forsøgsdeltagerne.

Baseret på resultaterne fra forsøget har projektet til formål at udforme en tabel med styrkeberegninger. Det vil sige en tabel over hvor mange personer, der skal inkluderes i et forsøg for at detektere en given effekt. Denne viden kan anvendes ved fremtidig brug af metoden.

På sigt kan forsøget bidrage til smerteforskningen ved at kvalitetssikre metoden, som kan anvendes i klinisk afprøvning af nyt smertestillende medicin.

Forsøgsdeltagerne:
15 raske mandlige deltagere, der ikke lider af smerter.
Forsøgsdeltagere indkaldes til en screening, hvor inklusions- og eksklusions- kriterier gennemgås. Forsøgsdeltagere, som opfylder disse kriterier, gennemgår en oplæringssession efterfulgt af 2x4 sessioner. Følgende undersøgelser gennemføres:

Procedure 1: Screeningsession
Under procedure 1, screeningssessionen, vil hver forsøgsdeltager ved ankomst blive vist til rette i et afsides rum, hvor vedkommende gennemgår en grundlæggende screening, der skal sikre, at personen opfylder kriterierne for at deltage i forsøget. Denne grundlæggende screening indeholder følgende:

- Inklusions- og eksklusions-kriterier
- Sygehistorie
  - Tidligere og nuværende brug af medicin
- Fysisk eksamination
- Højde, vægt og alder

Procedure 2: Oplæringssession
Under procedure 2, oplæringssessionen, gennemgår forsøgsdeltagerne procedurerne (de anvendte tests, procedure 3-12) for selve forsøget. Forsøgsdeltagerne vil få en fornemmelse af de forskellige stimulationer, således at de kan skelne mellem disse på hhv. ubehandlet og UVB-bestrålet hud. Denne session anvendes også til at bestemme forsøgsdeltagerens MED (Minimal Erythema Dose), dvs. bestemmelsen af den mængde UVB-lys, der skal til, for at den enkelte forsøgsdeltager får et rødt, irriteret område på huden. Graden af bestråling afhænger af hudtypen, derfor bestemmes den individuelle MED. Denne session er en ren oplæringssession, hvor metoder prøves, og forsøgsdeltagernes reaktioner observeres.

Screening og oplæring vil tage maksimalt 2 timer.

Bestrålingen og målingerne, som udføres før bestrålingen samt 24, 48 og 72 timer efter, er beskrevet i procedurerne 3-12 og vil tage meksimalt 2 timer.

Procedure 3: UVB-modelen, UVB-lys stimulering (5 minutter)
Indersiden af overarm bestråles med UVB-lys til fremkaldelse af en førstegradsforbrænding i et cirkulært område med en diameter på 5 cm. Målinger foretages førsamt 24, 48 og 72 timer efter stimulering med UVB-lys. Til selve bestrålingen anvendes 3xMED for at opnå den ønskende førstegradsforbrænding af huden. Ved stimulering med UVB-lys opnås førstegradsforbrændingen af huden, hvorefter området som bliver ekstra smerteføIsomt (hyperalgesi) kan bestemmes. Ved en førstegradsforbrænding af huden opnås områder med primær og sekundær hyperalgesi (områder som er ekstra smerteføIsomt).

Procedure 4: Rødme (5 minutter)
Rødmen i huden måles med et specielt apparat (colormeter), som giver en værdi for hundens rødme.

Procedure 5: Måling af den overfladiske blodgennemstrømning (5 minutter)
Den overfladiske blodgennemstrømning måles brug af et laser Doppler-system. Apparatet producerer et signal, som er proportionalt med blodgennemstrømning. Middelblodgennemstrømningen beregnes inden for et præ-defineret areal.

Procedure 6: Von Frey stik - Kvantificering af forøget smerteføelse(5 minutter)
Det primære område defineres som det bestrålede område. Det sekundære område kvantificeres med en let mekanisk stimulering med von Frey, som er et nylon filament, og en blød pensel. Områder, som føles smertefulde (ændring i opfattelsen af stimuleringerne), optegnes og defineres som det sekundære hyperalgesi-område.
Det definerede område overføres herefter til en klar transparent film (på forhånd mærket med deltagernummer og tidspunkt), og overfladearealet af den sekundære hyperalgesi kan derefter beregnes ved digitalisering. Det er ikke tilladt for deltagerne at se det testede område under proceduren.

Procedure 7: Nålestiktesten (5 minutter)
Nålestikket prikkes mod huden (pinprick) til bestemmelse af hyperalgasi-intensiteten (områder som er ekstra smerteføelsomt) i huden. Indenfor området med primær hyperalgesi, ca. 5 cm, bestemmes intensiteten af nålestik-stimuli ved brug af gradueret nålestik (diameter 0,6 mm, Aalborg Universitet) med 8 forskellige fikserede vægte. Hver vægt påføres 3 gange i 2 sek. med 10 sek. interval på omtrent samme sted. Forsøgsdeltagerne afgiver en værdi på en elektrisk smerteskala (visuel analog skala (eVAS)). Smerteskalaen går fra nul til ti, hvor ”0 cm” indikerer ”ingen smerter” og ”10 cm” indikerer ”maksimal smerte”. Forsøgsdeltagerne instrueres i, at midten af skalaen er smertetærsklen, og at styrken af de ikke-smertefulde prik placeres på den nederste halvdel af skalaen, mens styrken af de smertefulde prik- placeres på den øverste halvdel af skalaen. Værdierne omsættes til en numerisk værdi, som aflæses og registreres i CRF’en (case report form), et struktureret skema til opsamling af patientdata. Det er ikke tilladt for forsøgsdeltagerne at se denne værdi. Herefter kan en stimulus-respons-funktion etableres.

Procedure 8: Børste til bestemmelsen af intensiteten af smerter udløst ved at børste huden (allodyni) 5 minutter)
En børste stryges mod huden i området med primær hyperalgesi (det bestrålede areal, som er ekstra smerteføelsomt) til bestemmelse af graden af allodyni, (nedsat smertetærskel). Dvs. et område som har øget smerteføelse i en så høj grad at, en ellers ikke-smertefuldstimulus virker smertefuld. Børsten stryges 6 gange med 10 sek. interval, hvorefter forsøgsdeltagerne afgiver en værdi på en elektrisk smerteskala (eVAS), som omsættes til en numerisk værdi. Denne værdi aflæses og registreres i CRF’en (case report form), et struktureret skema til opsamling af patientdata. Det er ikke tilladt for forsøgsdeltagerne at se værdien.

Procedure 9: Trykalgometer til måling af smertetærsklen og smertetolerancetærsklen ved tryk (5 minutter)
Til bestemmelse af smertetærsklen for tryk og anvendes et trykalgometer; et apparat der opfører mekanisk tryk og registeret smertetærsklen. Apparatet presses mod det UVB-bestrålede område på huden og trykket påføres ved hjælp af en sonde. Forsøgsdeltagerne skal reagere, når de første gang føler, at trykket er smertefuldt, og trykket bliver derefter registreret. Alle forsøgsdeltagere får besked på at trykke på en knap, når de føler, at trykket lige netop er smertefuldt, dette tryk angives som tryk-smertetærsklen. Det er ikke tilladt for forsøgsdeltageren at se værdierne.

Procedure 10: Hudtemperatur (5 minutter)
Hudtemperaturen bliver målt med et infrarødt termometer.

Procedure 11: Varmestimulering til bestemmelse af den termiske smertetolerance tærskel) (5 minutter)
Til bestemmelse af den termiske smertetolerance anvendes en termostat. Et område af den bestrålede hud varmes op med termostaten, som er computerstyret. Den maksimale temperatur vil af sikkerhedsmæssige årsager være 52 °C. Når forsøgsdeltagerne opfatter varmestimuleringen som svarende til den termiske smertetolerancel, stoppes stimuleringen ved at forsøgspersonen trykker på en knap. Herved forsvinder varmen øjeblikkeligt. Varmestimuleringen ophører automatisk, hvis en forsøgsdeltager ikke har trykket stop inden 52 °C. Termostaten indstiller temperaturen af huden til ca. 32 °C, inden selve stimuleringen startes. Det betyder, at udgangstemperaturen er den samme for alle forsøgsdeltagerne. Inden forsøget påbegyndes, bestemmes temperaturen af huden for hver enkelt forsøgsdeltager. Varmestimuleringen udføres ved samme temperatur ca. 32 °C. Det vil sige, at hvis en af forsøgsdeltagerne har en lavere temperatur end den ønskede, bliver deltageren dækket med en trøje eller lignende, indtil temperaturen opnås. I tilfældet af at forsøgsdeltageren har en højere temperatur, vil en kold klud blive brugt til at nedkøle huden. Deltagernes termiske smertetolerance-værdi aflæses og registreres i CRF'en (case report form), et struktureret skema til opsamling af patientdata. Det er ikke tilladt for forsøgsdeltagerne at se de registreretværdier.

Procedure 12: Sikkerhed
Procedurerne forventes at fremkalde smerte hos forsøgsdeltagerne, men yderligere bivirkninger forventes ikke. Forsøgsdeltagerne vil være under observation igennem forsøget og vil være placeret i sengeleje under alle målinger.
Varigheden af hver undersøgelsesdag (Session1,2,3,4) vil være på maksimalt 2 timer. Forsøgsdeltagerne vil udgå af forsøget, hvis betydelige bivirkninger indtræffer. Frafaldne forsøgsdeltagere vil blive erstattet af nye.

Eventuelle bivirkninger og ulemper:
De anvendte metoder er alle blevet testet før, og de vil blive udført i henhold til normale kliniske procedurer. Der er ikke tidligere rapporteret om længerevarende bivirkninger ved de anvendte metoder og de anvendte stimulationer kan til enhver tid afbrydes. Forsøgets ulemper er, at forsøgsdeltagerne vil opleve en mild til moderat smerte under stimulationerne, og efterfølgende vil de i det bestrålede område få en rødme, som vil forsvinde efter et par dage.

Forsøget er godkendt af Den Videnskabsetiske Komité for Region Nordjylland, sagsnummer N-20100063.

Betaling for deltagelse til forsøgspersonerne udgør 150 kr./time. Beløbet er skattepligtigt og det vil derfor blive oplyst til Skat som B-indkomst.

Dette projekt er økonomisk støttet af Højteknologifonden med 7 mio. kr. Beløbet indgår i en forskningsfond, som er undergivet offentlig revision.

Deltagerinformation

Videreudvikling og validering af en human biomarkør til kutan inflammatorisk smerte (UVB-model) – et metodisk studie i raske forsøgspersoner

Vi vil spørge, om du vil deltage i et videnskabeligt forsøg, der udføres ved Center for Sanse-Motorisk Interaktion, Aalborg Universitet.

Før du beslutter, om du vil deltage i forsøget, skal du fuldt ud forstå, hvad forsøget går ud på, og hvorfor vi gennemfører forsøget. Vi vil derfor bede dig om at læse denne deltagerinformation grundigt.

Du vil blive inviteret til en samtale om forsøget, hvor denne deltagerinformation vil blive uddybet, og hvor du kan stille de spørgsmål, du har, til forsøget. Du er velkommen til at tage et familiemedlem, en ven eller en bekendt med til samtalen.

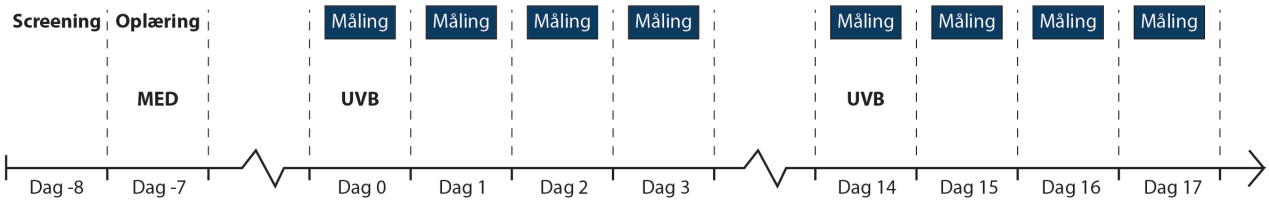
Hvis du beslutter dig for at deltage i forsøget, vil vi bede dig om at underskrive en samtykkeerklæring. Husk, at du har ret til betænkningstid, inden du underskriver samtykkeerklæringen.
Det er frivilligt at deltage i forsøget. Du kan når som helst og uden at give en grund trække dit samtykke tilbage.

Beskrivelse af forsøget:
Knap 20 %, eller hver femte voksne dansker, har kroniske smerter, og antallet af smertepatienter vokser med 6.000- 7.000 personer om året. Det nuværende behandlingsregime, der benyttes til kroniske smertepatienter, er ikke optimalt grundet manglende viden om smertemekanismerne. Derfor er der behov for en mere målrettet indsats, som kan forbedre livskvaliteten for patienter med kroniske smerter.
Udviklingen af humane smertemodeller, der reversibelt og kortvarigt simulerer kroniske, milde smerter i raske personer, kan bidrage til en mere effektiv smerteforskning samt udvikling og afprøvning af nye smertelindrende midler. Ved hjælp af smertemodeller kan virkningen og effekten af smertelindrende medicin testes under fiktive smerteforhold i ellers raske forsøgsdeltagere, så medicinering af faktiske smertepatienter kan optimeres.

Formålet med dette projekt er at:
Videreudvikle og validere en eksperimentel smertemodel (UVB-model), der benytter bestråling med ultraviolet B lys (UVB) til at fremkalde en inflammatorisk smertereaktion, som kan simulere den smerte, som den kroniske smertepatient oplever. Formålet er at undersøge, om smertemodellens simulering af smerter kan genskabes over tid; forekommer der variation 1) over 24, 48, 72 timer efter i samme forsøgsdeltager, 2) over flere dage (to uges interval) i samme forsøgsdeltager, 3) og imellem forsøgsdeltagere.

Udover at undersøge styrken, har projektet også til formål at udforme en tabel med såkaldte styrkeberegninger, dvs. en tabel over hvor mange personer, der skal inkluderes i et forsøg for at detektere en given effekt. Denne viden kan anvendes ved fremtidig brug af metoden.
På sigt kan forsøget bidrage til smerteforskningen ved at kvalitetssikre metoden, som kan anvendes i klinisk afprøvning af ny smertestillende medicin.

Forsøgets gang:
15 raske mandlige forsøgsdeltagere, der ikke lider af smerter, deltager i forsøget.
Figur 1 illustrerer forløbet af forsøget, som starter med en screeningssession (procedure 1) efterfulgt af en oplæringssession (procedure 2) med bestemmelse af Mimimal Erythema Dose (MED), dvs. den mængde UVB-lys, der skal til, for at den enkelte person får et rødt, irriteret område på huden (svarende til en førstegradsforbrænding). UVB-stråling forgår på dag 0 (session1; procedure 3-12), hvor de forskellige målinger bliver taget før bestrålingen (procedure 3-12). Målingerne bliver taget i de efterfølgende tre dage (session2-4; procedure 4-12). Alle procedurerne bliver gentaget fra dag 14 og forsøget slutter på dag 17 (session1-4; procedure 3-12).



Procedure 1: Screening
Når de mulige forsøgsdeltagere har modtaget skriftlig og mundtlig deltagerinformation, læst, diskuteret og accepterede denne med 24 timers betænkningstid, kan de afgive deres informerede samtykke. Forsøgsdeltagerne, som har afgivet informeret samtykke, vil efterfølgende gennemgår en grundlæggende screening, som indeholder følgende:

- Inklusions- og eksklusions- kriterier
- Sygehistorie
  - Tidligere og nuværende brug af medicin
- Fysisk eksamination
- Højde, vægt og alder

Procedure 2: Oplæringssessionen og bestemmelse af MED
Forsøgsdeltagere gøres bekendt med procedurerne (de anvendte tests, procedure 3-12) og lærer at skelne mellem normal smerteføelse (perception) og forøget smerteføelse (hyperalgesi), hvilket er kritisk for undersøgelsen. Denne session vil maksimalt tage to timer. Graden af bestråling afhænger af hudtypen. Derfor bestemmes den individuelle Minimal Erythema Dose (MED), dvs. den mængde UVB-lys, der skal til for at den enkelte person får et rødt, irriteret område.
Denne session er en ren oplæringssession, hvor procedurerne prøves, og forsøgsdeltagernes reaktioner observeres.

Screening og oplæring vil tage maksimalt 2 timer.

Session 1-4 udgør selve undersøgelsesdagene med bestrålingen efterfungt af tests, som udføres før og bestrålingen samt 24, 48 og 72 timer efter (procedure 3-12). Hver session vil tage maksimalt 2 timer.

Procedure 3: UVB-modelen, UVB-lys stimulering (5 minutter)
Indersiden af overarm anvendes til bestråling med 3xMED UVB-lys til fremkaldelse af en førstegradsforbrænding i et cirkulært område med en diameter på 5 cm. Ved stimulering med UVB-lys opnås førstegradsforbrændingen af huden, hvorefter området som bliver ekstra smerteølsomt (hyperalgesi) kan bestemmes.

Procedure 4: Måling af rødme (5 minutter)
Huden rødme måles med et specielt apparat (colormeter).

Procedure 5: Måling af den overfladiske blodgennemstrømning (5 minutter)
Den overfladiske blodgennemstrømning måles ved brug af et laser Doppler-system. Apparatet producerer et signal, som er proportionalt med blodgennemstrømning.

Procedure 6: Von Frey stik - Kvantificering af forøget smerteølelse (5 minutter)
Det primære område defineres som det bestrålede område. Det sekundære område kvantificeres med en let mekanisk stimulering med von Frey, som er et nylon filament. Områder, som føles smertefulde (ændring i opfattelsen af stimuleringerne), optegnes og defineres som det sekundære hyperalgesi-område. Det definerede område overføres herefter til et transparent ark.

Procedure 7: Pinprick stimulus-respons funktion (5 minutter)
Nålestik prikkes mod huden (pinprick) til bestemmelse af hyperalgasi intensiteten (områder som er ekstra smerteølsomt) i huden. Indenfor området medprimær hyperalgesi, ca. 5 cm, bestemmes intensiteten af nålestik-stimuli ved brug af gradueret nålestik med 8 forskellige fikserede vægte. Hver vægt påføres 3 gange i 2 sek. med 10 sek. interval på omtrent samme sted. Forsøgsdeltagerne afgiver en værdi på en elektrisk smerteskala (visuel analog skala (eVAS)). Smerteskalaen går fra nul til ti, hvor ”0 cm” indikerer ”ingen smerter” og ”10 cm” indikerer ”maksimal smerte”. Forsøgsdeltagerne instrueres i, at midten af skalaen er smertetærsklen, og at styrken af de ikke-smertefulde prik placeres på den nederste halvdel af skalaen, mens styrken af de smertefulde prik-placeres på den øverste halvdel af skalaen.

Procedure 8: Børste til bestemmelse af intensiteten af smerter udløst ved at børste huden (allodyni) 5 minutter)
En børste stryges mod huden i området med primær hyperalgesi (område som er ekstra smerteølsomt) til bestemmelse af området med allodyni, (nedsat smertetærskel). Dvs. et område som har øget smerteølelse i en så høj grad, at én ellers ikke-smertefuld stimulus virker smertefuld. Børsten stryges 6 gange med 10 sek. interval, hvorefter forsøgsdeltagerne afgiver en værdi på en elektrisk smerteskala (eVAS), som omsættes til en numerisk værdi.

Procedure 9: Trykalgometer til måling af smertetærsklen og smertetolerancetærsklen ved tryk (5 minutter)
Til bestemmelse af smertetærsklen ved tryk anvendes et trykalgometer, et apparat der opfører mekanisk tryk og registeret smertetærsklen. Apparatet presses mod det UVB-bestrålede område på huden og trykket påføres ved hjælp af en sonde. Forsøgsdeltagerne skal reagere, når de første

gang føler, at trykket er smertefuldt, og trykket bliver derefter registreret. Alle forsøgsdeltagere får besked på at trykke på en knap, når de føler, at trykket lige netop er smertefuldt, dette tryk angives som tryk-smertetærsklen.

Procedure 10: Måling af hudtemperatur (5 minutter)
Hudtemperaturen bliver målt med et infrarødt termometer.

Procedure 11: Varmestimulering til bestemmelse af den termiske smertetolerance tærskel (5 minutter)
Til bestemmelse af den termiske smertetolerance anvendes en termostat. Det UVB-bestrålede område på huden varmes op med termostaten, som er computerstyret. Den maksimale temperatur vil af sikkerhedsmæssige årsager være 52 °C. Når forsøgsdeltagerne opfatter varmestimuleringen som svarende til den termiske smertetolerance, stoppes stimuleringen ved at forsøgspersonen trykker på en knap. Herved forsvinder varmen øjeblikkeligt. Varmestimuleringen ophører automatisk, hvis en forsøgsdeltager ikke har trykket stop inden 52 °C.. Termostaten indstiller temperaturen af huden til ca. 32 °C, inden selve stimuleringen startes.

Procedure 12: Sikkerhed
Procedurerne forventes at fremkalde smerte hos forsøgsdeltagerne, men yderligere bivirkninger forventes ikke. Forsøgspersonerne vil være under observation igennem forsøget og vil være placeret i sengeleje under alle målinger.
Varigheden af hver undersøgelsesdag (Session1,2,3,4) vil være på maksimalt 2 timer. Forsøgsdeltagerne vil udgå af forsøget, hvis betydelige bivirkninger indtræffer. Frafaldne forsøgsdeltagere vil blive erstattet af nye.

Mulige risici og eventuelle bivirkninger:
Forsøgets ulemper er, at forsøgsdeltagerne vil opleve en mild til moderat smerte under stimulationerne, og efterfølgende vil de i det bestrålede område få rødme, som vil forsvinde efter et par dage.

Der kan være risici ved forsøget, som vi endnu ikke kender. Vi beder dig derfor om at fortælle, hvis du oplever problemer med dit helbred under forsøgets forløb. Hvis vi skulle observere bivirkninger, som vi ikke allerede har fortalt dig om, vil du naturligvis blive orienteret med det samme, og du vil få mulighed for at tage stilling til, om du ønsker at fortsætte i forsøget.

Der er ikke nogle umiddelbare fordele ved deltagelse i forsøget for forsøgspersonerne, men forsøget vil bidrage til en videreudvikling og validering af en ikke-invasiv og sikker human eksperimentel smertemodel, der kan anvendes inden for smerteforskning samt til udvikling og afprøvning af smertestillende medikamenter. På længere sigt kan det muligvis lede til udvikling af bedre behandlingsregimer og dermed en mere effektiv behandling af patienter med kroniske smerter. Den anvendte smertestimulation kan afbrydes på ethvert tidspunkt. Ulempen ved disse forsøg er minimal sammenlignet med bidraget fra den opnåede viden til den medicinske verden.

Deltagelse i forsøget:
Det er frivilligt at deltage, og du kan når som helst trække dit tilsagn om deltagelse tilbage.

Afslutning af forsøget:
Forsøget kan afsluttes på ethvert tidspunkt hvis:

- Du, efter forsøgslederens eller den klinisk ansvarliges vurdering, reagerer uventet på procedurerne i forsøget.
- Du på anden vis ikke er egnet til videre deltagelse i forsøget.
- Der opstår forhold, der kan påvirke forsøgets kvalitet.

Forsøget som helhed vil blive stoppet, hvis det skulle vise sig, at forsøgspersonerne generelt ikke tolererer procedurerne i forsøget eller finder forsøget for udmattende.

Din deltagelse i forsøget vil blive kompenseret med en betaling på 150 kr. i timen. Beløbet er skattepligtigt, og det vil derfor blive oplyst til Skat som B-indkomst.

Dette projekt er økonomisk støttet af Højteknologifonden med kr. 7 mio. Beløbet indgår i en forskningsfond, som er undergivet offentlig revision.

Adgang til forsøgsresultater:
Projektets resultater vil blive søgt offentliggjort i anerkendte tidsskrifter uanset udfaldet af forsøget. Forsøget er godkendt af "Den Videnskabsetiske Komité for Region Nordjylland", sagsnummer N-20100063 .

Vi håber, at du med denne information har fået tilstrækkeligt indblik i, hvad det vil sige at deltage i forsøget, og at du føler dig rustet til at tage beslutningen om din eventuelle deltagelse. Vi beder dig også om at læse det vedlagte materiale "Forsøgspersonens rettigheder i et biomedicinsk forskningsprojekt".

Hvis du vil vide mere om forsøget, er du meget velkommen til at kontakte undertegnede.

Med venlig hilsen

Asiah Rahi & Line Christensen
Specialestuderende på medicin med industriel specialisering

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AALBORG UNIVERSITY

Samtykkeerklæring

Informeret samtykke til deltagelse i et biomedicinsk forskningsprojekt.

Videreudvikling og validering af en human biomarkør til kutan inflammatorisk smerte (UVB-model) – et metodisk studie i raske personer

Erklæring fra forsøgspersonen:
Jeg har modtaget skriftlig og mundtlig information, hvorved jeg har opnået tilstrækkeligt viden om formål, metode, samt fordele og ulemper ved forsøget, til at jeg kan give mit samtykke til at deltage i forsøget.

Jeg ved, at det er frivilligt at deltage, og at jeg altid kan trække mit samtykke tilbage uden at miste mine nuværende eller fremtidige rettigheder til behandling.

Jeg giver samtykke til at deltage i forskningsstudiet og har fået en kopi af dette samtykkeark samt en kopi af den skriftlige information om forsøget til eget brug.

Forsøgspersonens navn: _____

Dato: _____ Underskrift:_____

Ønsker De at blive informeret om forskningsstudiets resultat samt eventuelle konsekvenser for Dem?:

Ja _____ (sæt x) Nej _____ (sæt x)

Erklæring fra den forsøgsansvarlige:
Jeg erklærer, at forsøgspersonen har modtaget mundtlig og skriftlig information om forsøget og har haft mulighed for at stille spørgsmål til mig. Efter min overbevisning er der givet tilstrækkelig information til, at der kan træffes beslutning om deltagelse i forsøget.

Den forsøgsansvarliges navn: Lars Arendt-Nielsen, professor, dr.med., ph.d.

Klinisk ansvarlig: Hans Christian Hoeck

Dato: _____ Underskrift:_____

Projektidentifikation: (Fx komiteens Projekt-ID, versions nr./dato eller lign.)

Opslag til rekruttering af forsøgspersoner

Videreudvikling og validering af en human biomarkør til kutan inflammatorisk smerte (UVB-model) – et metodisk studie i raske personer

Humane eksperimentelle smertemodeller anvendes i dag til udvikling og klinisk afprøvning af smertelindrende medicin i raske forsøgspersoner.

Projektet har til formål at undersøge smerteoverfølsomhed i huden, som induceres ved UVB-bestråling.

Ved deltagelse i forsøget vil du efter en screenings- og oplærings-session gennemgå 2x4 sessioner, hvor et lille område på din overarm bestråles med ultraviolet B lys. Forskellige metoder anvendes derefter til systematisk mekanisk stimulering (pensel, nylon filament, samt et tryk- og et varmeapparat) til bestemmelse af det område, som bliver ekstra smerteføl somt efter bestrålingen.

Forsøget forløber over 2x4 sessioner med to ugers mellemrum. Sessionerne finder sted umiddelbart efter bestråling samt 24, 48, 72 timer efter. For din deltagelse i forsøget udbetales en kompensation på 150 kr. i timen for forsøgsdeltagelse.

Forsøget forventes udført i perioden februar – april. ~~september-oktober.~~

Er du interesseret, så ring eller skriv til:

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Mail: lrbc07@hst.aau.dk

Forsøgspersonens rettigheder i et biomedicinsk forskningsprojekt

Som deltager i et biomedicinsk forskningsprojekt skal du vide at:

- Din deltagelse i forskningsprojektet er helt frivillig og kan kun ske efter, at du har fået både skriftlig og mundtlig information om forskningsprojektet og underskrevet samtykkeerklæringen.
- Du til enhver tid mundtligt, skriftligt eller ved anden klar tilkendegivelse kan trække dit samtykke til deltagelse tilbage og udtræde af forskningsprojektet. Såfremt du trækker dit samtykke tilbage, påvirker dette ikke din ret til nuværende eller fremtidig behandling eller andre rettigheder, som du måtte have.
- Du har ret til at tage et familiemedlem, en ven eller en bekendt med til informationssamtalen.
- Du har ret til betænkningstid, før du underskriver samtykkeerklæringen.
- Oplysninger om dine helbredsforhold, øvrige rent private forhold og andre fortrolige oplysninger om dig, som fremkommer i forbindelse med forskningsprojektet, er omfattet af tavshedspligt.
- Opbevaring af oplysninger om dig, herunder oplysninger i dine blodprøver og væv, sker efter reglerne i lov om behandling af personoplysninger og sundhedsloven.
- Der er mulighed for at få aktindsigt i forsøgsprotokoller efter offentlighedslovens bestemmelser. Det vil sige, at du kan få adgang til at se alle papirer vedrørende din deltagelse i forsøget, bortset fra de dele, som indeholder forretnings¬hemme¬ligheder eller fortrolige oplysninger om andre.
- Du har mulighed for at klage og få erstatning efter reglerne i lov om klage- og erstatningsadgang inden for sundhedsvæsenet.

(Dette tillæg udgives af Den Centrale Videnskabsetiske komité og kan vedhæftes den skriftlige information om det biomedicinske forskningsprojekt. Spørgsmål til et projekt skal rettes til den regionale komité, som har godkendt projektet)

OPSLAG

VI SØGER MANDLIGE DELTAGERE TIL FORSØGET

”VIDEREUDVIKLING OG VALIDERING AF EN HUMAN BIOMARKØR TIL KUTAN INFLAMMATORISK SMERTE (UVB-MODEL) – ET METODISK STUDIE I RASKE PERSONER ”

Humane eksperimentelle smertemodeller anvendes i dag til udvikling og klinisk afprøvning af smertelindrende medicin i raske forsøgspersoner.

Projektet har til formål at undersøge smerteoverfølsomhed i huden, som induceres ved UVB-bestråling af raske mandlige forsøgspersoner.

Ved deltagelse i forsøget vil du efter en screenings- og oplærings-session gennemgå 2x4 sessioner, hvor et lille område på din overarm bestråles med ultraviolet B lys. Forskellige metoder anvendes derefter til systematisk mekanisk stimulering (pensel, nylon filament, samt et tryk- og et varmeapparat) til bestemmelse af det område som bliver ekstra smerteføls omt efter bestrålingen.

Forsøget forløber over 2x4 sessioner med to ugers mellemrum. Sessionerne finder sted umiddelbart før bestråling samt 24, 48, 72 timer efter. For din deltagelse i forsøget udbetales en kompensation på 1000 kr. før skat. Forsøget forventes udført i perioden marts - april.

Er du interesseret, så ring eller skriv til:

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Den Videnskabsetiske Komité for
Region Nordjylland
Niels Bohrs Vej 30
9220 Aalborg Ø

Aalborg Universitet, 27. maj 2011

Vedr.: Anmeldelse af videnskabeligt forsøg

Vedlagt fremsendes hermed anmeldelse af forsøget " Videreudvikling og validering af en human biomarkør til kutan inflammatorisk smerte (UVB-model) – et metodisk studie i raske forsøgspersoner."

Vi beder venligst om fremsendelse af en elektronisk faktura, som bedes påført følgende oplysninger:

EAN nummer	5798 000 420 915
Medarbejder-id	
Rekvisitionsnummer	0915-31339

Såfremt der måtte være behøv for yderligere information, er De velkommen til at kontakte mig på telefon 23 86 19 99 eller e-mail arah07@hst.aau.dk.

Med venlig hilsen

F. Asiah Rahi

Lars Arendt-Nielsen
Professor
Center for Sanse-Motorisk Interaktion, Aalborg Universitet

Bilag: Anmeldelsesskema
Protokol
Kopi af dokumentation for den forsøgsansvarliges identifikation

CENTER FOR SANSE-MOTORISK INTERAKTION - SMI
INSTITUT FOR SUNDHEDSVIDENSKAB OG TEKNOLOGI
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Den Videnskabsetiske Komité for
Region Nordjylland
Niels Bohrs Vej 30
9220 Aalborg Ø

Aalborg Universitet, 27. maj 2011

Vedr.: Revidering af protokol med registreringsnummer: N-20100063

Vedlagt fremsendes hermed tillægsprotokol for forsøget " Videreudvikling og validering af en human biomarkør til kutan inflammatorisk smerte (UVB-model) – et metodisk studie i raske forsøgspersoner." Tilføjelser er angivet med rødt og slettet tekst er angivet ved gennemstreg. Datoen for forsøget afslutning er ændret på siderne 10 og 23, forsøget ønskes forlænget pga. tekniske problemer.

Vi beder venligst om fremsendelse af en elektronisk faktura, som bedes påført følgende oplysninger:

EAN nummer	5798 000 420 915
Medarbejder-id	
Rekvisitionsnummer	0915-31339

Såfremt der måtte være behøv for yderligere information, er De velkommen til at kontakte mig på telefon 23 86 19 99 eller e-mail arahi06@student.aau.dk.

Med venlig hilsen

F. Asiah Rahi

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Center for Sanse-Motorisk Interaktion, Aalborg Universitet

Bilag: 8 kopi af tillægsprotokol med rettelser
Anmeldelsesskema

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Appendix B - Approvals

REGION NORDJYLLAND

Professor
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Frederik Bajers Vej 7D3
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N-20100063
Videreudvikling og validering af en human biomarkør til kutan inflammatorisk smerte (UVB-model) – et metodisk studie i raske forsøgspersoner

Den Videnskabsetiske Komité for Region Nordjylland

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Journalnummer
N-20100063

15. september 2010

Den Videnskabsetiske Komité for Region Nordjylland har på møde den 31. august 2010 behandlet ovennævnte projekt.

Komitéen havde følgende bemærkninger:

- Deltagerinformationen er lang. Komitéen anbefaler, at forsker sorterer ud i hvilke informationer, der skal fremgå af Deltagerinformationen og evt. skematiserer forløbet yderligere ved hjælp af den eksisterende figur.
- Af Deltagerinformationen fremgår det, at deltagelse i forsøget ikke vil få indflydelse på fremtidig behandling. Dette skal slettes.
- Der mangler CV for forsøgsansvarlig.

Vi anmoder om tilbagemelding vedr. ovenstående punkter. Rettelser eller tilføjelser i forhold til ovenstående skal markeres i det materiale, som efterfølgende indsendes til komitéen.

Alle henvendelser vedrørende projektet bedes rettet til komitéens sekretariat. Komitéens registreringsnummer **N-20100063** bedes anført.

Med venlig hilsen

Karina Østergaard Schøler
Fuldmægtig

REGION NORDJYLLAND

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Ref.: jgp

Journalnummer
N-20100063

25. oktober 2010

N-20100063
Videreudvikling og validering af en human biomarkør til kutan inflammatorisk smerte (UVB-model) – et metodisk studie i raske forsøgspersoner

Komitéen har behandlet sagen på sit møde den 31. august 2010 og efterfølgende modtaget revideret materiale. På denne baggrund er truffet følgende

Afgørelse:
Projektet godkendes i henhold til lov om et videnskabsetisk komitésystem, lov nr. 402 af 28. maj 2003 med senere ændringer.

Godkendelsen gælder for de anmeldte forsøgssteder, den anmeldte forsøgsansvarlige i Danmark samt for den angivne forsøgsperiode.

Godkendelsen gælder til den **31. januar 2011** og omfatter følgende dokumenter:
Protokol af **28. September 2011**
Deltagerinformation af **28. September 2011**
Samtykkeerklæring af **28. September 2011**

Iværksættelse af projektet i strid med godkendelsen kan straffes med bøde eller fængsel, jf. komitélovens § 29.

Ændringer:

Foretages der væsentlige ændringer i protokolmaterialet under gennemførelsen af projektet, skal disse anmeldes til komiteen i form af tillægsprotokoller. Ændringerne må først iværksættes efter godkendelse fra komitéen, jf. komitélovens § 23, stk. 1, nr. 1.

Anmeldelse af tillægsprotokoller skal ske elektronisk på www.drvk.dk med det allerede tildelte anmeldelsesnummer og adgangskode.

Væsentlige ændringer er bl.a. ændringer, der kan få betydning for forsøgspersonernes sikkerhed, fortolkning af den videnskabelige dokumentation, som projektet bygger på samt gennemførelsen eller ledelsen af projektet. Det kan fx være ændringer i in- og eksklusionskriterier, forsøgsdesign, antal forsøgspersoner, forsøgsprocedurer, behandlingsvarighed, effektparametre, ændringer om de forsøgsansvarlige eller forsøgssteder samt indholdsmæssige ændringer i det skriftlige informationsmateriale til forsøgspersonerne.

Hvor nye oplysninger betyder, at forskeren overvejer at ændre proceduren eller stoppe forsøget, skal komiteen orienteres om det.

Bivirkninger og hændelser:

Komiteen skal omgående underrettes, hvis der under projektet optræder alvorlige bivirkninger eller alvorlige hændelser, jf. komitélovens § 22, stk. 3.

Én gang årligt i hele forsøgsperioden skal komitéen have tilsendt en liste over alle alvorlige bivirkninger og alvorlige hændelser, som er indtruffet i forsøgsperioden sammen med en rapport om forsøgspersonernes sikkerhed, jf. komitélovens § 22, stk. 4.

Materialet skal være på dansk. Listen over alvorlige bivirkninger og alvorlige hændelser kan dog være på engelsk, hvis der er vedlagt et dansk resumé.

Afslutning:

Den forsøgsansvarlige skal senest 90 dage efter afslutningen af projektet underrette komiteen herom, jf. komitélovens § 22, stk. 5.

Afbrydes projektet tidligere end planlagt, skal en begrundelse herfor sendes til komiteen senest 15 dage efter, at beslutningen er truffet, jf. komitélovens § 22, stk. 5.

Hvis projektet ikke påbegyndes, skal dette samt årsagen hertil meddeles komiteen.

Komiteen beder om kopi af den afsluttende forskningsrapport eller publikation, jf. komitélovens § 22, stk. 2. Vi skal i den forbindelse gøre opmærksom på, at der er pligt til at offentliggøre såvel negative som positive forsøgsresultater, jf. komitélovens § 14, stk. 1, nr. 6.

Tilsyn:

Komiteen fører tilsyn med, at projektet udføres i overensstemmelse med godkendelsen, jf. komitélovens § 22, stk. 1.

Følgende komitémedlemmer deltog i mødebehandlingen:

- Udviklingskonsulent Pernille Buhelt
- Bibliotekar Susanne Hjort Hansen
- Advokat Per Nielsen
- Cand. theol Ninni Lodahl Gjessing
- Overlæge Henrik Krarup
- Professor Henrik Carl Schønheyder
- Overlæge Erik Søgaard-Andersen

Med venlig hilsen

Karina Østergaard Schøler
Fuldmægtig

REGION NORDJYLLAND

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Journalnummer
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25. januar 2011

N-20100063
Videreudvikling og validering af en human biomarkør til kutan inflammatorisk smerte (UVB-model) – et metodisk studie i raske forsøgspersoner

Den Videnskabsetiske Komité for Region Nordjylland har på møde den 17. januar 2011 behandlet **tillægsprotokol 1** til ovennævnte projekt.

Komitéen kunne godkende tillægsprotokollen som værende i overensstemmelse med bestemmelserne i lov nr. 402 af 28. maj 2003.

I medfør af lov nr. 402 af 28. maj 2003 har de videnskabsetiske komitéer til opgave at følge op på de godkendte projekter. I den forbindelse gør vi særligt opmærksom på følgende bestemmelser i loven om den forsøgsansvarliges forpligtelser:

§ 22, stk. 4: Den forsøgsansvarlige skal en gang årligt i hele forsøgsperioden indsende en liste til komitéen over alle alvorlige bivirkninger og alvorlige hændelser, som er indtruffet i perioden, samt give oplysninger om forsøgspersonernes sikkerhed.

§ 23, stk. 1, nr. 3: Senest 90 dage efter afslutningen af et biomedicinsk forskningsprojekt underretter den forsøgsansvarlige komitéen om, at projektet er afsluttet. Når et projekt afbrydes tidligere end planlagt, er fristen for underretning 15 dage fra det tidspunkt, hvor beslutningen om at afbryde projektet blev truffet. Afbrydelsen skal begrundes.

Resultatet af projektet, eventuelt i form af en artikel, rapport eller lignende indsendes til komitéen når det foreligger.

Alle henvendelser vedrørende projektet bedes rettet til komitéens sekretariat. Komitéens registreringsnummer **N-20100063** bedes anført.

Med venlig hilsen

Karina Østergaard Schøler
Fuldmægtig

DATATILSYNET

Lars Arendt-Nielsen
Center for Sanse-
Motorisk Interaktion
Aalborg Universitet
Frederik Bajers Vej
7D3
9220 Aalborg

12. oktober2010

Vedrørende anmeldelse til Datatilsynet af følgende behandling: Videreudvikling og validering af en human biomarkør til kutan inflammatorisk smerte (UVB-model) – et metodisk studie i raske forsøgspersoner

Datatilsynet
Borgergade 28, 5.
1300 København K

CVR-nr. 11-88-37-29

Telefon 3319 3200
Fax 3319 3218

E-post
dt@datatilsynet.dk
www.datatilsynet.dk

J.nr. 2010-41-5321
Sagsbehandler
Louise Black Mogensen
Direkte 3319 3231

Datatilsynet kvitterer hermed for modtagelsen af anmeldelsen, som nu vil blive vurderet af tilsynet.

Hvis det ved tilsynets behandling viser sig, at der skal foretages rettelse i anmeldelsen, vil De selv kunne gøre dette på følgende adresse på internettet:

http://anmeld.datatilsynet.dk/frontend/5.asp

De skal indtaste følgende password: x7tj6ijd

Hvis De har indsendt anmeldelsen på papir, kan De på den nævnte adresse se den indtastede anmeldelse, og De vil kunne foretage eventuelle rettelser via internettet.

De skal ikke rette, før Datatilsynet har bedt om dette. Kontakt Datatilsynets sagsbehandler, hvis De bliver opmærksom på noget, der skal rettes, inden De hører fra tilsynet.

Vi vil kontakte Dem igen, når vi har set nærmere på anmeldelsen.

Med venlig hilsen

Louise Black Mogensen

DATATILSYNET

Professor, dr.med., ph.d. Lars Arendt-Nielsen
Center for Sanse-Motorisk Interaktion
Aalborg Universitet
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20. oktober 2010

Datatilsynet
Borgergade 28, 5.
1300 København K

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www.datatilsynet.dk

J.nr. 2010-41-5321

Sagsbehandler
Louise Black Mogensen
Direkte 3319 3231

Vedrørende anmeldelse af: "Videreudvikling og validering af en human biomarkør til kutan inflammatorisk smerte (UVB-model) - et metodisk studie i raske forsøgspersoner"

Ovennævnte projekt er den 12. oktober 2010 anmeldt til Datatilsynet efter persondatalovens[1] § 48, stk. 1. Der er samtidigt søgt om Datatilsynets tilladelse.

Det fremgår af anmeldelsen, at De er dataansvarlig for projektets oplysninger. Behandlingen af oplysningerne ønskes påbegyndt 1. november 2010 og forventes at ophøre 4. januar 2011.

Oplysningerne vil blive behandlet på følgende adresse: Center for Sanse-Motorisk Interaktion, Aalborg Universitet, Frederik Bajers vej 7C1, lokale 221, 9220 Aalborg Ø.

TILLADELSE

Datatilsynet meddeler hermed tilladelse til projektets gennemførelse, jf. persondatalovens § 50, stk. 1, nr. 1. Datatilsynet fastsætter i den forbindelse nedenstående vilkår:

Generelle vilkår

Tilladelsen gælder indtil: 4. januar 2011

Ved tilladelsens udløb skal De særligt være opmærksom på følgende:

Hvis De ikke inden denne dato har fået tilladelsen forlænget, går Datatilsynet ud fra, at projektet er afsluttet, og at personoplysningerne er slettet, anonymiseret, tilintetgjort eller overført til arkiv, jf. nedenstående vilkår vedrørende projektets afslutning. Anmeldelsen af Deres projekt fjernes derfor fra fortegnelsen over anmeldte behandlinger på Datatilsynets hjemmeside.

[1] Lov nr. 429 af 31. maj 2000 om behandling af personoplysninger med senere ændringer.

Datatilsynet gør samtidig opmærksom på, at al behandling (herunder også opbevaring) af personoplysninger efter tilladelsens udløb er en overtrædelse af persondataloven, jf. § 70.

1. Professor, dr.med., ph.d. Lars Arendt-Nielsen er ansvarlig for overholdelsen af de fastsatte vilkår.

2. Oplysningerne må kun anvendes til brug for projektets gennemførelse.

3. Behandling af personoplysninger må kun foretages af den dataansvarlige eller på foranledning af den dataansvarlige og på dennes ansvar.

4. Enhver, der foretager behandling af projektets oplysninger, skal være bekendt med de fastsatte vilkår.

5. De fastsatte vilkår skal tillige iagttages ved behandling, der foretages af databehandler.

6. Lokaler, der benyttes til opbevaring og behandling af projektets oplysninger, skal være indrettet med henblik på at forhindre uvedkommende adgang.

7. Behandling af oplysninger skal tilrettelægges således, at oplysningerne ikke hændeligt eller ulovligt tilintetgøres, fortabes eller forringes. Der skal endvidere foretages den fornødne kontrol for at sikre, at der ikke behandles urigtige eller vildledende oplysninger. Urigtige eller vildledende oplysninger eller oplysninger, som er behandlet i strid med loven eller disse vilkår, skal berigtiges eller slettes.

8. Oplysninger må ikke opbevares på en måde, der giver mulighed for at identificere de registrerede i et længere tidsrum end det, der er nødvendigt af hensyn til projektets gennemførelse.

9. En eventuel offentliggørelse af undersøgelsens resultater må ikke ske på en sådan måde, at det er muligt at identificere enkeltpersoner.

10. Eventuelle vilkår, der fastsættes efter anden lovgivning, forudsættes overholdt.

Elektroniske oplysninger

11. Identifikationsoplysninger skal krypteres eller erstattes af et kodenummer el. lign. Alternativt kan alle oplysninger lagres krypteret**.** Krypteringsnøgle, kodenøgle m.v. skal opbevares forsvarligt og adskilt fra personoplysningerne.

12. Adgangen til projektdata må kun finde sted ved benyttelse af et fortroligt password. Password skal udskiftes mindst én gang om året, og når forholdene tilsiger det.

13. Ved overførsel af personhenførbare oplysninger via Internet eller andet eksternt netværk skal der træffes de fornødne sikkerhedsforanstaltninger mod, at oplysningerne kommer til uvedkommendes kendskab. Oplysningerne skal som minimum være forsvarligt krypteret under hele transmissionen. Ved anvendelse af interne net skal det sikres, at uvedkommende ikke kan få adgang til oplysningerne.

14. Udtagelige lagringsmedier, sikkerhedskopier af data m.v. skal opbevares forsvarligt aflåst og således, at uvedkommende ikke kan få adgang til oplysningerne.

Manuelle oplysninger

15. Manuelt projektmateriale, udskrifter, fejl- og kontrollister, m.v., der direkte eller indirekte kan henføres til bestemte personer, skal opbevares forsvarligt aflåst og på en sådan måde, at uvedkommende ikke kan gøre sig bekendt med indholdet.

Oplysningspligt over for den registrerede

16. Hvis der skal indsamles oplysninger hos den registrerede (ved interview, spørgeskema, klinisk eller paraklinisk undersøgelse, behandling, observation m.v.) skal der uddeles/fremsendes nærmere information om projektet. Den registrerede skal heri oplyses om den dataansvarliges navn, formålet med projektet, at det er frivilligt at deltage, og at et samtykke til deltagelse til enhver tid kan trækkes tilbage. Hvis oplysningerne skal videregives til brug i anden videnskabelig eller statistisk sammenhæng, skal der også oplyses om formålet med videregivelsen samt modtagerens identitet.

17. Den registrerede skal endvidere oplyses om, at projektet er anmeldt til Datatilsynet efter persondataloven, samt at Datatilsynet har fastsat nærmere vilkår for projektet til beskyttelse af den registreredes privatliv.

Indsigtsret

18. Den registrerede har ikke krav på indsigt i de oplysninger, der behandles om den pågældende.

Videregivelse

19. Videregivelse af personhenførbare oplysninger til tredjepart må kun ske til brug i andet statistisk eller videnskabeligt øjemed.

20. Videregivelse må kun ske efter forudgående tilladelse fra Datatilsynet. Datatilsynet kan stille nærmere vilkår for videregivelsen samt for modtagerens behandling af oplysningerne.

***Æ*ndringer i projektet**

21. Væsentlige ændringer i projektet skal anmeldes til Datatilsynet (som ændring af eksisterende anmeldelse). Ændringer af mindre væsentlig betydning kan meddeles Datatilsynet.

22. *Ændring af tidspunktet for projektets afslutning skal altid anmeldes.*

Ved projektets afslutning

23. *Senest ved projektets afslutning skal oplysningerne slettes, anonymiseres eller tilintetgøres, således at det efterfølgende ikke er muligt at identificere enkeltpersoner, der indgår i undersøgelsen.*

24. Alternativt kan oplysningerne overføres til videre opbevaring i Statens Arkiver (herunder Dansk Dataarkiv) efter arkivlovens regler.

25. Sletning af oplysninger fra elektroniske medier skal ske på en sådan måde, at oplysningerne ikke kan genetableres.

Ovenstående vilkår er gældende indtil videre. Datatilsynet forbeholder sig senere at tage vilkårene op til revision, hvis der skulle vise sig behov for det.

Opmærksomheden henledes specielt på, at Datatilsynets vilkår også skal iagttages ved behandling af oplysninger på de deltagende centre mv., jf. de generelle vilkår nr. 4.

Datatilsynet gør opmærksom på, at denne tilladelse alene er en tilladelse til at behandle personoplysninger i forbindelse med projektets gennemførelse. Tilladelsen indebærer således ikke en forpligtelse for myndigheder, virksomheder m.v. til at udlevere eventuelle oplysninger til Dem til brug for projektet.

En videregivelse af oplysninger *fra* statistiske registre, videnskabelige projekter m.v. kræver dog, at den dataansvarlige har indhentet særlig tilladelse hertil fra Datatilsynet, jf. persondatalovens § 10, stk. 3.

Anmeldelsen offentliggøres i fortegnelsen over anmeldte behandlinger på Datatilsynets hjemmeside **www.datatilsynet.dk**.

Persondataloven kan læses/hentes på Datatilsynets hjemmeside under punktet "Lovgivning".

Advarsel – ved brug af Excel, PowerPoint m.v.
Den dataansvarlige skal til enhver tid sikre sig, at dokumenter og andre præsentationer, som publiceres eller på anden måde gøres tilgængelig for andre på internettet, usb-nøgle eller på andet elektronisk medie, ikke indeholder personoplysninger.

Der skal vises særlig agtpågivenhed i forbindelse med brug af grafiske præsentationer i Excel og PowerPoint, da de uforvarende kan indeholde indlejrede persondata i form af regneark, tabeller mv. Præsentationer, der gøres tilgængelig på internettet, bør derfor omformateres til Portable Digital Format (PDF), da dette fjerner eventuelle indlejrede Excel-tabeller.

Med venlig hilsen

Louise Black Mogensen

DATATILSYNET

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Aalborg Universitet
Fredrik Bajers Vej 7 D3
9220 Aalborg Øst

Sendt til: arahi06@student.aau.dk

7. marts 2011

Forlængelse af tilladelse fra Datatilsynet – j.nr. 2010-41-5321

Datatilsynet
Borgergade 28, 5.
1300 København K

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J.nr. 2010-41-5321
Sagsbehandler
Suzanne Stenkvist
Direkte 3319 3256

Datatilsynet har den 10. februar 2011 modtaget Deres anmodning om forlængelse af tilladelsen til at behandle personoplysninger i det videnskabelige projekt med ovennævnte journalnummer.

Projektets titel er: " idereudvikling og validering af en human biomarkør til kutan inflammatorisk smerte (UVB-model) - et metodisk studie i raske forsøgspersoner".

Det fremgår, at tilladelsen ønskes forlænget til 1. juli 2011.

Anmodningen giver ikke Datatilsynet anledning til bemærkninger.

Datatilsynets tilladelse forlænges hermed til: **1. juli 2011**.

Behandlingen af personoplysningerne kan fortsætte indtil denne dato på de af Datatilsynet tidligere fastsatte vilkår.

Ved tilladelsens udløb skal De særligt være opmærksom på følgende:

Hvis De ikke inden denne dato har fået tilladelsen forlænget, går Datatilsynet ud fra, at projektet er afsluttet, og at personoplysningerne er slettet, anonymiseret, tilintetgjort eller overført til arkiv, jf. de tidligere fastsatte vilkår om projektets afslutning. Anmeldelsen af projektet fjernes derfor fra fortegnelsen over anmeldte behandlinger på Datatilsynets hjemmeside.

Datatilsynet gør samtidig opmærksom på, at al behandling (herunder også opbevaring) af personoplysninger efter tilladelsens udløb er en overtrædelse af persondataloven, jf. § 70.

Den ændrede anmeldelse offentliggøres i Datatilsynets fortegnelse over anmeldte behandlinger på tilsynets hjemmeside.

Med venlig hilsen

Suzanne Stenkvist

Appendix C - Case report form (CRF)

Generelle oplysninger

Deltager Nr. _____

Alder (18-30 år) _____

Samtykke Ja ☐ Nej ☐

Screening

Højde _____

Vægt _____

BMI _____

Dansker	Ja ☐	Nej ☐
Rød Hårfarve	Ja ☐	Nej ☐
Addiktiv adfærd	Ja ☐	Nej ☐
Smitsom-sygdom	Ja ☐	Nej ☐
Akut smertetilstand	Ja ☐	Nej ☐
Neurologisk sygdom	Ja ☐	Nej ☐
Muskuloskeletal sygdom	Ja ☐	Nej ☐
Psykisk sygdom	Ja ☐	Nej ☐
Hud sygdom på det bestrålede område	Ja ☐	Nej ☐

Oplæringssession

Alkohol 24t inden Ja ☐ Nej ☐

Koffein 24t inden Ja ☐ Nej ☐

Smertestillende medicin 24t inden Ja ☐ Nej ☐

Erythema før UVB A1 A2 A3 A4 A5

Erythema efter UVB A1 A2 A3 A4 A5

MED _____

Session 1A

Alkohol 24t inden Ja ☐ Nej ☐

Koffein 24t inden Ja ☐ Nej ☐

Smertestillende medicin 24t inden Ja ☐ Nej ☐

Måling før UVB bestråling

Erythema E

Rødme Ja ☐ Nej ☐

Hvis ja, er arealet optegnet Ja ☐ Nej ☐

Superficiel blodgennemstrømning Ja ☐ Nej ☐

Hudtemperatur _____ °C

Von frey i primær område prik mærkes ved _____ g

Von frey i sekundær område er areal optegnet Ja ☐ Nej ☐

Pensel _____ eVas

	0,8	1,6	3,2	6,4	12,8
mN/ eVAS	25,6	50,1	60,0		

Pinprick

Pinprick i sekundær område er areal optegnet Ja ☐ Nej ☐

PPT _____ kPA

HPT _____ °C

UVB 3x MED

3xMED påført Ja ☐ Nej ☐

Session 2A

Alkohol 24t inden Ja ☐ Nej ☐

Koffein 24t inden Ja ☐ Nej ☐

Smertestillende medicin 24t inden Ja ☐ Nej ☐

Erythema E

Rødme Ja ☐ Nej ☐

Hvis ja, er arealet optegnet Ja ☐ Nej ☐

Superficiel blodgennemstrømning Ja ☐ Nej ☐

Hudtemperatur °C

Von frey i primær område prik mærkes ved g

Von frey i sekundær område er areal optegnet Ja ☐ Nej ☐

Pensel eVas

	0,8	1,6	3,2	6,4	12,8
Pinprick mN/ eVAS	25,6	50,1	60,0		

Pinprick i sekundær område er areal optegnet Ja ☐ Nej ☐

PPT kPA

HPT °C

Session 3A

Alkohol 24t inden	Ja ☐	Nej ☐
Koffein 24t inden	Ja ☐	Nej ☐
Smertestillende medicin 24t inden	Ja ☐	Nej ☐

Erythema		E				
Rødme	Ja ☐	Nej ☐				
Hvis ja, er arealet optegnet	Ja ☐	Nej ☐				
Superficiel blodgennemstrømning	Ja ☐	Nej ☐				
Hudtemperatur		°C				
Von frey i primær område prikk mærkes ved		g				
Von frey i sekundær område er areal optegnet	Ja ☐	Nej ☐				
Pensel		eVas				
		0,8	1,6	3,2	6,4	12,8
Pinprick	mN/ eVAS	25,6	50,1	60,0		
Pinprick i sekundær område er areal optegnet	Ja ☐	Nej ☐				
PPT		kPA				
HPT		°C				

Session 4A

Alkohol 24t inden	Ja ☐	Nej ☐
Koffein 24t inden	Ja ☐	Nej ☐
Smertestillende medicin 24t inden	Ja ☐	Nej ☐

Erythema		E				
Rødme	Ja ☐	Nej ☐				
Hvis ja, er arealet optegnet	Ja ☐	Nej ☐				
Superficiel blodgennemstrømning	Ja ☐	Nej ☐				
Hudtemperatur		°C				
Von frey i primær område prik mærkes ved		g				
Von frey i sekundær område er areal optegnet	Ja ☐	Nej ☐				
Pensel		eVas				
		0,8	1,6	3,2	6,4	12,8
Pinprick		mN/ eVAS	25,6	50,1	60,0	
Pinprick i sekundær område er areal optegnet	Ja ☐	Nej ☐				
PPT		kPA				
HPT		°C				

Session 1B

Alkohol 24t inden	Ja ☐	Nej ☐
Koffein 24t inden	Ja ☐	Nej ☐
Smertestillende medicin 24t inden	Ja ☐	Nej ☐

Måling før UVB bestråling

Erythema	E					
Rødme	Ja ☐	Nej ☐				
Hvis ja, er arealet optegnet	Ja ☐	Nej ☐				
Superficiel blodgennemstrømning	Ja ☐	Nej ☐				
Hudtemperatur	°C					
Von frey i primær område prik mærkes ved	g					
Von frey i sekundær område er areal optegnet	Ja ☐	Nej ☐				
Pensel	eVas					
		0,8	1,6	3,2	6,4	12,8
Pinprick	mN/ eVAS	25,6	50,1	60,0		
Pinprick i sekundær område er areal optegnet	Ja ☐	Nej ☐				
PPT	kPA					
HPT	°C					

UVB 3x MED

3xMED påført	Ja ☐	Nej ☐

Session 2B

Alkohol 24t inden	Ja ☐	Nej ☐
Koffein 24t inden	Ja ☐	Nej ☐
Smertestillende medicin 24t inden	Ja ☐	Nej ☐

Erythema		E				
Rødme	Ja ☐	Nej ☐				
Hvis ja, er arealet optegnet	Ja ☐	Nej ☐				
Superficiel blodgennemstrømning	Ja ☐	Nej ☐				
Hudtemperatur		°C				
Von frey i primær område prik mærkes ved		g				
Von frey i sekundær område er areal optegnet	Ja ☐	Nej ☐				
Pensel		eVas				
		0,8	1,6	3,2	6,4	12,8
Pinprick		mN/ eVAS	25,6	50,1	60,0	
Pinprick i sekundær område er areal optegnet	Ja ☐	Nej ☐				
PPT		kPA				
HPT		°C				

Session 3B

Alkohol 24t inden	Ja ☐	Nej ☐
Koffein 24t inden	Ja ☐	Nej ☐
Smertestillende medicin 24t inden	Ja ☐	Nej ☐

Erythema		E				
Rødme	Ja ☐	Nej ☐				
Hvis ja, er arealet optegnet	Ja ☐	Nej ☐				
Superficiel blodgennemstrømning	Ja ☐	Nej ☐				
Hudtemperatur		°C				
Von frey i primær område prik mærkes ved		g				
Von frey i sekundær område er areal optegnet	Ja ☐	Nej ☐				
Pensel		eVas				
		0,8	1,6	3,2	6,4	12,8
Pinprick	mN/ eVAS	25,6	50,1	60,0		
Pinprick i sekundær område er areal optegnet	Ja ☐	Nej ☐				
PPT		kPA				
HPT		°C				

Session 4B

Alkohol 24t inden	Ja ☐	Nej ☐
Koffein 24t inden	Ja ☐	Nej ☐
Smertestillende medicin 24t inden	Ja ☐	Nej ☐

Erythema		E				
Rødme	Ja ☐	Nej ☐				
Hvis ja, er arealet optegnet	Ja ☐	Nej ☐				
Superficiel blodgennemstrømning	Ja ☐	Nej ☐				
Hudtemperatur		°C				
Von frey i primær område prik mærkes ved		g				
Von frey i sekundær område er areal optegnet	Ja ☐	Nej ☐				
Pensel		eVas				
		0,8	1,6	3,2	6,4	12,8
Pinprick		mN/ eVAS	25,6	50,1	60,0	
Pinprick i sekundær område er areal optegnet	Ja ☐	Nej ☐				
PPT		kPA				
HPT		°C				

Appendix D - Requisition form

AALBORG UNIVERSITET

Institut: _____

Udbetaling af vederlag for deltagelse i forsøg/ Payment for participation in experiment

Udfyldes med blokbogstaver eller på skrivemaskine/Please use capital letters or typewriter

Navn/name:	**CPR. NR.**	
Adresse/Address:	**Beløbet bliver overført til din NEM konto**	
Postnr./Postal Code **By/City**	**ONLY foreign citizens: Bank registration no**	
	ONLY foreign citizens: Bank account no	

Foreign citizens:		
Date of valid work permit:	**Your nationality:**	**Your aliens no: (if not an EU citizen)**

Local Ethics Committee's Case Number (must be stated): VN-_____

Dato / Date	Forsøgets art / type of experiment	beløb / amount

- Det udbetalte beløb indberettes som B-indkomst ved skatteårets udgang/The amount will be reported to the taxation authorities by the end of the tax year.

Dato/Date: _____

_____

Underskrift/Signature (forsøgsperson/subject):

_____

Underskrift/Signature(projektleder/project leader):

NAME IN BLOCK LETTERS:

uk			modt./eft.reg.:				
art	1	4	7	0	1	5	
omk. sted						att.:	
proj. nr.							
fin. kilde			indkøbskontor:				
formål							
analyse							
disp. nr.					anvist:		

Appendix E - Pilot study - Raw data

Subject	MED (min)	Skin temp. (°C) BL	24h	48h	72h	Erythema (E) BL	24h	48h	72h	Mean Blood flow primary	secondary	von Frey area	pin Prick area
1	6	23.5	33.5	33.3	33.3	10.05	23.23	21.23	19.25	59.9	54.0	xxx	9.34
										1032.6	51.5	14.3	11.1
										1000.4	59.5	5.34	8.77
										834.1	54.7		
2	4	32.2	33.2	33.4	33.1	6.64	22.97	23.52	24.23	111.0	96.7	2.83	4.22
										908.8	93.0	7.35	4.39
										683.0	94.5	4.39	5.89
										586.2	91.9		
3	10	31.7	30.9	32.0	32.9	10.87	23.81	23.51	20.46	78.7	71.8	3.41	3.32
										851.5	82.7	6.53	5.69
										255.7	82.2	3.25	5.92
										327.0	81.6		
4	4	32.6	34.3	31.2	33.8	10.11	26.66	22.27	23.11	73.7	69.1	10.5	4.22
										xxx	xxx	9.39	4.39
										475.1	67.1	4.12	5.89
										586.6	112.2		

Subject	von Frey (g) BL	24h	48h	72h	pin Prick (eVas) BL	24h	48h	72h	Brush (eVas) BL	24h	48h	72h	PPT (kPA) BL	24h	48h	72h	HPT (°C) BL	24h	48h	72h
1	6	7	7	8	0.4	0.9	2.6	2.1	0.8	0.8	0.6	0.6	562	270	225	191	41.4	37.4	36.6	38.0
					0.6	1.4	3.2	2.6	0.8	0.8	0.6	0.6								
					0.9	2.1	3.7	2.6	0.8	0.8	0.6	0.6								
					1.5	2.9	3.9	3.2												
					1.5	3.4	4.2	3.7												
					1.9	4.2	4.7	3.9												
					2.6	5.3	5.5	4.7												
					2.9	5.8	6.3	5.3												
2	6	6	5	6	0.8	1.3	0.4	0.6	0.1	0.1	0.1	0.3	1069	448	402	484	45.4	36.4	38.6	39.3
					0.8	2.0	1.1	0.9	0.1	0.1	0.1	0.3								
					1.1	2.6	1.1	1.8	0.1	0.1	0.1	0.3								
					1.4	3.1	1.9	2.7												
					2.0	4.1	2.8	3.6												
					2.3	5.2	3.6	4.5												
					2.9	6.2	4.4	4.9												
					3.4	7.4	5.2	6.1												
3	8	6	5	8	0.2	0.2	0.3	0.1	0.2	0.1	0.1	0.2	784	314	372	408	46.6	37.3	36.7	37.9
					0.2	0.2	0.3	0.4	0.2	0.1	0.1	0.2								
					0.2	0.2	0.3	0.4	0.2	0.1	0.1	0.2								
					0.2	0.2	0.3	0.4												
					0.2	0.2	0.3	0.6												
					0.2	0.3	0.8	0.9												
					0.2	0.3	1.0	1.1												
					0.2	0.6	1.0	1.6												
4	8	4	6	6	0.1	2.8	2.1	1.1	0.1	0.1	0.2	0.2	468	328	420	266	44.1	39.5	38.6	XXX
					0.3	2.8	2.1	1.1	0.1	0.1	0.2	0.2								
					0.6	2.4	2.8	1.1	0.1	0.1	0.2	0.2								
					0.6	3.0	3.6	2.1												
					0.6	3.0	4.7	3.2												
					0.8	3.7	5.7	3.2												
					2.2	4.3	6.7	4.0												
					2.2	4.9	7.3	4.0												

Appendix F - Radiometer calibrations

Preliminary measurements
Wednesday 17/11 -2010 MED attachment

Intensity (mJ/cm^2)	Time (s)	Radiometer (W/m^2)	Calculation mJ=(W·s)/10	Average
200 Highest circle	34	55	187	
200 Highest circle	34	58	197	
200 Highest circle	34	57	194	
				193
80 Lowest circle	34	20	68	
80 Lowest circle	34	19	65	
80 Lowest circle	34	19	65	
				66
80 Highest circle	13	57	74	
80 Highest circle	13	57	74	
80 Highest circle	13	53	69	
				72

Preliminary measurements
Thursday 18/11 – 2010 MED attachment

Intensity (mJ/cm^2)	Time (s)	Radiometer (W/m^2)	Calculation mJ=(W·s)/10	Average
200 Highest circle	33	54,9	181,2	
200 Highest circle	33	56,8	187	
200 Highest circle	33	57	188	
				185
80 Lowest circle	33	17,9	59	
80 Lowest circle	33	17,7	58	
80 Lowest circle	33	17,6	58	
				58
80 Highest circle	13	49,3	64	
80 Highest circle	13	52,9	69	
80 Highest circle	13	51,8	67	
				67

Measurements sent to the company

Thursday 18/11 – 2010 MED attachment

Intensity (mJ/cm^2)	Time (s) applied	Radiometer (W/m^2)	Calculation mJ=(W·s)/10	Average
10	1	34,8	3,5	
10	1	34,8	3,5	
10	1	35,1	3,5	3,5
20	3	36,2		
20	3	36,3		
20	3	36,4		
30	5	37,4		
30	5	37,9		
30	5	38,4		
40	6	38,3		
40	6	38,6		
40	6	38,9		
50	8	38,2	30,6	
50	8	38,8	31,4	
50	8	39,1	31,3	31,0
60	10	38,8		
60	10	39,2		
60	10	39,5		
70	11	39,0		
70	11	39,0		
70	11	39,3		
80	13	39,0		
80	13	39,2		
80	13	39,2		
90	15	38,8		
90	15	39,0		
90	15	39,1		
100	16	38,4	61,4	
100	16	38,7	61,9	
100	16	38,9	62,2	61,8
110	18	38,5		
110	18	38,7		
110	18	38,7		
120	20	38,3		
120	20	38,4		
120	20	38,5		
130	21	38,1		
130	21	38,3		
130	21	38,3		
140	23	37,9		
140	23	38,3		
140	23	38,2		
150	25	37,9	94,8	
150	25	38,1	95,25	
150	25	38,0	95,0	95,0

160	26	37,7		
160	26	37,6		
160	26	37,5		
170	28	37,4		
170	28	37,6		
170	28	37,6		
180	30	37,2		
180	30	37,1		
180	30	37,1		
190	31	37,4		
190	31	37,1		
190	31	37,1		
200	33	37,3	123,1	
200	33	37,2	122,8	
200	33	37,0	122,1	122,7
210	35	36,7		
210	35	36,7		
210	35	36,7		
220	36	36,7		
220	36	37,4		
220	36	37,0		
230	38	36,4		
230	38	36,4		
230	38	36,1		
240	40	36,1		
240	40	35,9		
240	40	36,1		
250	41	36,6	150,1	
250	41	36,6	150,1	
250	41	36,2	148,2	149,4
260	43	36,0		
260	43	36,0		
260	43	35,7		
270	45	35,6		
270	45	35,7		
270	45	35,7		
280	46	35,6		
280	46	35,4		
280	46	35,4		
290	48	35,3		
290	48	35,3		
290	48	35,1		
300	50	35,0	175,0	
300	50	35,0	175,0	
300	50	35,1	175,5	175,2
310	51	35,2		
310	51	35,1		
310	51	35,1		
320	53	35,2		
320	53	35,1		

320	53	35,1		
330	55	35,0		
330	55	34,9		
330	55	34,7		
340	56	34,8		
340	56	34,8		
340	56	35,1		
350	58	35,1	203,6	
350	58	35,0	203,0	
350	58	34,6	200,7	202,2
360	60	34,6		
360	60	34,5		
360	60	34,4		
370	61	34,4		
370	61	34,5		
370	61	34,5		
380	63	34,6		
380	63	34,5		
380	63	34,6		
390	65	34,4		
390	65	34,5		
390	65	34,3		
400	66	34,4	227,4	
400	66	34,4	227,4	
400	66	34,4	227,4	227,4

Measurements sent to the company

Friday 19/11 – 2010 MED attachment

Intensity (mJ/cm^2)	Tid (s) applied	Radiometer (W/m^2)	Calculation mJ=(W·s)/10	Average
20	2	32,3	6,5	
20	2	32,9	6,7	
20	2	33,3	6,7	6,6
30	4	34,0		
30	4	34,5		
30	4	35,2		
40	5	35,1		
40	5	35,5		
40	5	35,7		
50	7	35,8	25,1	
50	7	36,2	25,3	
50	7	36,2	25,3	25,2
60	9	35,6		
60	9	36,2		
60	9	36,6		
70	10	36,3		
70	10	36,6		
70	10	36,8		
80	12	36,6		
80	12	37,0		
80	12	37,3		
90	14	37,2		
90	14	36,1		
90	14	37,3		
100	15	37,0	55,5	
100	15	36,5	54,8	
100	15	36,3	54,5	54,9
150	24	35,5	85,2	
150	24	36,0	86,4	
150	24	36,1	86,6	86,1
200	32	36,0	115,2	
200	32	36,3	116,2	
200	32	36,3	116,2	115,9
250	40	36,2	144,8	
250	40	36,3	145,2	
250	40	36,1	144,4	144,8
300	49	34,8	170,5	
300	49	35,0	171,5	
300	49	35,5	174,0	172,0
350	57	35,2	200,6	
350	57	35,1	200,1	
350	57	35,2	200,6	200,4
400	65	27,4	178,1	
400	65	20,6	133,9	
400	65	20,6	133,9	148,6

Measurements sent to the company

Friday 19/11 – 2010 UVB attachment

Intensity (mJ/cm^2)	Tid (s) applied	Radiometer (W/m^2)	Calculation mJ=(W·s)/10	Average
20	2	44,6	8,9	
20	2	44,9	9,0	
20	2	45,3	9,1	9,0
30	2	45,9		
30	2	46,3		
30	2	46,3		
40	3	46,3		
40	3	46,9		
40	3	47,7		
50	4	47,4	19,0	
50	4	47,5	19,0	
50	4	46,8	18,7	18,9
60	5	48,0		
60	5	48,4		
60	5	48,9		
70	5	47,3		
70	5	48,1		
70	5	48,6		
80	6	48,7		
80	6	49,2		
80	6	49,3		
90	7	49,0		
90	7	49,2		
90	7	49,2		
100	8	48,9	39,1	
100	8	49,5	39,6	
100	8	49,7	39,8	39,5
150	12	49,6	59,5	
150	12	49,9	59,9	
150	12	49,9	59,9	59,8
200	17	49,3	83,8	
200	17	50,3	85,5	
200	17	50,3	85,5	84,9
250	21	49,8	109,6	
250	21	50,1	105,2	
250	21	50,1	105,2	106,7
300	26	49,8	129,5	
300	26	50,0	130,0	
300	26	49,8	129,5	129,7
350	30	49,7	149,1	
350	30	49,7	149,1	
350	30	49,6	148,8	149,0
400	35	49,4	172,9	
400	35	49,5	173,3	
400	35	49,4	172,9	173,0

Preliminary measurements

Monday 6/12 - 2010 MED attachment

Intensity (mJ/cm^2)	Time (s)	Radiometer (W/m^2)	Calculation mJ=(W·s)/10	Average
200 Highest circle	34	75,8	257,72	
200 Highest circle	34	76,1	258,74	
200 Highest circle	34	75,7	257,38	
				257,947
80 Lowest circle	34	28,3	96,22	
80 Lowest circle	34	28,4	96,56	
80 Lowest circle	34	28,3	96,22	
				96,00
80 Highest circle	13	76,5	99,45	
80 Highest circle	13	78,8	102,44	
80 Highest circle	13	79,8	103,74	
				101.88

Intensity (mj/cm^2)	Time (s)	Radiometer (W/m^2)	Calculation	Average
200 Highest circle	32	76,3	244,16	
200 Highest circle	32	76,7	245,44	
200 Highest circle	32	76,2	243,84	
				244,48
80 Lowest circle	32	28,6	91,52	
80 Lowest circle	32	28,8	92,16	
80 Lowest circle	32	28,8	92,16	
				91.95
80 Highest circle	12	72,5	87	
80 Highest circle	12	74,4	89,28	
80 Highest circle	12	74,3	89,16	
				88.48

Measurements perform when determining MED

D.D	((W/m^2)·s)/10	mJ/cm^2
Mandag d.21/2	56,6 x 17	96,2
Mandag d. 28/2	59,5 x 17	101,15
Tirsdag 1/3	54,2 x 17	92,1
Torsdag 3/3	52,0 x 17	88,4
Torsdag 3/3	58,3 x 17	99,1
Torsdag 3/3	50,9 x17	86,5
Torsdag 3/3	50,2 x 17	85,3
Torsdag 10/3	53,1 x 17	90,3
Mandag 14/3	52,3 x 17	88,9
Tirsdag 15/3	54,0 x 17	91,8
Tirsdag 15/3	58,4 x17	99,8
Onsdag 16/3	53,6 x 17	91,1
Onsdag 16/3	54,8 x 17	93,2
Tirsdag 22/3	54,7 x 17	93,0
Onsdag 23/3	57,5 x 17	97,8
Torsdag 24/3	56,4 x 17	95,9

CD with radiometer measurements

Appendix G - How to do statistical calculations

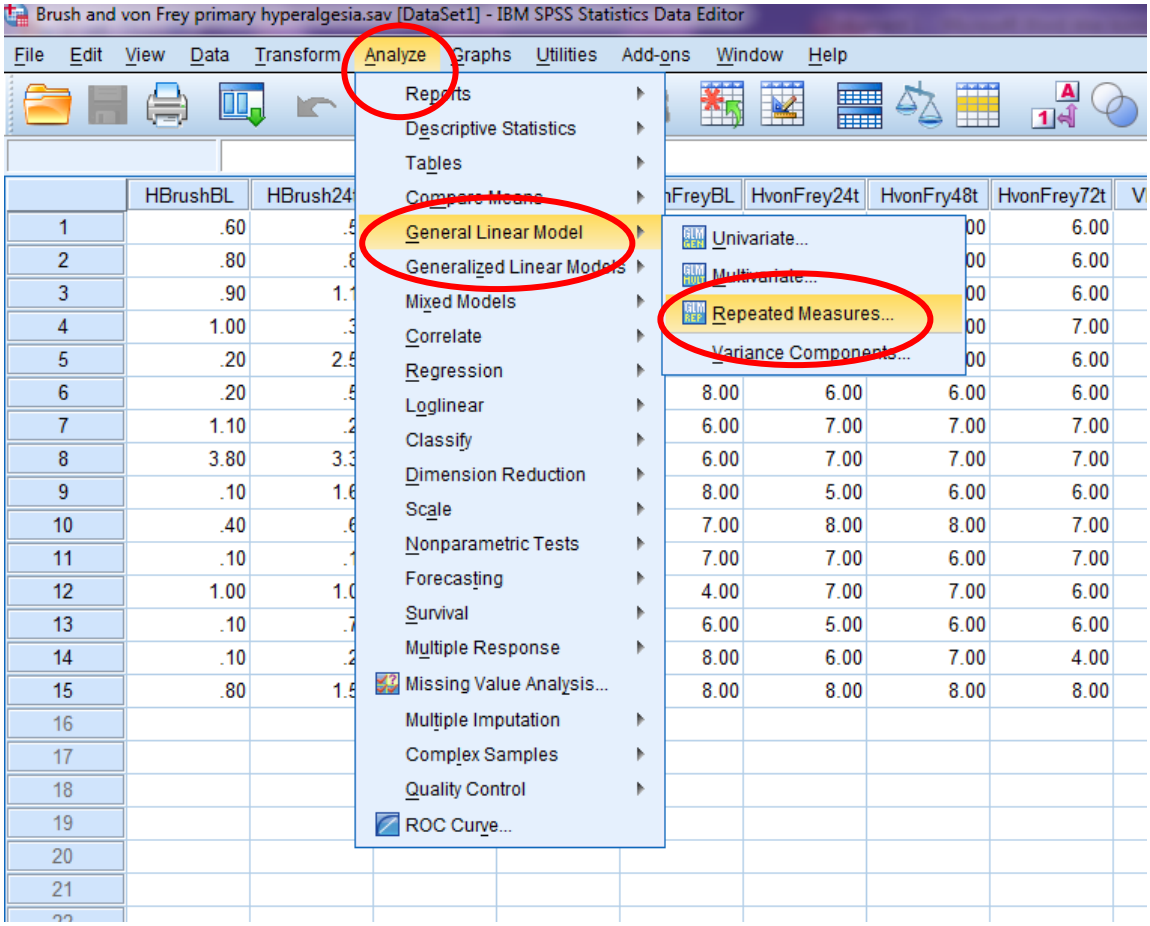
The following chapter is a guidance on how to analyze the obtained data from a study multiple measurements in SPSS or Microsoft Excel when SPSS fail to do the task. The following test will explain Repeated measures ANOVA, calculation of ICC and CV values and Sample size calculations for a cross-over and parallel study. Output of the examples use in this guidance can be found in appendix H of the project "Development and validation of a human bio-marker for cutaneous inflammatory pain (the UVB-model) - a methodological study in healthy volunteers".

Repeated measures ANOVA (RM ANOVA)

Like any ANOVA, repeated measures ANOVA tests the equality of means. However, Repeated Measures ANOVA is used when all members of a random sample are measured under a number of different conditions. As the sample is exposed to each condition in turn, the measurement of the dependent variable is repeated. Using a standard ANOVA in this case is not appropriate because it fails to model the correlation between the repeated measures. Repeated-measures designs can be thought of as an extension of the paired-samples t-test to include comparison between *more than two repeated measures i.e. compares three or more matched groups.* An appropriate *post hoc* (e.g. Bonferroni) for multiple comparisons is used to assess systematic bias between measurements.

How to do the RM ANOVA in SPSS

Click on the menu **Analyze** → choose **General Linear Model** → choose **Repeated Measures**

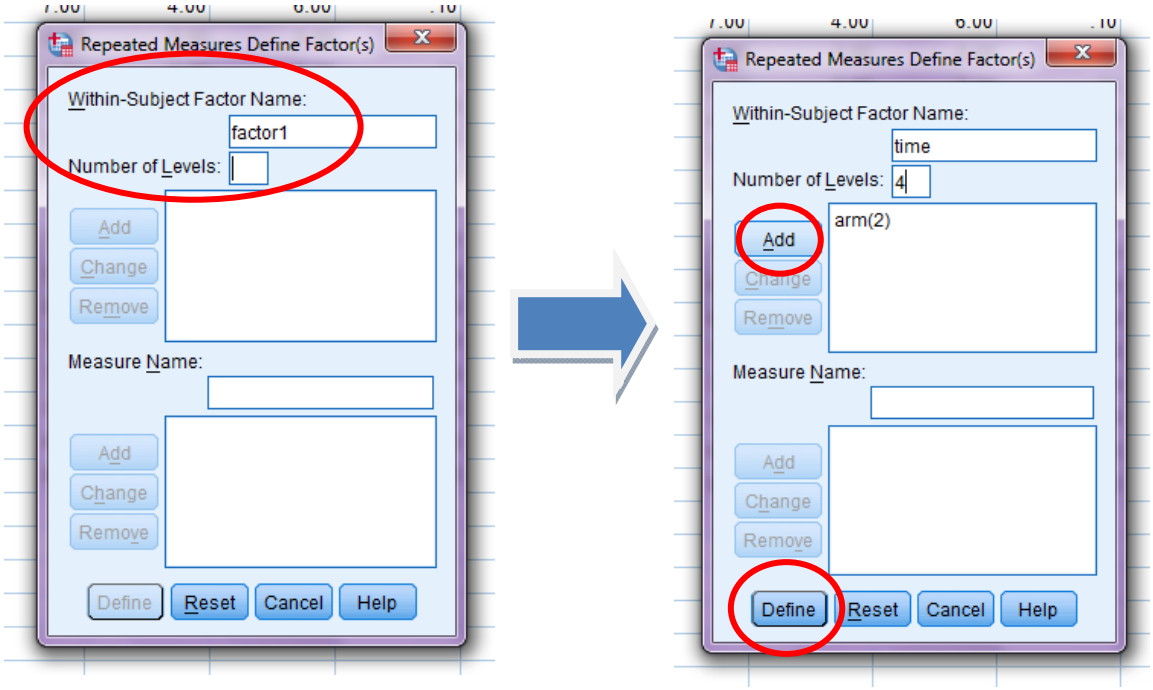


Then you will get this window: *Repeated Measures Define Factors*

You will have to define the **within-subject factors**
i.e.

- *Time (4: baseline, 24h, 48h and 72h)*
- *Arm (2:right and left)*

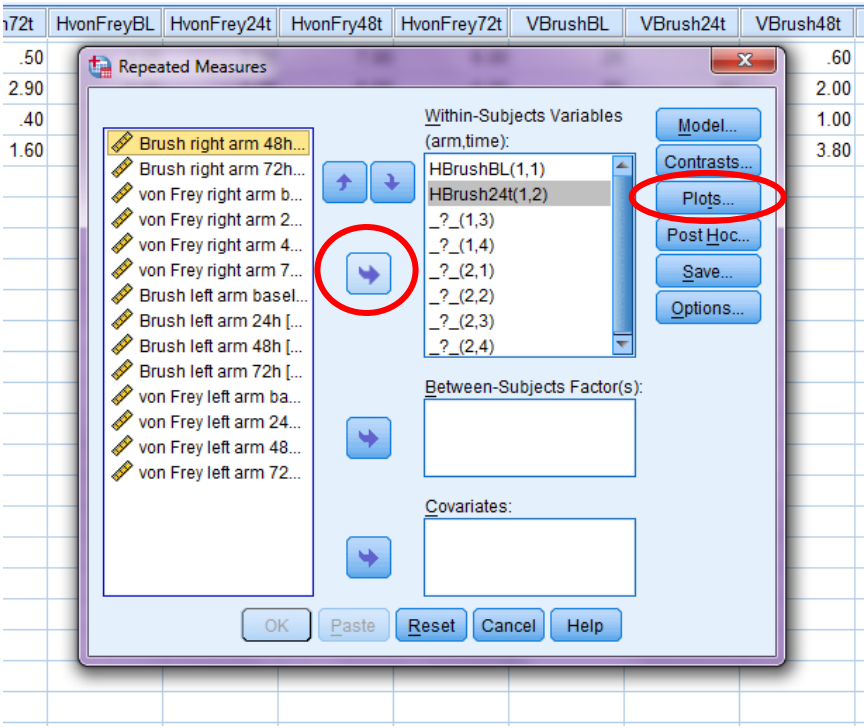
Write the **name** of the factor *i.e. arm* → then the **number** of factor *i.e. 2 (right and left arm)*→ click on **Add**



Click on the **Define** option and a new window will appear: *Repeated Measures*

You have to choose the **within-subjects variables**.
i.e. Brush test.
By marking the **variable** in the row to the left and clicking on the **arrow** →the **variable** will be moved to the list on the right.
i.e. brush data (eVas-score) from right arm at baseline, 24h, 48h, 72h and left arm at baseline, 24h, 48h, 72h

If you want a plot, click on the **Plots** option**.**



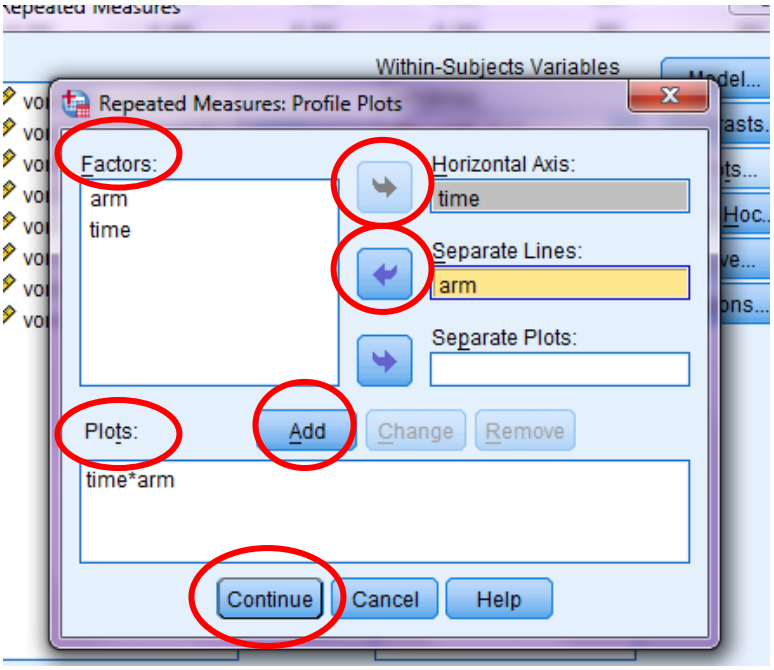
This window will appear: *Repeated Measures Profile Plots*

You have to choose the **factors** you want to plot.

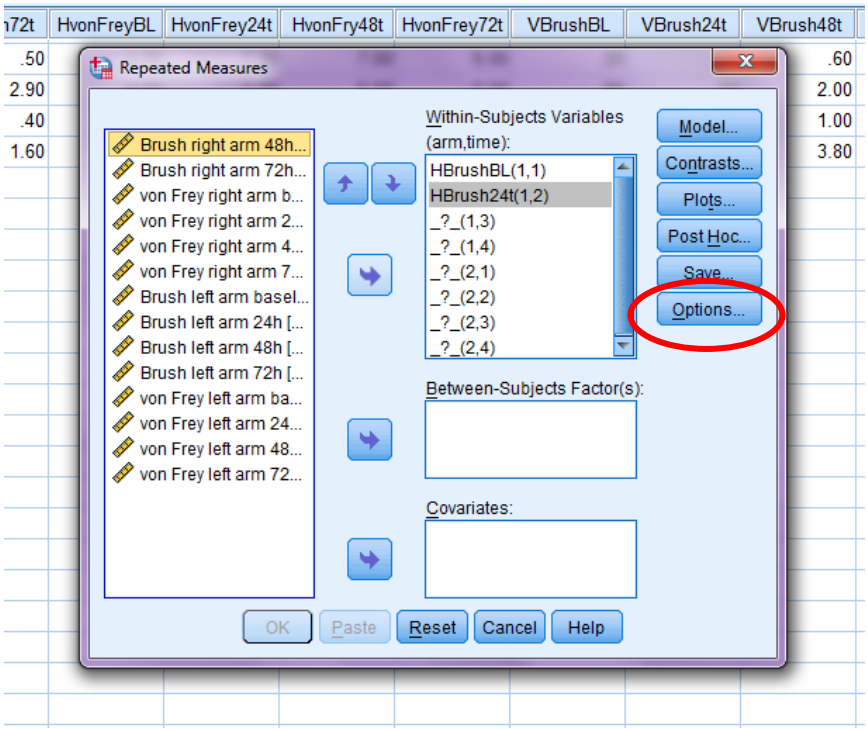
Mark the factors and move them to the boxes named
Horizontal Axies and **Separate lines**
→ by clicking on the **arrows**,
i.e. time and arm
→ and click on **Add**

The name of your plot should then appear in the lower box "**plots**:" *i.e. time*arm*

Click on the **Continue** option
→You will return to the Repeated measures window



Now click on the **Options**



This window will appear: *Repeated Measures Options*

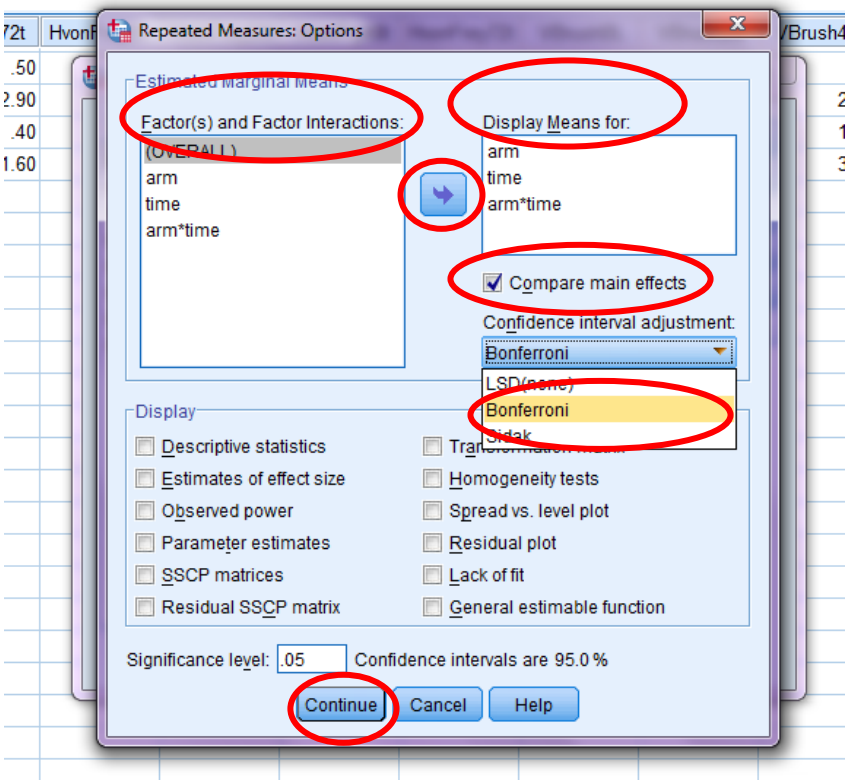
You have to choose the **factors**, which you would like to **display the means for** by marking the factors in the box on the left side

→ then click on the **arrow** to move the factors to the box on the right side

→ You have to mark the square **Compare main effect** with a check mark **(√)**

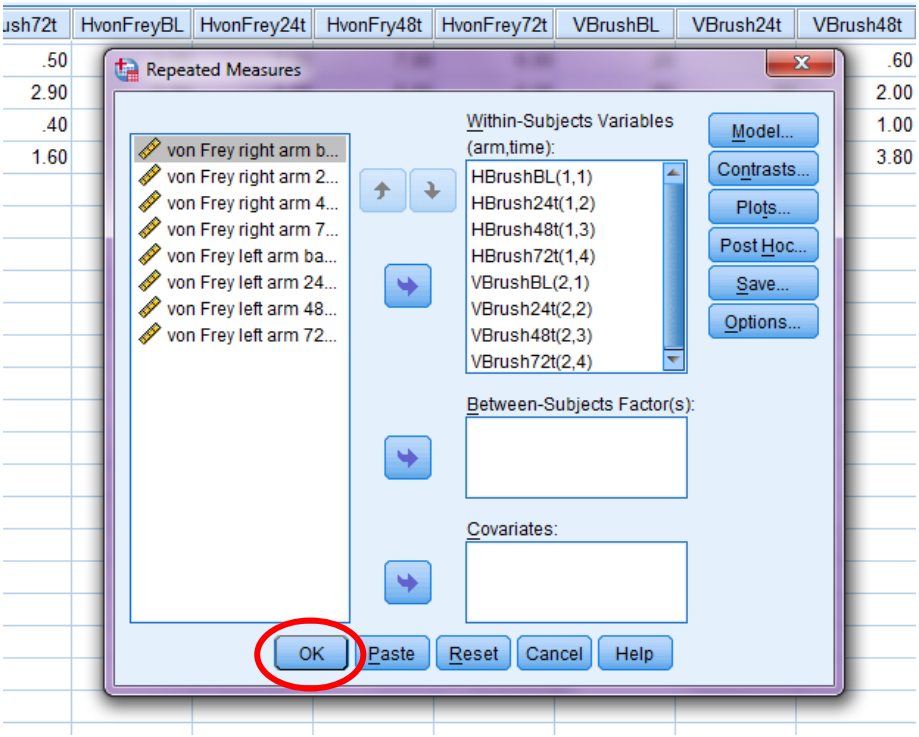
→ Choose a confidence interval adjustment below i.e. **Bonferroni**

→ Click on **Continue**



You will return to the window: *Repeated measures*

Click on **OK** to finish the test and get your output

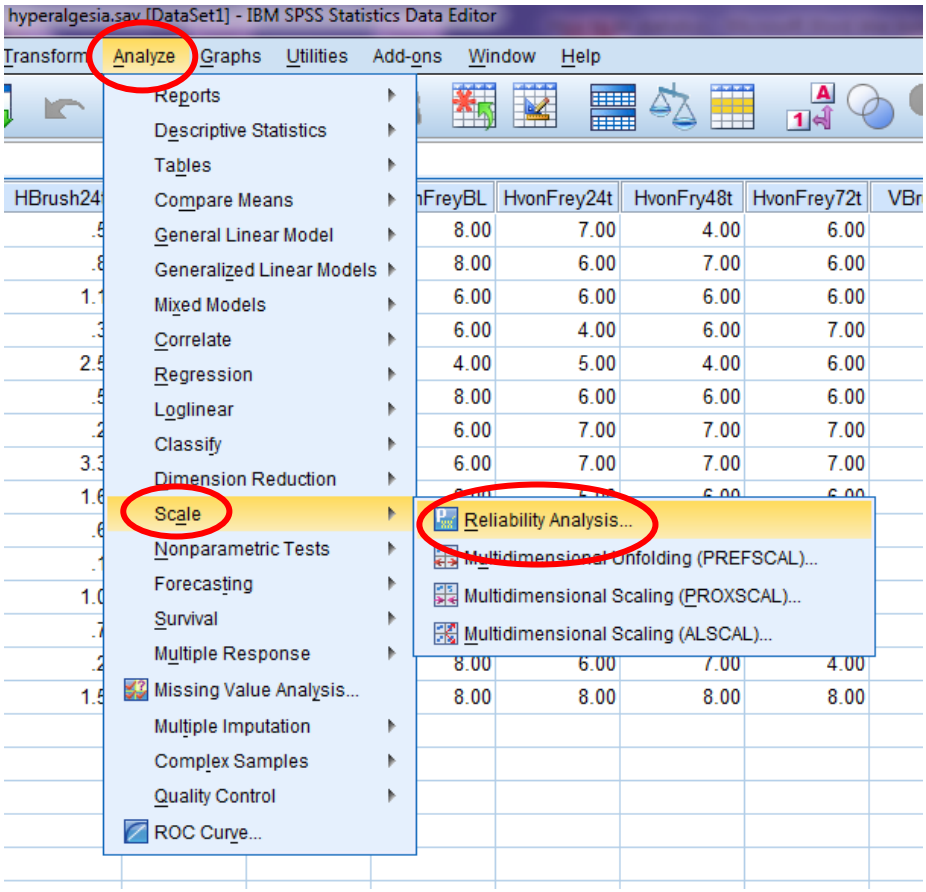


Intraclass Correlation Coefficient ICC)

ICC is calculated to assess the rating reliability by comparing the variability of different ratings of the same subject to the total variation across all ratings and all subjects i.e. agreements between two or more raters. ICC evaluates each person's ability to reproduce a response across sessions and illustrates the difference between a person's scores at repeated experimental sessions i.e. measurement of *intra-individual variance.*

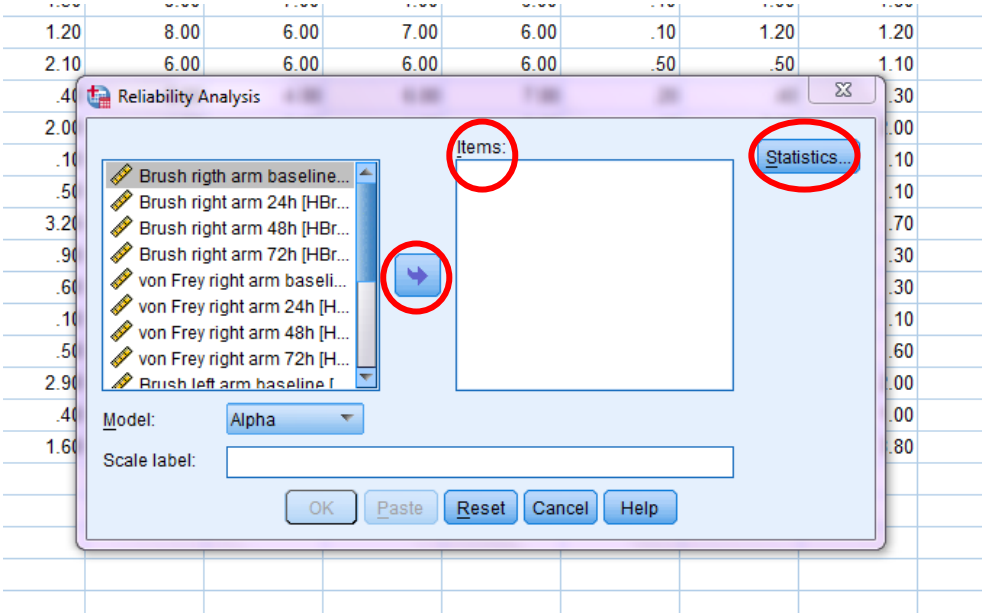
How to calculate ICC in SPSS

Click on the menu **Analyze** → choose **Scale** → choose **Reliability Analysis**

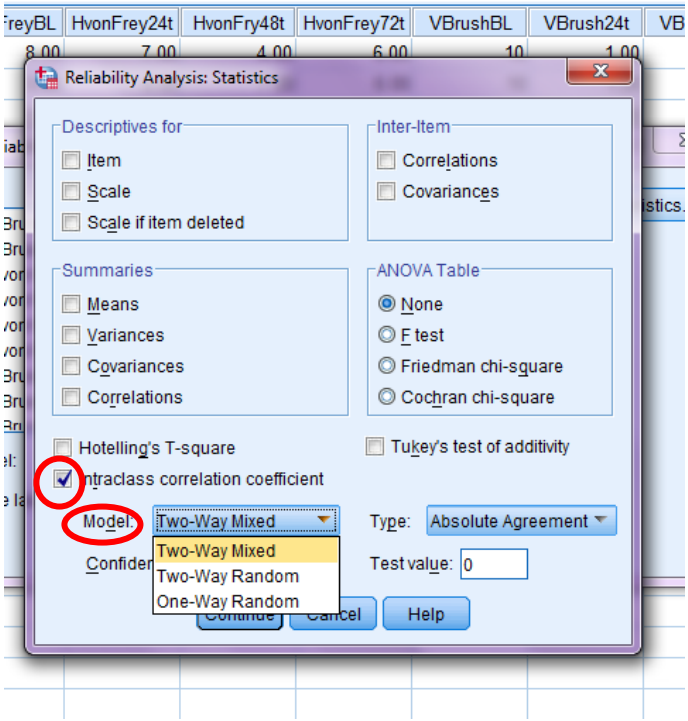


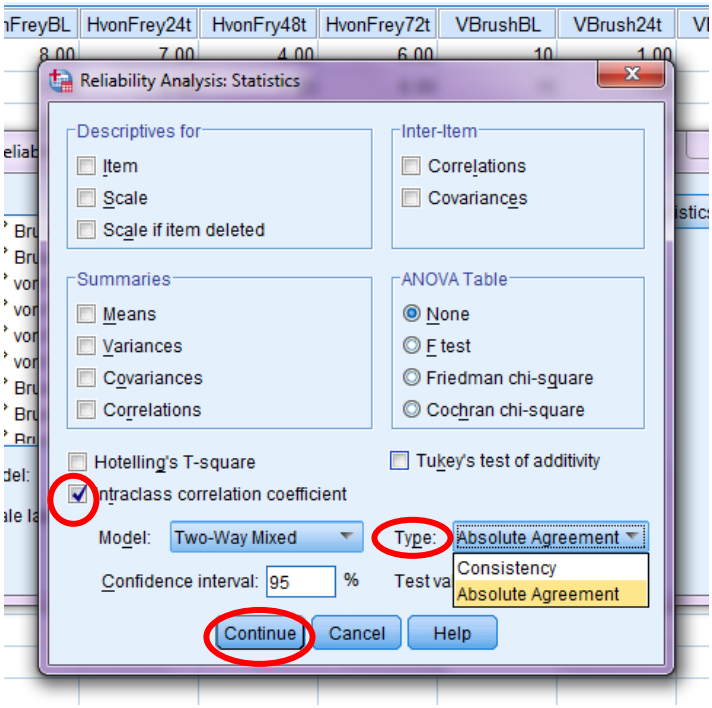
Then you will get this window: *Reliability Analysis*

You have to choose the **items** you want to compare by marking the items in the box on the left
→ Click on the **arrow**
→ the items will be moved to the box on the right. *i.e. Brush data from 24h and 48h*
→ Click on the **Statistic** option



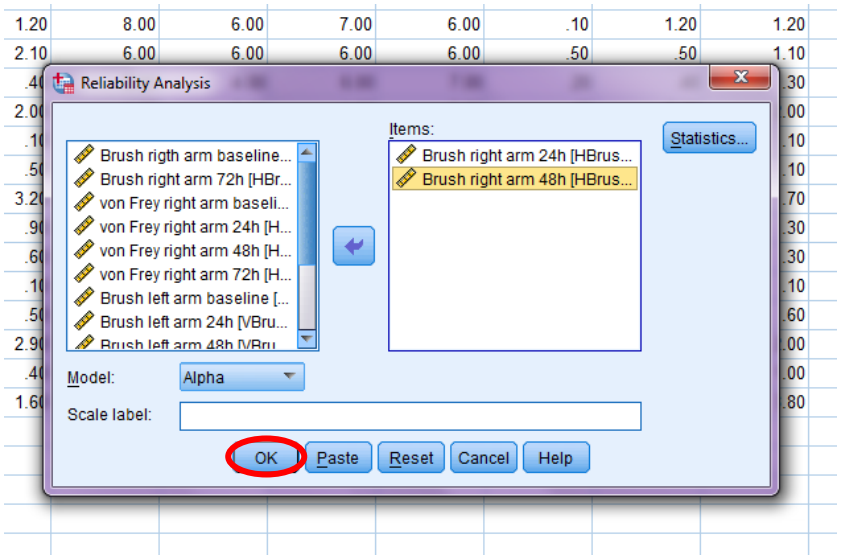
You will get this window: *Reliability Analysis Statistics*
You have to choose **Interclass correlation coeffcient** by marking the square with a check mark (**√**)
Choose a **Model** →**Two-way Mixed**
Choose a **Type** → **Absolute Agreement**
→Click on **Continue**





Then you will return to the window: *Reliability analysis*

Click on **OK** option to finish the test and get your output



Coefficient of variation (CV)

CV is calculated by determining the **standard deviation (SD)** of the data and dividing it by the **mean (μ)** and multiply by **100**. **CV**= $\frac{\mathbf{SD}}{\mu} \times \mathbf{100}\%$

CV reflects the overall variability of a model, i.e. the overall *inter-individual variance*.
SPSS is not able to calculate CV, Microsoft Excel can be used.

First you have to find the **SD** of your data by typing **=STDAF(**xx:xx**)/KVROD(2) in the fx box**

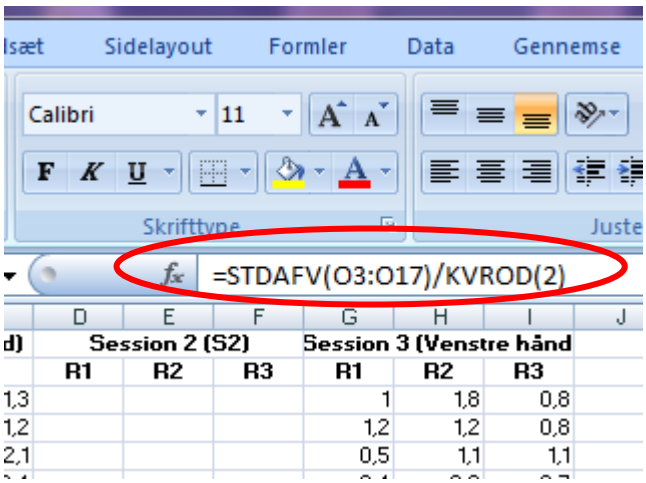
xx and xx is the names of the column containing the data that you want calculate a SD for.
e.g. you data can range from A1-A15 then you write A1:A15

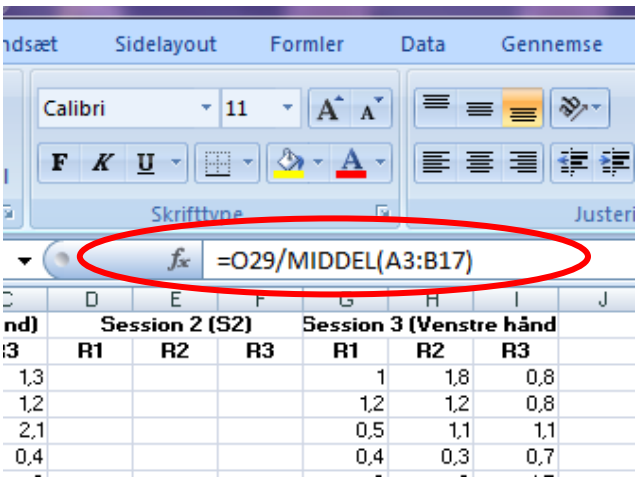
Then you divide the SD with the **mean** of your date and multiply with **100** by typing in

= yy**/MIDDEL(**xx:xx**) in the fx box**

yy in this calculation is the name of squared in the excel ark with the SD calculation
(eg. O29)

xx and xx is the names of the first and last squares in the excel art with the data





Calculations of sample size in a cross over study and the minimal detectable difference (MDD)

The sample size calculation for a **paired samples t-test** is used to calculate the sample size for a cross-over study. The paired samples t-test is used to investigate whether or not the difference between two *paired* population means is zero i.e. the difference between two responses measured on the same statistical unit as a mean value of zero. The paired-samples t-test will give you the **standard deviation (s)** between the two measures, which is pate of the sample size calculation:

$$n = \frac{s^2}{\delta_2}(t_{\alpha(2),v} + t_{\beta(1),v})^2$$

δ is **the minimal detectable difference** and it is calculated by:

$$\delta = \sqrt{\frac{s^2}{n}(t_{\alpha,v} + t_{\beta(1),v})}$$

S = the standard deviation from the paired t-test

n = the number of subject

t =(**α2,v**) =
α = significant level required
2 =represents a two-tailed t-test
v= the degree for freedom =**n-1** (number of subjects minus one)

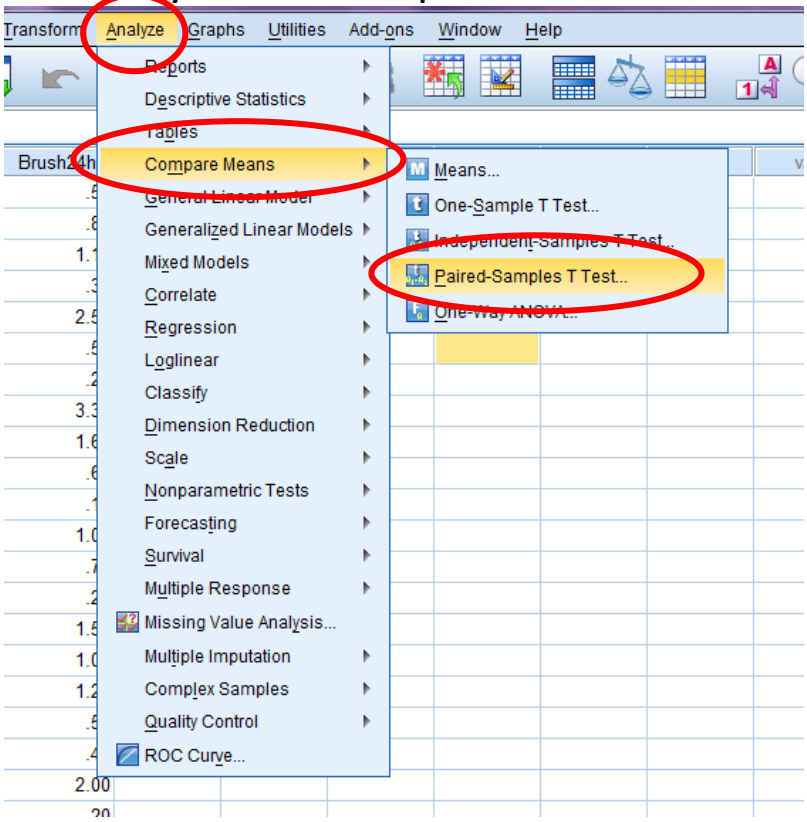
When you know **α** and **v** the **t** can be looked up in a statistical table

t=(**β1,v**)=
β= the power required
v= the degree for freedom = **n-1** (number of subjects minus one)

When you know **β** and **v** the **t** can be looked up in a statistical table

First you have to perform a **paired samples t-test** in SPSS to find the **standard deviation (S)** between two the two measurements.

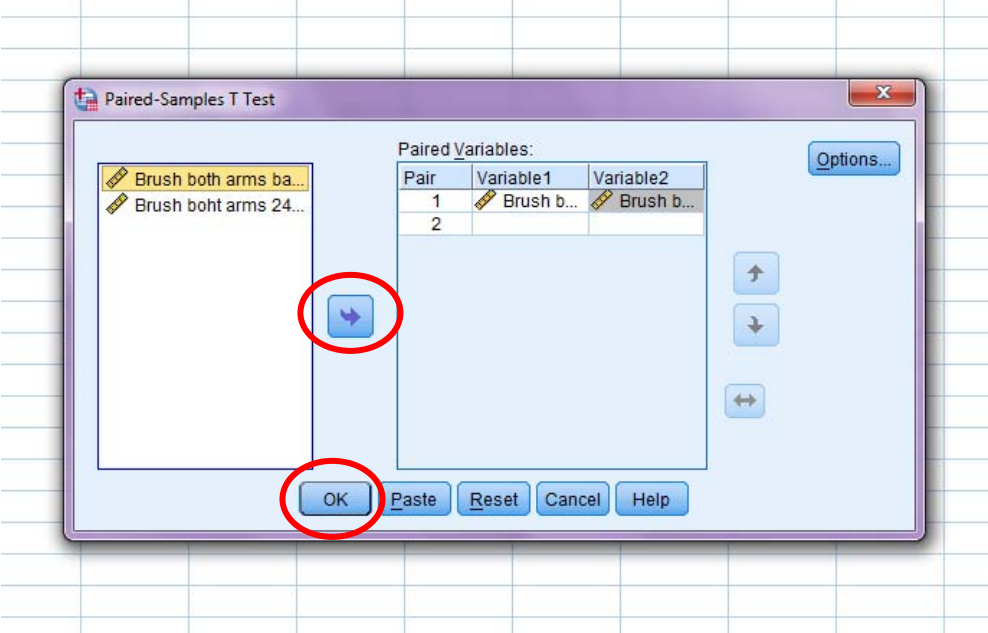
Click on **Analyze** → choose **Compare Means** →choose **Paired-Samples T Test**



Then you will get this window: *Paired-Samples T Test*

Choose the variables you want to compare, by marking the **variable**
→ Clicking on the **arrow**
The variable will be moved from the box on the left into the box on the right
i.e. brush measurements from baseline and 24h post-treatment

→ click **Ok** to finish the test and get your out-put



You have to choose your **α** *(e.g. 0.05)* and **β** *(e.g. 80%)* and then you must look up the values for **t(α,v)**and **t(β,v)**in the statistical **table**

Now you have all the values for calculating the minimal detectable difference and the sample-size.

ν	$\alpha(2)$: 0.50 $\alpha(1)$: 0.25	0.20 0.10	0.10 0.05	0.05 0.025	0.02 0.01	0.01 0.005	0.005 0.0025	0.002 0.001	0.001 0.0005
1	1.000	3.078	6.314	12.706	31.821	63.657	127.321	318.309	636.619
2	0.816	1.886	2.920	4.303	6.965	9.925	14.089	22.327	31.599
3	0.765	1.638	2.353	3.182	4.541	5.841	7.453	10.215	12.924
4	0.741	1.533	2.132	2.776	3.747	4.604	5.598	7.173	8.610
5	0.727	1.476	2.015	2.571	3.365	4.032	4.773	5.893	6.869
6	0.718	1.440	1.943	2.447	3.143	3.707	4.317	5.208	5.959
7	0.711	1.415	1.895	2.365	2.998	3.499	4.029	4.785	5.408
8	0.706	1.397	1.860	2.306	2.896	3.355	3.833	4.501	5.041
9	0.703	1.383	1.833	2.262	2.821	3.250	3.690	4.297	4.781
10	0.700	1.372	1.812	2.228	2.764	3.169	3.581	4.144	4.587
11	0.697	1.363	1.796	2.201	2.718	3.106	3.497	4.025	4.437
12	0.695	1.356	1.782	2.179	2.681	3.055	3.428	3.930	4.318
13	0.694	1.350	1.771	2.160	2.650	3.012	3.372	3.852	4.221
14	0.692	1.345	1.761	2.145	2.624	2.977	3.326	3.787	4.140
15	0.691	1.341	1.753	2.131	2.602	2.947	3.286	3.733	4.073
16	0.690	1.337	1.746	2.120	2.583	2.921	3.252	3.686	4.015
17	0.689	1.333	1.740	2.110	2.567	2.898	3.222	3.646	3.965
18	0.688	1.330	1.734	2.101	2.552	2.878	3.197	3.610	3.922
19	0.688	1.328	1.729	2.093	2.539	2.861	3.174	3.579	3.883
20	0.687	1.325	1.725	2.086	2.528	2.845	3.153	3.552	3.850
21	0.686	1.323	1.721	2.080	2.518	2.831	3.135	3.527	3.819
22	0.686	1.321	1.717	2.074	2.508	2.819	3.119	3.505	3.792
23	0.685	1.319	1.714	2.069	2.500	2.807	3.104	3.485	3.768
24	0.685	1.318	1.711	2.064	2.492	2.797	3.091	3.467	3.745
25	0.684	1.316	1.708	2.060	2.485	2.787	3.078	3.450	3.725
26	0.684	1.315	1.706	2.056	2.479	2.779	3.067	3.435	3.707
27	0.684	1.314	1.703	2.052	2.473	2.771	3.057	3.421	3.690
28	0.683	1.313	1.701	2.048	2.467	2.763	3.047	3.408	3.674
29	0.683	1.311	1.699	2.045	2.462	2.756	3.038	3.396	3.659
30	0.683	1.310	1.697	2.042	2.457	2.750	3.030	3.385	3.646
31	0.682	1.309	1.696	2.040	2.453	2.744	3.022	3.375	3.633
32	0.682	1.309	1.694	2.037	2.449	2.738	3.015	3.365	3.622
33	0.682	1.308	1.692	2.035	2.445	2.733	3.008	3.356	3.611
34	0.682	1.307	1.691	2.032	2.441	2.728	3.002	3.348	3.601
35	0.682	1.306	1.690	2.030	2.438	2.724	2.996	3.340	3.591
36	0.681	1.306	1.688	2.028	2.434	2.719	2.990	3.333	3.582
37	0.681	1.305	1.687	2.026	2.431	2.715	2.985	3.326	3.574
38	0.681	1.304	1.686	2.024	2.429	2.712	2.980	3.319	3.566
39	0.681	1.304	1.685	2.023	2.426	2.708	2.976	3.313	3.558
40	0.681	1.303	1.684	2.021	2.423	2.704	2.971	3.307	3.551
41	0.681	1.303	1.683	2.020	2.421	2.701	2.967	3.301	3.544
42	0.680	1.302	1.682	2.018	2.418	2.698	2.963	3.296	3.538
43	0.680	1.302	1.681	2.017	2.416	2.695	2.959	3.291	3.532
44	0.680	1.301	1.680	2.015	2.414	2.692	2.956	3.286	3.526
45	0.680	1.301	1.679	2.014	2.412	2.690	2.952	3.281	3.520
46	0.680	1.300	1.679	2.013	2.410	2.687	2.949	3.277	3.515
47	0.680	1.300	1.678	2.012	2.408	2.685	2.946	3.273	3.510
48	0.680	1.299	1.677	2.011	2.407	2.682	2.943	3.269	3.505
49	0.680	1.299	1.677	2.010	2.405	2.680	2.940	3.265	3.500
50	0.679	1.299	1.676	2.009	2.403	2.678	2.937	3.261	3.496

Calculation of sample size in parallel study

The sample size is calculated by **comparing the means** of two groups.
Both SPSS and Microsoft Excel are able to do these calculations and it is only a matter of which one you prefer.

The formula is:

$$n = \frac{2 * SD^2}{E^2} * k$$

SD = the standard division between the two variables

E= the minimal change in the mean, which would be clinically useful or otherwise interesting (e.g. 30%)

K= a magic number from a statistical **table**, which depends on the power (e.g. 80%) and the significance levels (e.g. 0.05) required.

First you have to choose the power and level of significance you want to achieve and afterwards look up **K** the magic number for these values in the table.

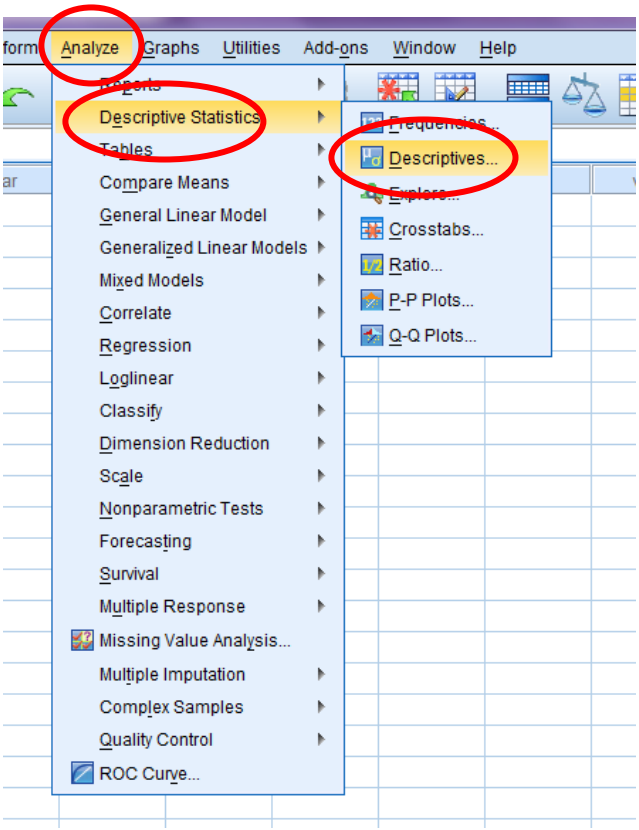
SIGNIFICANCE LEVEL (α)	POWER ($1 - \beta$)			
	70%	80%	90%	95%
0.05	6.2	7.8	10.5	13.0
0.01	9.6	11.7	14.9	17.8

Then you have to define **E** the minimal change in the mean you would like to detect.
(if you have a **baseline variable** and a **post-treatment variable** you could find the **mean** of your baseline measurement and **subtract** the **chance** you want to detect, the mean can be calculated in both SPSS and microstoft Excel)

Then you calculated the **SD** of the two variables in SPSS or Microsoft Excel

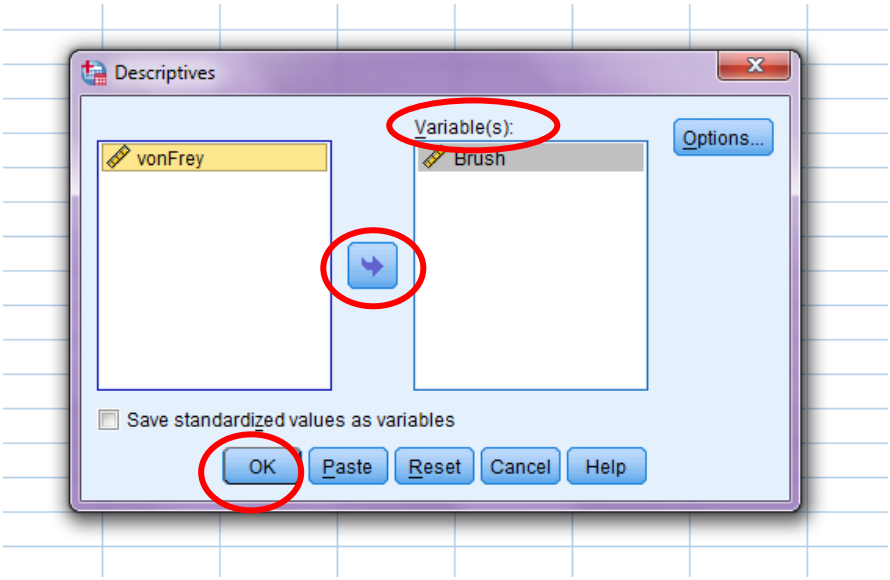
How to calculate SD and mean in SPSS

Click on the menu **Analyze** → choose **Descriptive Statistics** → choose **Descriptives**



Then you will get the window: *Descriptives*

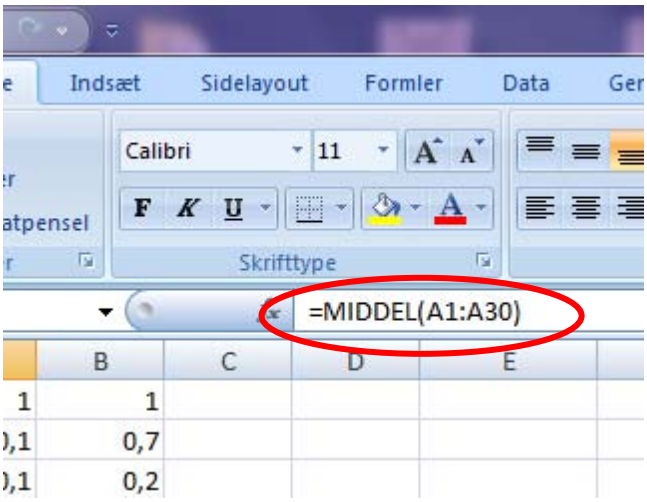
Choose the **variable** by marking it in the left box
→ Click on the **arrow,** which will move the variable to the right box called **variables**
→ Click on the **OK** button and you to finish the test and get your output



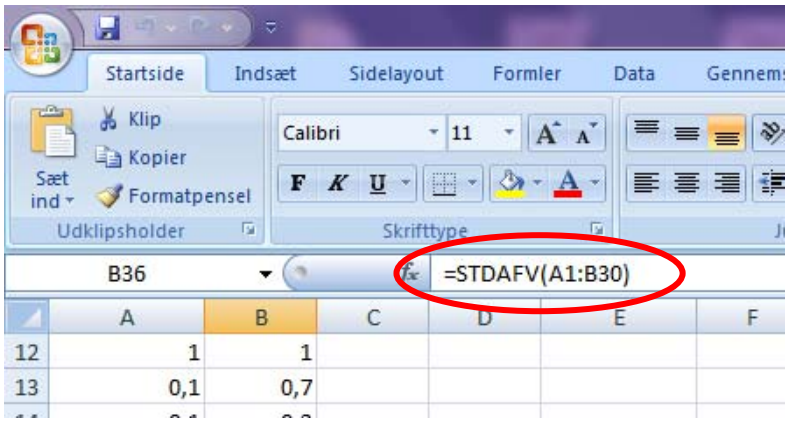
How to calculate SD and mean in Microsoft Excel

Mean is calculated by typing in **=MIDDEL (**xx:xx**) in the fx box**

xx and xx is the names of the first and last squares in the excel ark which contains the data you want to calculate the mean of.



The SD is calculated by typing in fx **=STDAFV(**xx:xx**)**
xx and xx is the names of the first and the last squares in the excel ark which contains the data you want to calculate the SD for.



Appendix H - Statistical output

Analysis of the measurements for the assessment of inflammation

Erythema

Within-Subjects Factors

arm	time	Dependent Variable
right	BL	Erythema
	24h	Erythema
	48h	Erythema
	72h	Ertyhema
left	BL	Erythema
	24h	Erythema
	48h	Erythema
	72h	Erythema

This table illustrates the analyzed data. Erythema was measured on both arms at baseline and 24h, 48h and 72h after the irradiation.

Mauchly's Test of Sphericity

Within Subjects Effect	Sig.
arm	
time (BL,24h,48h,72h)	.616
arm * time	.078

This table illustrates the sphericity of the data. There is significant sphericity in this data.

Tests of Within-Subjects Effects

Source		Sig.
arm	Greenhouse-Geisser	.875
time (BL,24h,48h,72h)	Greenhouse-Geisser	.000
arm * time	Greenhouse-Geisser	.268

This table illustrates the test for any significant difference between the arms and between the times. There is no significant difference between the arms, which means that the degree of erythema was similar on both arms. There is a significant difference between the times, which means that the degree of erythema was different within the timeslots. This table also shows that there is no interaction between the arm and time, which means that a similar degree of erythema can be achieved at a given time point regardless the arm.

Pairwise Comparisons

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
BL	24h	-12.657*	.466	.000	-14.086	-11.227
	48h	-12.294*	.349	.000	-13.365	-11.224
	72h	-12.554*	.498	.000	-14.082	-11.026
24h	BL	12.657*	.466	.000	11.227	14.086
	48h	.362	.443	1.000	-.996	1.721
	72h	.103	.491	1.000	-1.404	1.610
48h	BL	12.294*	.349	.000	11.224	13.365
	24h	-.362	.443	1.000	-1.721	.996
	72h	-.260	.377	1.000	-1.418	.898
72h	BL	12.554*	.498	.000	11.026	14.082
	24h	-.103	.491	1.000	-1.610	1.404
	48h	.260	.377	1.000	-.898	1.418

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

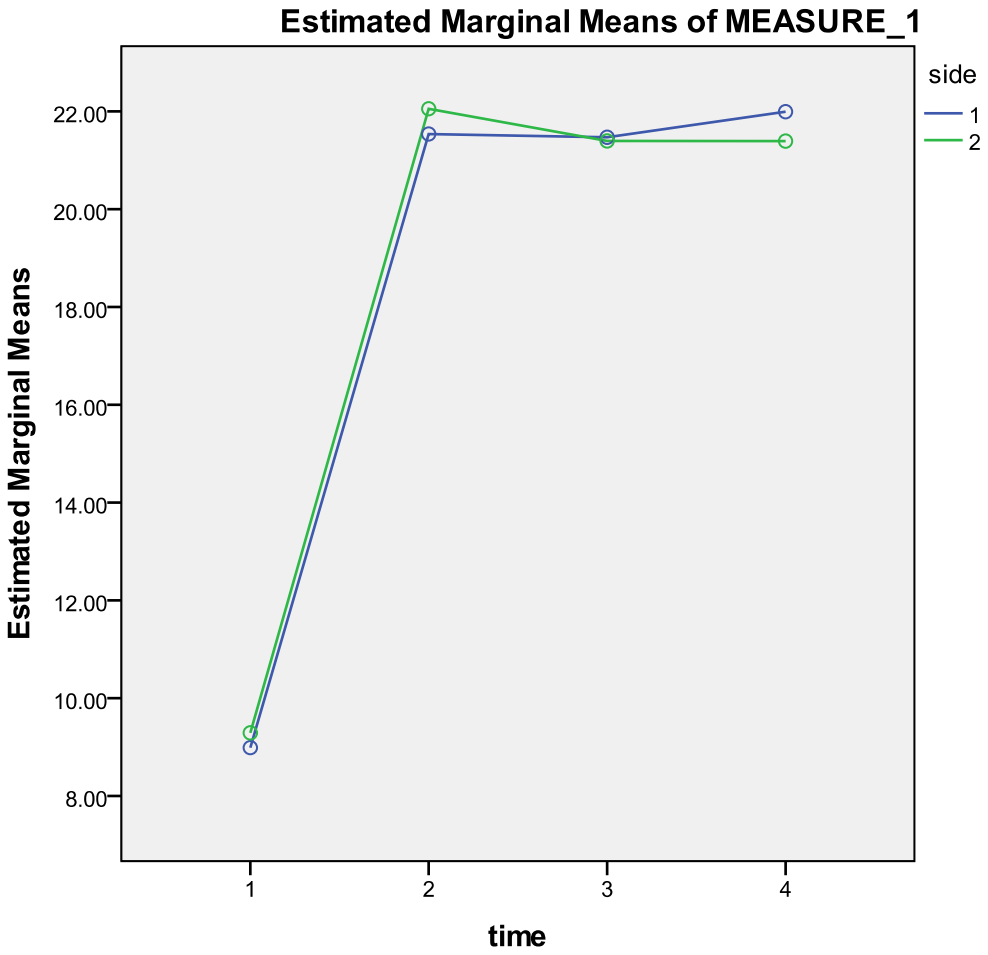
a. Adjustment for multiple comparisons: Bonferroni.

This table illustrates the significant difference between the baseline measurement and the measurements 24h, 48h and 72h after the irradiation. There is no significant difference between the measurements from 24h, 48h and 72h. This means that the degree of erythema is similar between 24 and 72 hours but it is significantly different from the baseline measurement.

arm * time

arm	time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
right	BL	8.989	.411	8.108	9.870
	24h	21.539	.479	20.512	22.565
	48h	21.472	.488	20.425	22.519
	72h	21.994	.593	20.723	23.265
left	BL	9.291	.481	8.260	10.323
	24h	22.055	.582	20.806	23.304
	48h	21.397	.448	20.437	22.357
	72h	21.394	.458	20.412	22.376

This table shows the mean values of erythema measurements on both arms during the different timeslots. The mean values for 24h, 48h and 72h have similar confidence intervels, which further indicates a similar degree of erythema during these timeslots.



This figure illustrates the change in the erythema from baseline until 72 h after the irradiation on both arms (1 = right arm, 2 = left arm). It is clear that the erythema at 24h, 48h and 72h are different from that at baseline, on both arms.

Skin temperature

Within-Subjects Factors

arm	time	Dependent Variable
right	BL	HtemperatureBL
	24h	Htemperature24
	48h	Htemperature48
	72h	Htemperature72
left	BL	VtemperatureBL
	24h	Vtemprature24t
	48h	Vtemperature48t
	72h	Vtemperature72t

This table illustrates the analyzed data. The skin temperature was measured on both arms at baseline and 24h, 48h and 72h after the irradiation.

Mauchly's Test of Sphericity

Within Subjects Effect	Sig.
arm	
time	.668
arm * time	.070

This table illustrates the sphericity of the data. There is a significant sphericity in this data.

Tests of Within-Subjects Effects

Source		Sig.
arm	Greenhouse-Geisser	.146
time (BL,24h,48h,72h)	Greenhouse-Geisser	.000
arm * time	Greenhouse-Geisser	.312

This table illustrates the test for any significant difference between the arms and between the times. There is no significant difference between the arms, which means that the degree of skin temperature was similar on both arms. There is a significant difference between the timeslots (BL, 24h, 48h and 72h), which means that the degree of skin temperature was different within the timeslots. This table also shows that there is no correlation between the side and time, which means that the same degree of skin temperature can be achieved at a given time point

Pairwise Comparisons

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
BL	24h	-1.640*	.289	.000	-2.526	-.754
	48h	-1.403*	.278	.001	-2.256	-.551
	72h	-1.103*	.260	.005	-1.901	-.305
24h	BL	1.640*	.289	.000	.754	2.526
	48h	.237	.220	1.000	-.437	.910
	72h	.537	.203	.117	-.087	1.161
48h	BL	1.403*	.278	.001	.551	2.256
	24h	-.237	.220	1.000	-.910	.437
	72h	.300	.195	.874	-.297	.897
72h	BL	1.103*	.260	.005	.305	1.901
	24h	-.537	.203	.117	-1.161	.087
	48h	-.300	.195	.874	-.897	.297

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

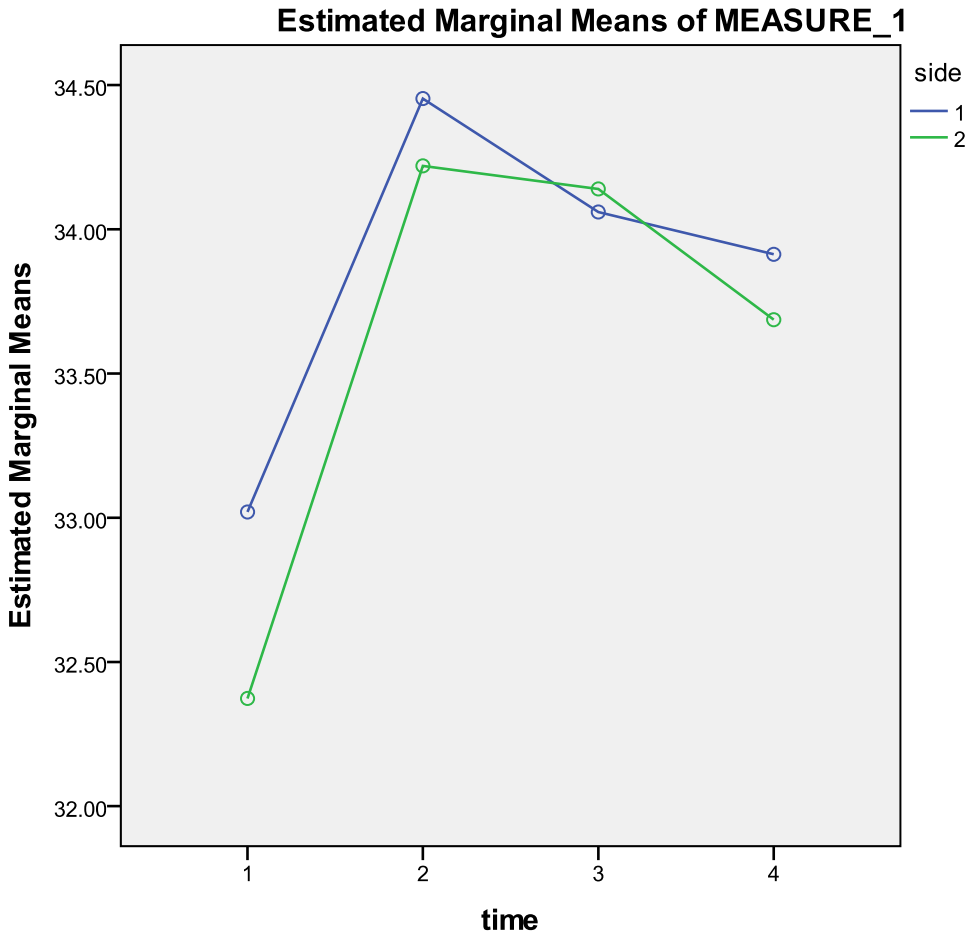
a. Adjustment for multiple comparisons: Bonferroni.

This table illustrates the significant difference between the baseline measurement and the measurements from 24h, 48h and 72h after the irradiation. There is no significant difference between the measurements from 24h, 48h and 72h. This means that the degree of skin temperature is similar between 24 and 72 hours but it is significantly different from the baseline measurement.

arm * time

arm	time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
right	BL	33.020	.296	32.386	33.654
	24h	34.453	.187	34.051	34.855
	48h	34.060	.243	33.538	34.582
	72h	33.913	.225	33.432	34.395
left	BL	32.373	.209	31.924	32.822
	24h	34.220	.220	33.749	34.691
	48h	34.140	.322	33.449	34.831
	72h	33.687	.375	32.883	34.491

This table shows the mean values of skin temperature on both arms during the different timeslots. The mean values for 24h, 48h and 72h have similar confidence intervels, which further indicates a similar degree of skin temperature during these timeslots.



This figure illustrates the change in the skin temperature from baseline until 72 h after the irradiation on both arms (1 = right arm, 2 = left arm). It is clear that skin temperature at 24h, 48h and 72h are different from that at baseline, on both arms.

Mean blood flow in the area of primary hyperalgesia.

Within-Subjects Factors

arm	time	Dependent Variable
right	24h	HPflux24
	48h	HPflux48
	72h	HPflux72
left	24h	VPflux24t
	48h	VPflux48t
	72h	VPflux72t

This table illustrates the analyzed data. The data is analyzed as the percentage of the mean increase in the blood flow. The blood flow in the primary area was measured on both arms at baseline and 24h, 48h and 72h after irradiation.

Mauchly's Test of Sphericity

Within Subjects Effect	Sig.
arm	
time (BL,24h,48h,72h)	.252
side * time	.351

This table illustrates the sphericity of the data. There is significant sphericity in this data.

Tests of Within-Subjects Effects

Source		Sig.
arm	Greenhouse-Geisser	.826
Time (BL, 24h,48h,72h)	Greenhouse-Geisser	.000
arm * time	Greenhouse-Geisser	.461

This table illustrates the test for any significant difference between the arms and between the times. There is no significant difference between the arms, which means that the degree of the increase in mean blood flow was similar on both arms. There is a significant difference between the times, which means that the degree of the increase in the mean blood flow was different within the timeslots. This table also shows that there is no interaction between the side and time, which means that a similar degree of increase in the mean blood flow can be achieved at a given time point regardless the arm.

Pairwise Comparisons

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
24h	48h	134.958*	36.280	.007	36.359	233.557
	72h	242.530*	48.270	.001	111.344	373.717
48h	24h	-134.958*	36.280	.007	-233.557	-36.359
	72h	107.572*	34.956	.025	12.570	202.575
72h	24h	-242.530*	48.270	.001	-373.717	-111.344
	48h	-107.572*	34.956	.025	-202.575	-12.570

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

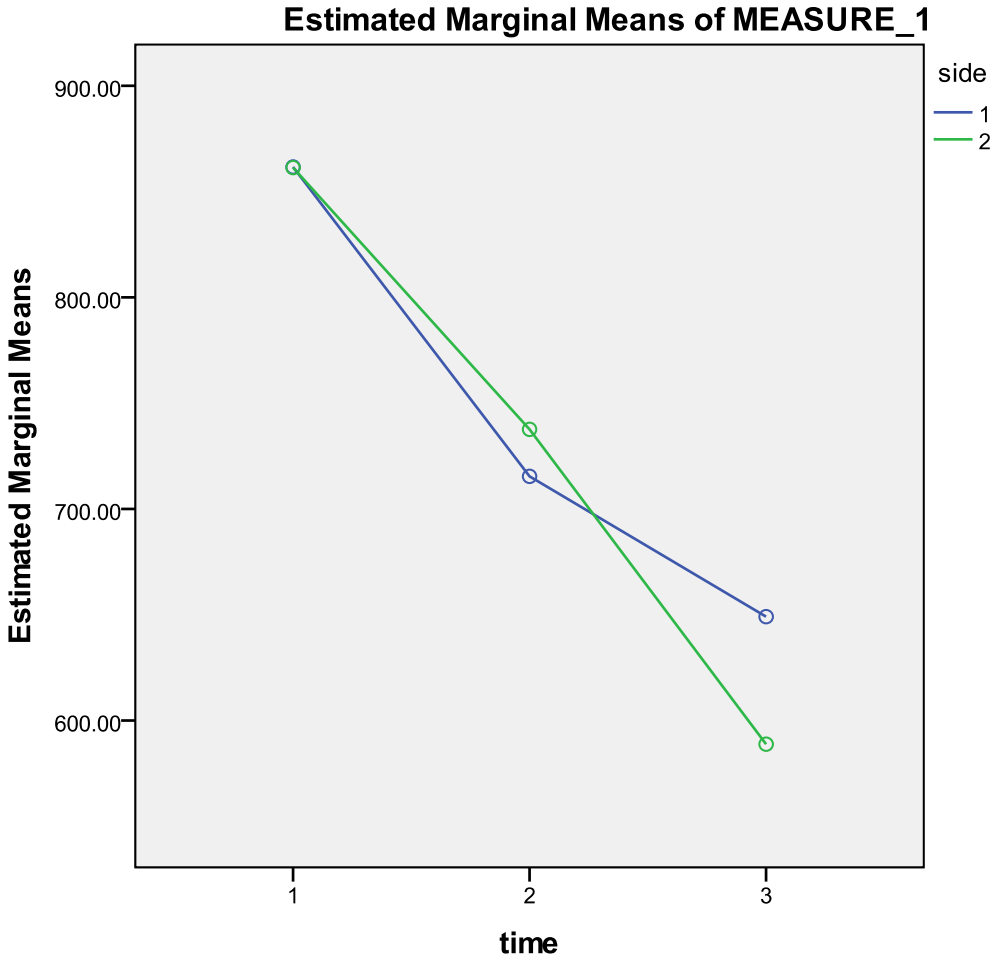
a. Adjustment for multiple comparisons: Bonferroni.

This table illustrates the significant difference between the measurements from 24h, 48h and 72h after the irradiation.

arm* time

arm	Time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
right	24h	861.601	71.463	708.327	1014.874
	48h	715.401	65.139	575.691	855.112
	72h	649.052	57.192	526.387	771.717
left	24h	861.309	79.115	691.625	1030.993
	48h	737.592	67.020	593.849	881.335
	72h	588.797	45.022	492.234	685.359

This table shows the mean values of the degree ofblood flow during the different timeslots and on both arms. The mean values for 24h, 48h and 72h do not have similar confidence intervels which further indicates that the degree of blood flow is decreasing during these timeslots.



This figure illustrates the change in the increase in the mean blood flow from 24h until 72 h after the irradiation on both arms (1 = right arm, 2 = left arm). It is clear that the degree of mean blood flow decreases from 24h to 27h after the irradiation, on both arms.

Analysis of the measurements for the mechanical and thermal stimuli

Brush

Within-Subjects Factors

arm	time	Dependent Variable
right	BL	HBrushBL
	24h	HBrush24t
	48h	HBrush48t
	72h	HBrush72t
left	BL	VBrushBL
	24h	VBrush24t
	48h	VBrush48t
	72h	VBrush72t

This table illustrates the analyzed data. The eVas score for brush evoked allodynia was measured on both arms at baseline and 24h, 48h and 72h after irradiation.

Mauchly's Test of Sphericity

Within Subjects Effect	Sig.
arm	
time (BL,24h,48h,72h)	.016
arm * time	.003

This table illustrates the sphericity of the data. The data shows significant sphericity between the times. There is not a significant sphericity in this data regarding the interaction between side and time.

Tests of Within-Subjects Effects

Source		Sig.
arm	Greenhouse-Geisser	.249
time BL, 24h, 48h, 72h	Greenhouse-Geisser	.009
arm * time	Greenhouse-Geisser	.312

This table illustrates the test for any significant difference between the arms and between the times. There is no significant difference between the arms, which means that the of degree brush evoked allodynia was similar on both arms. There is a significant difference between the time, which means that the degree of brush evoked allodynia was different within the timeslots (BL, 24h, 48h and 72h). This table also shows that there is no interaction between the arms and time, which means that a similar degree of brush evoked allodynia can be achieved at a given time point regardless the arm.

Pairwise Comparisons

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
BL	24h	-.347	.168	.345	-.861	.168
	48h	-.523*	.151	.022	-.986	-.061
	72h	-.367	.147	.157	-.819	.086
24h	BL	.347	.168	.345	-.168	.861
	48h	-.177	.100	.603	-.485	.132
	72h	-.020	.142	1.000	-.457	.417
48h	BL	.523*	.151	.022	.061	.986
	24h	.177	.100	.603	-.132	.485
	72h	.157	.075	.329	-.073	.386
72h	BL	.367	.147	.157	-.086	.819
	24h	.020	.142	1.000	-.417	.457
	48h	-.157	.075	.329	-.386	.073

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

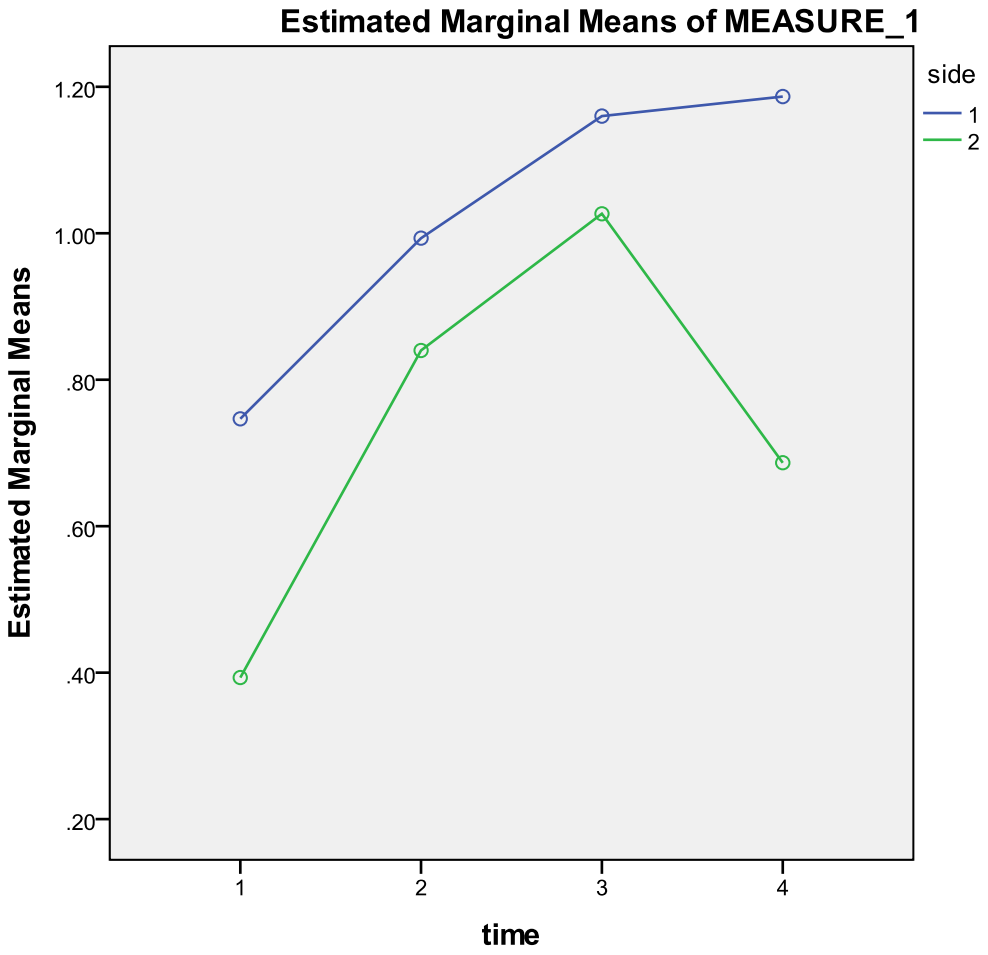
*. The mean difference is significant at the .05 level.

This table illustrates that the detected significant difference between the times is actually the difference between the baseline measurement and the measurements from 48h after the irradiation. This means that the brush induced allodynia was only detected at 48h. There is no significant difference between the measurements from 24h, 48h and 72h.

arm* time

side	time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
right	BL	.747	.239	.234	1.260
	24h	.993	.234	.491	1.496
	48h	1.160	.212	.706	1.614
	72h	1.187	.255	.640	1.733
left	BL	.393	.148	.075	.712
	24h	.840	.251	.301	1.379
	48h	1.027	.264	.460	1.594
	72h	.687	.106	.459	.914

This table shows the mean values of brush evoked allodynia on both arms during the different timeslots. The mean values for 24h, 48h and 72h have similar confidence intervels (CI) except the CI for 48h and 72h on the left arm. The related CI values indicate a similar degree of brush evoked allodynia during these timeslots.



This figure illustrates the change brush induced allodynia from baseline until 72 h after the irradiation on both arms (1 = right arm, 2 = left arm). As mentioned, the baseline measurements on both arms are only significantly different from the measurements at 48h.

von Frey

Within-Subjects Factors

side	time	Dependent Variable
right	BL	HvonFreyBL
	24h	HvonFrey24t
	48h	HvonFry48t
	72h	HvonFrey72t
left	BL	VvonFreyBL
	24h	VvonFrey24t
	48h	VvonFrey48t
	72h	VvonFrey72t

This table illustrates the analyzed data. The primary hyperalgasia was detected on both arms with von Frey filaments and the measurements were taken at baseline and 24h, 48h and 72h after irradiation.

Mauchly's Test of Sphericity

Within Subjects Effect	Sig.
arm	
time (BL, 24h, 48h,72h)	.038
side * time	.682

This table illustrates the sphericity of the data. There is significant sphericity in the interaction between arm and time, but not between the arms.

Tests of Within-Subjects Effects

Source		Sig.
arm	Greenhouse-Geisser	.025
time BL, 24h,48h,72h	Greenhouse-Geisser	.124
side * time	Greenhouse-Geisser	.600

This table illustrates the test for any significant difference between the arms and between the times. There is a significant difference between the arms, which means that the degree of von Frey evoked primary hyperalgesia was not similar on both arms. There is no significant difference between the times, which means that the degree of von Frey evoked primary hyperalgesia was not different within the timeslots. This table also shows that there is no interaction between the arms and time, which means that a similar degree of von Frey evoked primary hyperalgesia can be achieved at a given time point regardless the arm.

Pairwise Comparisons

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
BL	24h	.600	.277	.288	-.250	1.450
	48h	.600	.277	.288	-.250	1.450
	72h	.433	.365	1.000	-.686	1.553
24h	BL	-.600	.277	.288	-1.450	.250
	48h	.000	.239	1.000	-.734	.734
	72h	-.167	.216	1.000	-.831	.497
48h	BL	-.600	.277	.288	-1.450	.250
	24h	.000	.239	1.000	-.734	.734
	72h	-.167	.193	1.000	-.759	.426
72h	BL	-.433	.365	1.000	-1.553	.686
	24h	.167	.216	1.000	-.497	.831
	48h	.167	.193	1.000	-.426	.759

Based on estimated marginal means

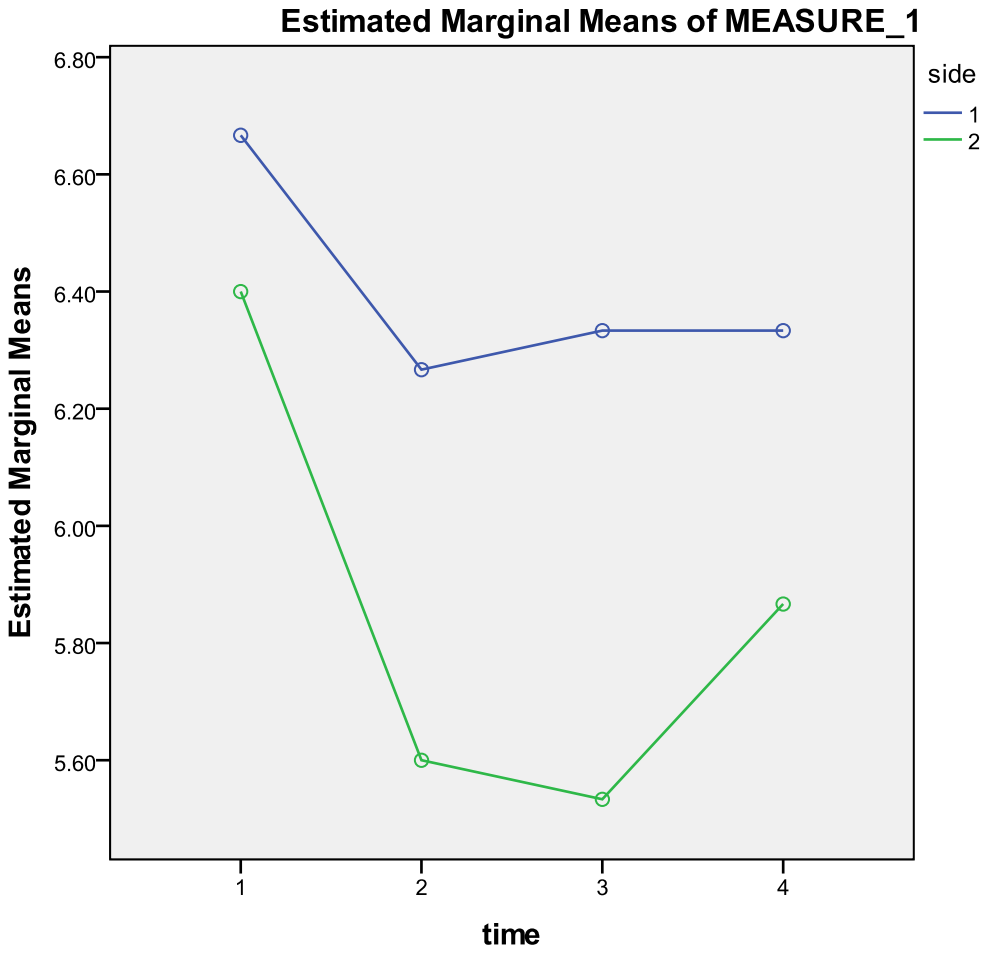
a. Adjustment for multiple comparisons: Bonferroni.

This table illustrates that there is no significant difference between the timeslots, i.e. BL, 24h, 48h and 72h.

arm * time

arm	time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
right	BL	6.667	.361	5.893	7.440
	24h	6.267	.300	5.623	6.911
	48h	6.333	.303	5.683	6.984
	72h	6.333	.232	5.835	6.832
left	BL	6.400	.335	5.681	7.119
	24h	5.600	.254	5.054	6.146
	48h	5.533	.236	5.026	6.040
	72h	5.867	.307	5.209	6.524

This table shows the mean values of the degree of von Frey evoked primary hyperalgesia on both arms during the different timeslots. The mean values for 24h, 48h and 72h have similar confidence intervels, which further indicates a similar degree of primary hyperalgesia during these timeslots.



This figure illustrates the change in the brush evoked allodynia from baseline until 72 h after the irradiation on both arms (1 = right arm, 2 = left arm). The degree of brush evoked allodynia was similar between the timeslots.

Pressure Pain Threshold (PPT)

Within-Subjects Factors

Arm	time	Dependent Variable
right	BL	HPPTBL
	24h	HPPT24t
	48h	HPPT48t
	72h	HPPT72t
left	BL	VPPTBL
	24h	VPPT24t
	48h	VPPT48t
	72h	VPPT72t

This table illustrates the analyzed data. PPT was measured on both arms at baseline and 24h, 48h and 72h after irradiation.

Mauchly's Test of Sphericity

Within Subjects Effect	Sig.
arm	
time	.000
	.005

This table illustrates the sphericity of the data. There is no significant sphericity in this data.

Tests of Within-Subjects Effects

Source		Sig.
arm	Greenhouse-Geisser	.020
time BL, 24h, 48h, 72h	Greenhouse-Geisser	.000
arm * time	Greenhouse-Geisser	.334

This table illustrates the test for any significant difference between the arms and between the times. There is a significant difference between the arms, which means that the degree of PPT was not similar on both arms. There is a significant difference between the times, which means that the degree of PPT was different within the timeslots. This table also shows that there is no interaction between the arms and times, which means that a similar degree of PPT can be achieved at a given time point regardless the arm.

Pairwise Comparisons

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
BL	24h	261.233*	41.426	.000	134.105	388.362
	48h	293.567*	38.025	.000	176.875	410.258
	72h	301.833*	30.848	.000	207.169	396.498
24h	BL	-261.233*	41.426	.000	-388.362	-134.105
	48h	32.333	17.103	.477	-20.153	84.819
	72h	40.600	19.154	.314	-18.179	99.379
48h	BL	-293.567*	38.025	.000	-410.258	-176.875
	24h	-32.333	17.103	.477	-84.819	20.153
	72h	8.267	10.092	1.000	-22.704	39.237
72h	BL	-301.833*	30.848	.000	-396.498	-207.169
	24h	-40.600	19.154	.314	-99.379	18.179
	48h	-8.267	10.092	1.000	-39.237	22.704

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

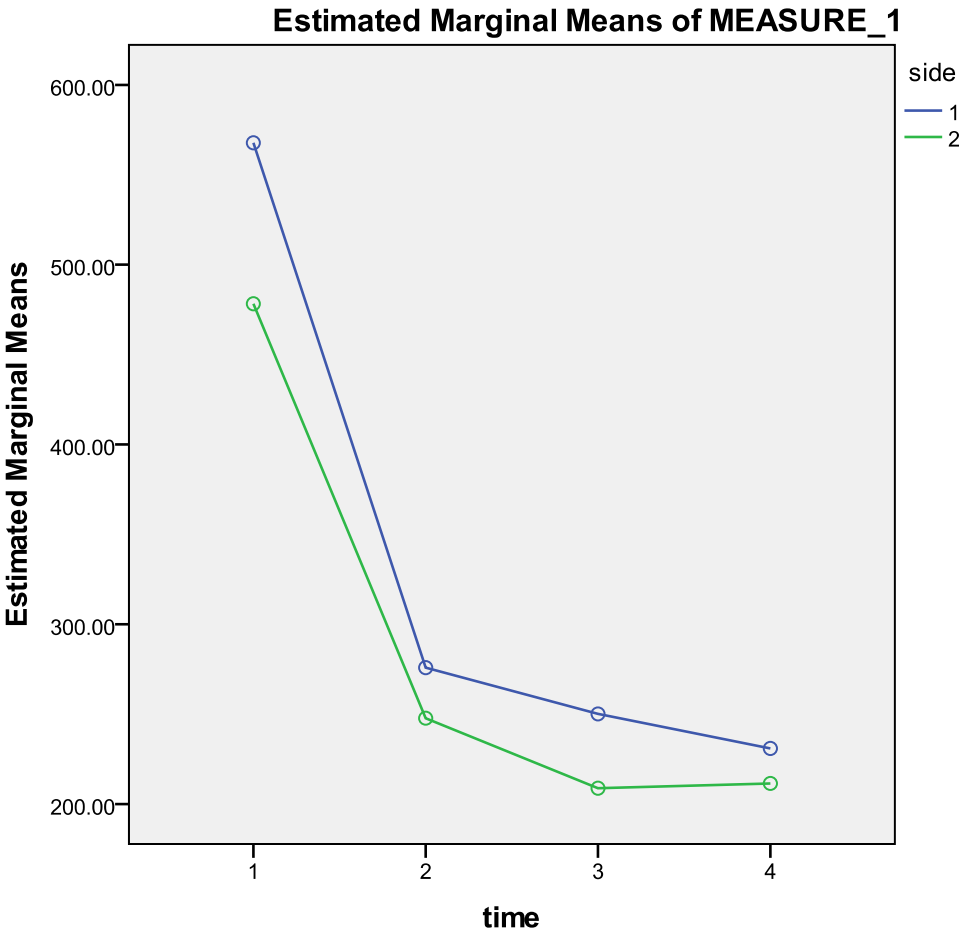
a. Adjustment for multiple comparisons: Bonferroni.

This table illustrates the significant difference between the baseline measurement and the measurements from 24h, 48h and 72h after the irradiation. There is no significant difference between the measurements from 24h, 48h and 72h. This means that the degree of PPT is similar between 24 and 72 hours but it is significantly different from the baseline measurement.

arm * time

arm	time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
rigth	BL	567.800	50.279	459.963	675.637
	24h	275.867	37.664	195.086	356.648
	48h	250.133	37.444	169.823	330.443
	72h	230.933	33.658	158.744	303.123
left	BL	478.267	45.548	380.576	575.957
	24h	247.733	24.418	195.363	300.104
	48h	208.800	25.920	153.207	264.393
	72h	211.467	28.650	150.019	272.915

This table shows the mean values of the PPT on both arms during the different timeslotsThe mean values for 24h, 48h and 72h have similar confidence intervels. which further indicates a similar degree of erythema during these timeslots.



This figure illustrates the change in the PPT from baseline until 72 h after the irradiation on both arms (1 = right arm, 2 = left arm). It is clear that PPT at 24h, 48h and 72h are different from that at baseline, on both arms.

Heat Pain Threshold (HPT)

Within-Subjects Factors

side	time	Dependent Variable
right	BL	HTPTTBL
	24h	HTPTT24t
	48h	HTPTT48t
	72h	HTPTT72t
left	BL	VTPTTBL
	24h	VTPTT24t
	48h	VTPTT48t
	72h	VTPTT72t

This table illustrates the analyzed data. HPT was measured on both arms at baseline and 24h, 48h and 72h after irradiation.

Mauchly's Test of Sphericity

Within Subjects Effect	Sig.
arm	
Time (BL,24h,48h,72h)	.000
arm * time	.000

This table illustrates the sphericity of the data. There is not significant sphericity in this data.

Tests of Within-Subjects Effects

Source		Sig.
arm	Greenhouse-Geisser	.741
Time (BL,24h,48h,72h)	Greenhouse-Geisser	.000
arm * time	Greenhouse-Geisser	.895

This table illustrates the test for any significant difference between the arms and between the times. There is no significant difference between the arms, which means that the HPT was similar on both arms. There is a significant difference between the times, which means that the HPT was different within the timeslot . This table also shows that there is no interaction between the arms and times, which means that a similar HPT can be achieved at a given time point regardless the arm.

Pairwise Comparisons

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
BL	24h	4.170*	.586	.000	2.373	5.967
	48h	3.977*	.514	.000	2.399	5.555
	72h	3.617*	.533	.000	1.981	5.252
24h	BL	-4.170*	.586	.000	-5.967	-2.373
	48h	-.193	.184	1.000	-.758	.371
	72h	-.553*	.174	.040	-1.087	-.020
48h	BL	-3.977*	.514	.000	-5.555	-2.399
	24h	.193	.184	1.000	-.371	.758
	72h	-.360*	.113	.040	-.707	-.013
72h	BL	-3.617*	.533	.000	-5.252	-1.981
	24h	.553*	.174	.040	.020	1.087
	48h	.360*	.113	.040	.013	.707

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

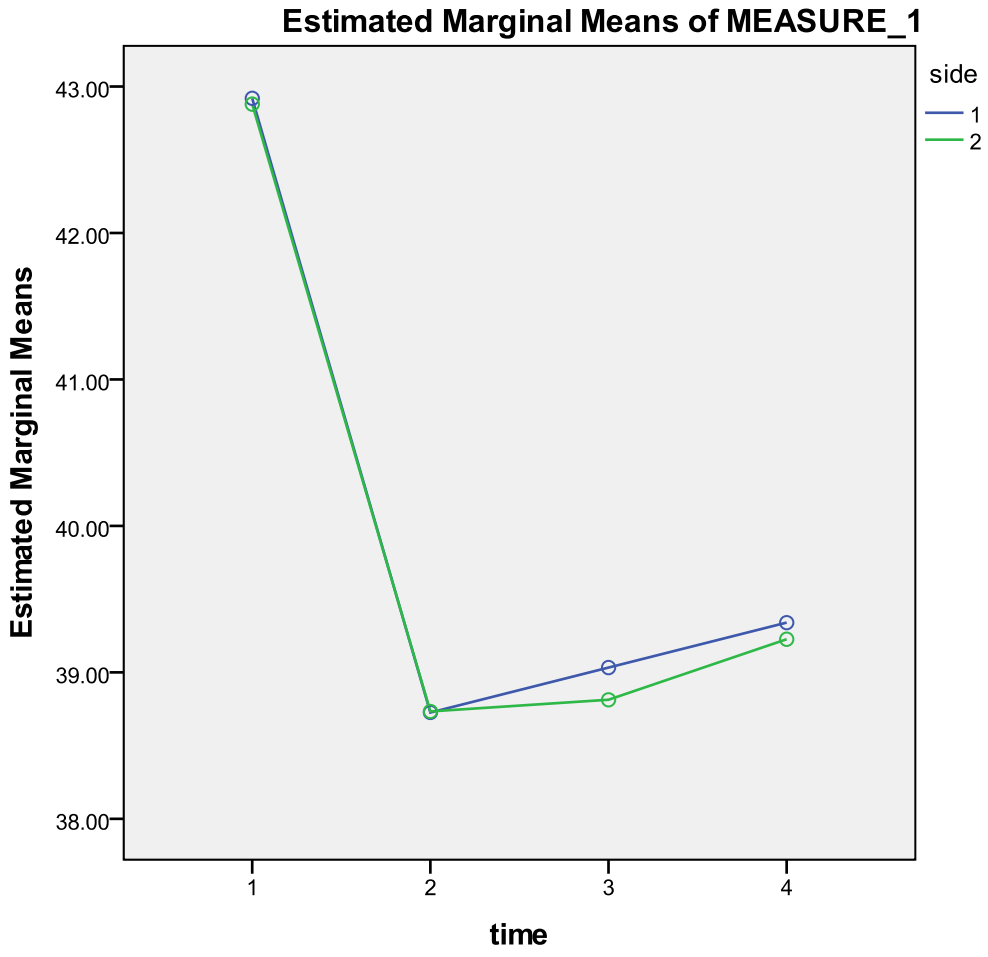
a. Adjustment for multiple comparisons: Bonferroni.

This table illustrates the significant difference between the baseline measurement and the measurements from the 24h, 48h and 72h after the irradiation. There is no significant difference between the measurements from 24h and 48h. This means that the degree of HPT is similar between 24 and 48 hours. Measurements at 72h are significantly different from those at BL, 24h and 48h.

arm * time

arm	time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
right	BL	42.920	.789	41.227	44.613
	24h	38.727	.622	37.392	40.062
	48h	39.033	.624	37.694	40.373
	72h	39.340	.718	37.799	40.881
left	BL	42.880	.874	41.005	44.755
	24h	38.733	.678	37.280	40.186
	48h	38.813	.728	37.252	40.375
	72h	39.227	.699	37.728	40.725

This table shows the mean values of the HPT on both arms during the different timeslots. The mean values for 24h, 48h and 72h have similar confidence intervels, which further indicates a similar degree of HTPduring these timeslots.



This figure illustrates the change in the HPT from baseline until 72 h after the irradiation on both arms (1 = right arm, 2 = left arm). It is clear that the HPT at 24h, 48h and 72h are different from that at baseline, on both arms.

Analysis of pin prick measurements

Within-Subjects Factors

arm	time	pinsize	Dependent Variable
right	BL	1	HpinP0.8BL
		2	HpinP1.6BL
		3	HpinP3.2BL
		4	HpinP6.4BL
		5	HpinP12.8BL
		6	HpinP25.6BL
		7	HpinP50.1BL
		8	HpinP60.0BL
	24h	1	HpinP0.824h
		2	HpinP1.624h
		3	HpinP3.224t
		4	HpinP6.424h
		5	HpinP12.824h
		6	HpinP25.624h
		7	HpinP50.124h
		8	HpinP60.024h
	48h	1	HpinP0.848h
		2	HpinP1.648h
		3	HpinP3.248h
		4	HpinP6.448h
		5	HpinP12.848t
		6	HpinP25.648h
		7	HpinP50.148h
		8	HpinP60.048h
	72h	1	HpinP0.872h
		2	HpinP1.672h
		3	HpinP3.272h
		4	HpinP6.472h
		5	HpinP12.872h
		6	HpinP25.672h
		7	HpinP50.172h
		8	HpinP60.172h
left	BL	1	VpinP0.8BL
		2	VpinP1.6BL
		3	VpinP3.2BL
		4	VpinP6.4BL
		5	VpinP12.8BL
		6	VpinP25.6BL

		7	VpinP50.1BL
		8	VpinP60.0BL
	24h	1	VpinP0.824h
		2	VpinP1.624h
		3	VpinP3.224h
		4	VpinP6.424h
		5	VpinP12.824h
		6	VpinP25.624h
		7	VpinP50.124h
		8	VpinP60.024h
	48h	1	VpinP0.848h
		2	VpinP1.648h
		3	VpinP3.248h
		4	VpinP6.448h
		5	VpinP12.848h
		6	VpinP25.648h
		7	VpinP50.148h
		8	VpinP60.048h
	72h	1	VpinP0.872h
		2	VpinP1.672h
		3	VpinP3.272h
		4	VpinP6.472h
		5	VpinP12.872h
		6	VpinP25.672h
		7	VpinP50.172h
		8	VpinP60.072h

This table illustrates the analyzed data. Primary hyperalgesia evoked by pin Prick was measured on both arms at baseline and 24h, 48h and 72h after irradiation.

Mauchly's Test of Sphericity

Within Subjects Effect	Sig.
arm	
time (BL,24h,48h,72h)	.000
pinsize	.000
arm * time	.711
arm * pinsize	.000
time * pinsize	
arm * time * pinsize	

This table illustrates the sphericity of the data. There is no significant sphericity in this data, except for the interaction between side and time.

Tests of Within-Subjects Effects

Source		Sig.
arm	Greenhouse-Geisser	.755
time (BL,24h,48h,72h)	Greenhouse-Geisser	.000
pinsize	Greenhouse-Geisser	.000
arm * time	Greenhouse-Geisser	.798
arm * pinsize	Greenhouse-Geisser	.686
time * pinsize	Greenhouse-Geisser	.000
arm * time * pinsize	Greenhouse-Geisser	.923

This table illustrates the test for any significant difference between the arms, times and sizes of pins. There is no significant difference between the arms , which means that the pin prick induced primary hyperalgesia was similar on both arms. There is a significant difference between the times, which means that the pin prick induced primary hyperalgesia was different within the timeslots. There is a significant difference between the pin sizes, which means that the pin prick induced primary hyperalgesia was different depending on the pin size. This table also shows that there is no interaction between the sides, times and pins which means that a similar degree of pin prick induced primary hyperalgesia can be achieved at a given time point, with a given pin, regardless the arm.

Pairwise Comparisons

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
BL	24h	-3.191*	.329	.000	-4.200	-2.182
	48h	-3.446*	.371	.000	-4.583	-2.309
	72h	-3.319*	.415	.000	-4.591	-2.046
24h	BL	3.191*	.329	.000	2.182	4.200
	48h	-.255	.212	1.000	-.906	.396
	72h	-.128	.249	1.000	-.891	.636
48h	BL	3.446*	.371	.000	2.309	4.583
	24h	.255	.212	1.000	-.396	.906
	72h	.127	.109	1.000	-.206	.461
72h	BL	3.319*	.415	.000	2.046	4.591
	24h	.128	.249	1.000	-.636	.891
	48h	-.127	.109	1.000	-.461	.206

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Adjustment for multiple comparisons: Bonferroni.

This table illustrates the significant difference between the baseline measurement and the measurements from the 24h, 48h and 72h after the irradiation. There is no significant difference between the measurements from 24h, 48h and 72h. This means that the degree of pin prick evoked primary hyperalgesia, with a given pin prick, is similar between 24 and 72 hours but it is significantly different from the baseline measurement.

Pairwise Comparisons

(I) pinsize	(J) pinsize	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	-.493*	.084	.001	-.815	-.172
	3	-1.066*	.100	.000	-1.451	-.680
	4	-1.710*	.158	.000	-2.317	-1.103
	5	-2.230*	.186	.000	-2.946	-1.514
	6	-2.843*	.206	.000	-3.635	-2.052
	7	-3.704*	.247	.000	-4.652	-2.756
	8	-4.127*	.256	.000	-5.109	-3.144
2	1	.493*	.084	.001	.172	.815
	3	-.572*	.055	.000	-.785	-.360
	4	-1.217*	.108	.000	-1.633	-.800
	5	-1.737*	.141	.000	-2.279	-1.195
	6	-2.350*	.167	.000	-2.993	-1.707
	7	-3.211*	.212	.000	-4.027	-2.394
	8	-3.633*	.230	.000	-4.516	-2.751
3	1	1.066*	.100	.000	.680	1.451
	2	.572*	.055	.000	.360	.785
	4	-.644*	.089	.000	-.986	-.303
	5	-1.164*	.114	.000	-1.603	-.726
	6	-1.778*	.144	.000	-2.332	-1.223
	7	-2.639*	.192	.000	-3.378	-1.899
	8	-3.061*	.216	.000	-3.890	-2.232
4	1	1.710*	.158	.000	1.103	2.317
	2	1.217*	.108	.000	.800	1.633
	3	.644*	.089	.000	.303	.986
	5	-.520*	.059	.000	-.747	-.293
	6	-1.133*	.093	.000	-1.490	-.776
	7	-1.994*	.145	.000	-2.551	-1.437
	8	-2.417*	.170	.000	-3.069	-1.765
5	1	2.230*	.186	.000	1.514	2.946
	2	1.737*	.141	.000	1.195	2.279
	3	1.164*	.114	.000	.726	1.603
	4	.520*	.059	.000	.293	.747
	6	-.613*	.049	.000	-.800	-.426
	7	-1.474*	.110	.000	-1.896	-1.053
	8	-1.897*	.141	.000	-2.440	-1.353
6	1	2.843*	.206	.000	2.052	3.635
	2	2.350*	.167	.000	1.707	2.993
	3	1.778*	.144	.000	1.223	2.332
	4	1.133*	.093	.000	.776	1.490
	5	.613*	.049	.000	.426	.800

	7	-.861*	.073	.000	-1.142	-.580
	8	-1.283*	.112	.000	-1.714	-.853
7	1	3.704*	.247	.000	2.756	4.652
	2	3.211*	.212	.000	2.394	4.027
	3	2.639*	.192	.000	1.899	3.378
	4	1.994*	.145	.000	1.437	2.551
	5	1.474*	.110	.000	1.053	1.896
	6	.861*	.073	.000	.580	1.142
	8	-.422*	.069	.001	-.689	-.156
8	1	4.127*	.256	.000	3.144	5.109
	2	3.633*	.230	.000	2.751	4.516
	3	3.061*	.216	.000	2.232	3.890
	4	2.417*	.170	.000	1.765	3.069
	5	1.897*	.141	.000	1.353	2.440
	6	1.283*	.112	.000	.853	1.714
	7	.422*	.069	.001	.156	.689

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Adjustment for multiple comparisons: Bonferroni.

This table illustrates a significant difference between the pin sizes in all the measurements from the timeslots. This means that the degree of pin pinck induced primary hyperalgesia is dependent on the size of the pin.

arm * pinsize

arm	pinsize	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
right	1	2.748	.400	1.890	3.607
	2	3.247	.386	2.419	4.075
	3	3.858	.356	3.096	4.621
	4	4.567	.312	3.898	5.235
	5	4.997	.332	4.285	5.708
	6	5.628	.327	4.926	6.330
	7	6.507	.315	5.832	7.182
	8	6.868	.294	6.238	7.499
left	1	2.743	.341	2.013	3.474
	2	3.232	.369	2.439	4.024
	3	3.764	.367	2.978	4.551
	4	4.345	.376	3.539	5.151
	5	4.955	.372	4.158	5.752
	6	5.550	.391	4.711	6.389
	7	6.393	.399	5.537	7.249
	8	6.877	.373	6.076	7.678

This table shows the mean values of the pin sizes on both arms. The mean values for 24h, 48h and 72h have similar confidence intervels, which further indicates that similar result can be actived by a give pin on both arms.

5. time * pinsize

Time	pinsize	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
BL	1	1.127	.271	.545	1.709
	2	1.283	.280	.684	1.883
	3	1.575	.323	.883	2.268
	4	1.930	.353	1.173	2.687
	5	2.230	.370	1.436	3.024
	6	2.737	.404	1.870	3.603
	7	3.560	.473	2.546	4.574
	8	3.787	.478	2.761	4.813
24h	1	3.320	.439	2.379	4.261
	2	3.967	.412	3.084	4.849
	3	4.410	.422	3.505	5.315
	4	5.177	.389	4.342	6.012
	5	5.720	.409	4.842	6.598
	6	6.217	.431	5.291	7.142
	7	7.257	.372	6.458	8.055
	8	7.690	.338	6.965	8.415
48h	1	3.467	.429	2.547	4.386
	2	4.000	.451	3.033	4.967
	3	4.730	.425	3.818	5.642
	4	5.460	.360	4.689	6.231
	5	6.073	.369	5.283	6.864
	6	6.693	.344	5.955	7.432
	7	7.420	.340	6.692	8.148
	8	7.953	.309	7.291	8.616
72h	1	3.070	.488	2.024	4.116
	2	3.707	.503	2.628	4.785
	3	4.530	.443	3.580	5.480
	4	5.257	.411	4.376	6.138
	5	5.880	.384	5.056	6.704
	6	6.710	.383	5.888	7.532
	7	7.563	.353	6.807	8.320
	8	8.060	.302	7.411	8.709

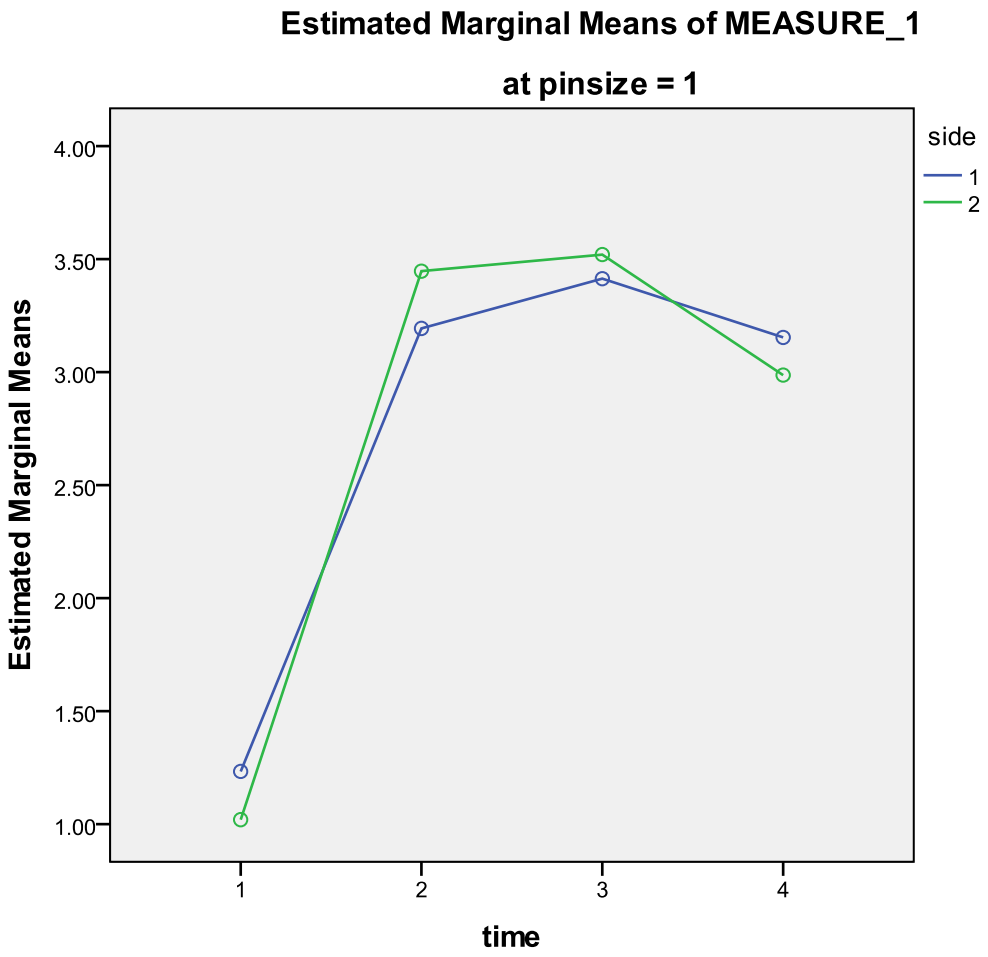
This table shows the mean values of the pin sizes during the diffent timeslots. The mean values for give pin at a given time have different confidence intervels, which further indicates a significant difference in the degree of pin prick induces primary hyperalgesia during the timeslots.

6. arm * time * pinsize

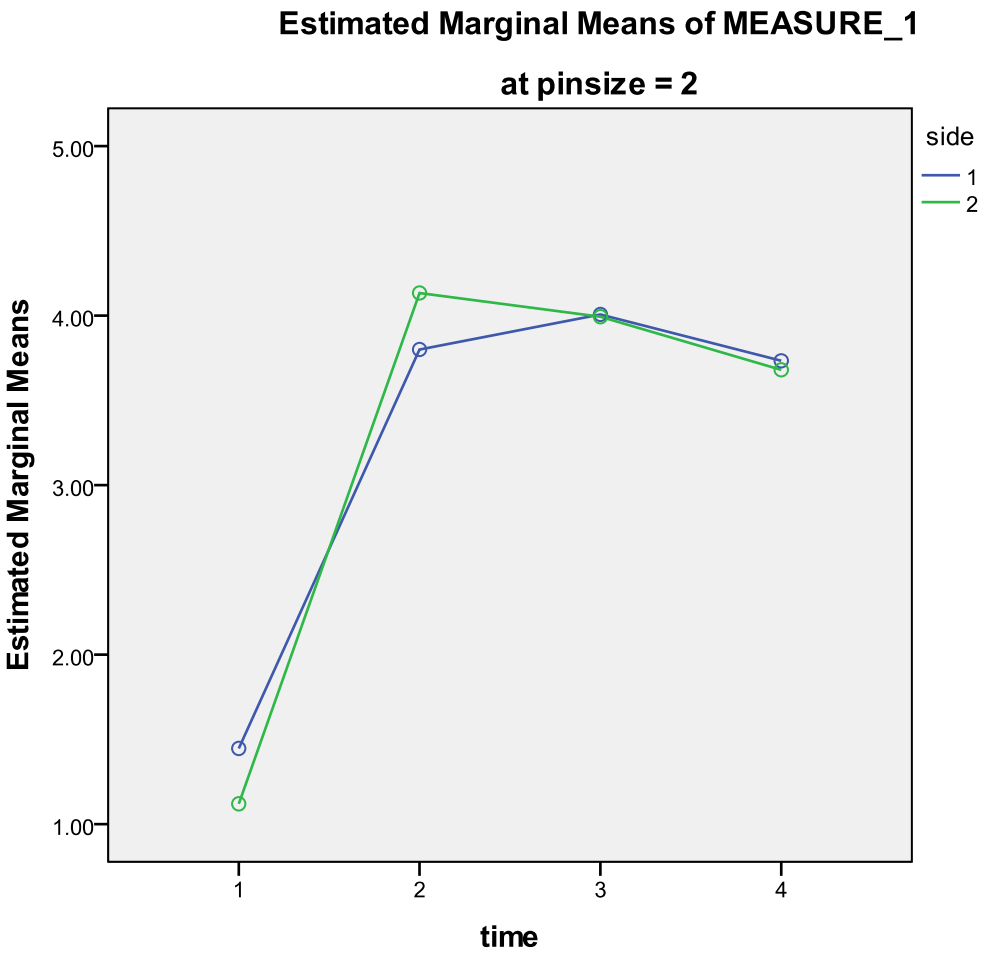
side	time	pinsize	Mean	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
Right	BL	1	1.233	.342	.501	1.966
		2	1.447	.375	.641	2.252
		3	1.787	.423	.880	2.693
		4	2.140	.417	1.246	3.034
		5	2.340	.432	1.414	3.266
		6	2.860	.475	1.842	3.878
		7	3.733	.478	2.707	4.760
		8	3.913	.502	2.837	4.990
	24h	1	3.193	.527	2.062	4.324
		2	3.800	.471	2.789	4.811
		3	4.393	.474	3.376	5.410
		4	5.227	.444	4.275	6.179
		5	5.693	.453	4.721	6.666
		6	6.213	.437	5.276	7.151
		7	7.293	.339	6.565	8.021
		8	7.687	.298	7.047	8.326
	48h	1	3.413	.427	2.497	4.330
		2	4.007	.410	3.128	4.885
		3	4.593	.433	3.664	5.523
		4	5.500	.324	4.805	6.195
		5	6.060	.311	5.393	6.727
		6	6.647	.299	6.005	7.288
		7	7.407	.322	6.716	8.098
		8	7.800	.335	7.083	8.517
	72h	1	3.153	.545	1.983	4.323
		2	3.733	.550	2.554	4.913
		3	4.660	.476	3.640	5.680
		4	5.400	.390	4.565	6.235
		5	5.893	.392	5.053	6.733
		6	6.793	.369	6.001	7.585
		7	7.593	.345	6.853	8.334
		8	8.073	.307	7.414	8.733
left	BL	1	1.020	.386	.192	1.848
		2	1.120	.378	.309	1.931
		3	1.364	.439	.423	2.305
		4	1.720	.500	.647	2.793
		5	2.120	.458	1.137	3.103
		6	2.613	.489	1.565	3.662
		7	3.387	.563	2.179	4.594
		8	3.660	.536	2.511	4.809

	24h	1	3.447	.440	2.503	4.390
		2	4.133	.454	3.160	5.106
		3	4.427	.485	3.387	5.466
		4	5.127	.463	4.133	6.120
		5	5.747	.475	4.727	6.766
		6	6.220	.525	5.094	7.346
		7	7.220	.497	6.154	8.286
		8	7.693	.467	6.692	8.695
	48h	1	3.520	.529	2.384	4.656
		2	3.993	.563	2.787	5.200
		3	4.867	.522	3.747	5.987
		4	5.420	.495	4.358	6.482
		5	6.087	.478	5.062	7.111
		6	6.740	.440	5.797	7.683
		7	7.433	.421	6.530	8.336
		8	8.107	.375	7.302	8.911
	72h	1	2.987	.510	1.893	4.080
		2	3.680	.514	2.578	4.782
		3	4.400	.502	3.324	5.476
		4	5.113	.500	4.042	6.185
		5	5.867	.442	4.920	6.814
		6	6.627	.451	5.659	7.595
		7	7.533	.422	6.629	8.438
		8	8.047	.361	7.273	8.820

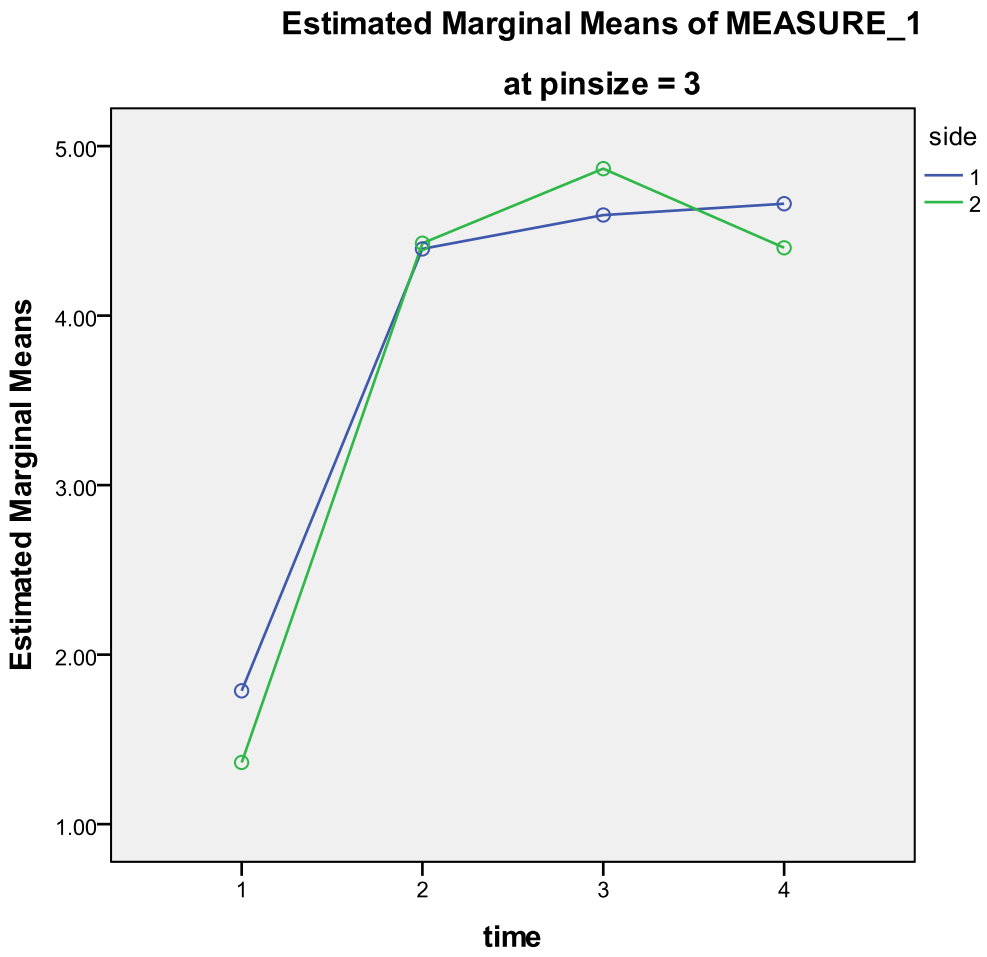
This table shows the mean values of the pin sizes during the diffent timeslots at a given arm. The mean values for give pin at a given time and arm have different confidence intervels, which further indicates a significant difference in the degree of pin prick induces primary hyperalgesia during the timeslots on both arms.



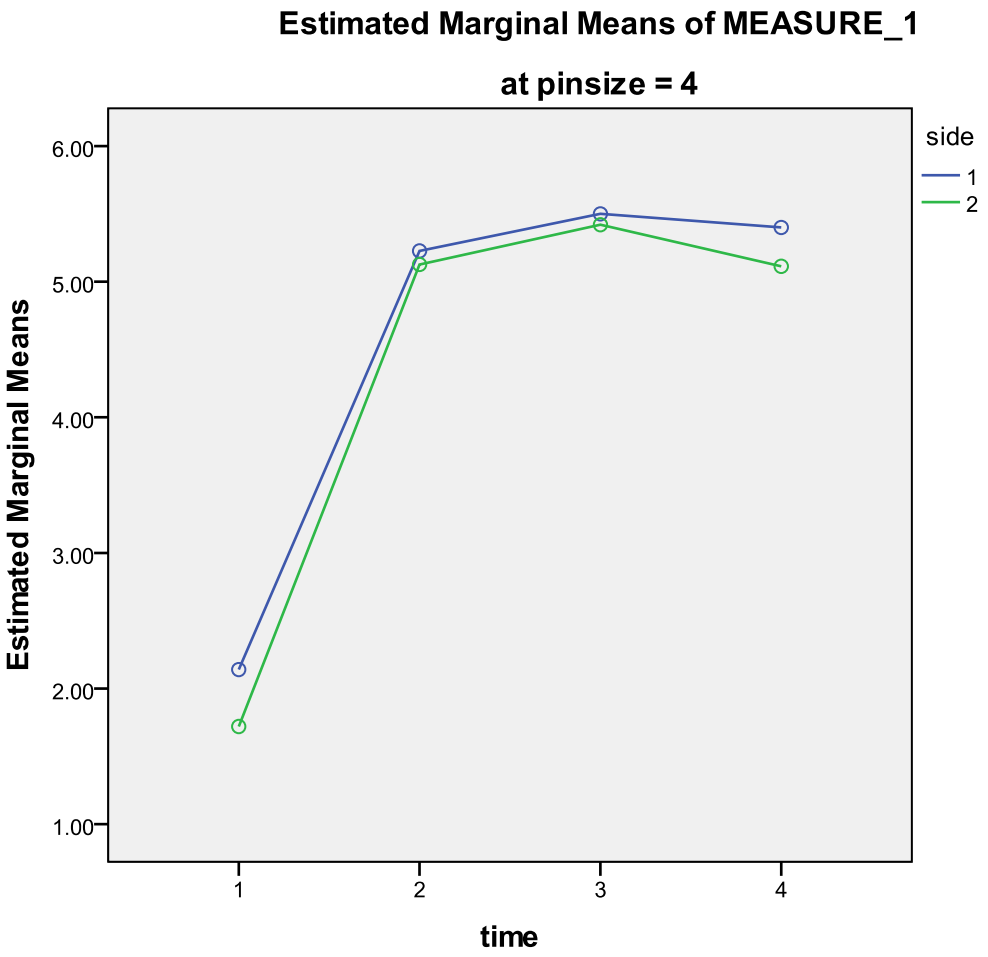
This figure illustrates the change in the primary hyperalgesia, induced by pin prick 0.8, from baseline until 72 h after the irradiation on both arms (1 = right arm, 2 = left arm). It is clear that the primary hyperalgesia at 24h, 48h and 72h are different from that at baseline, on both arms.



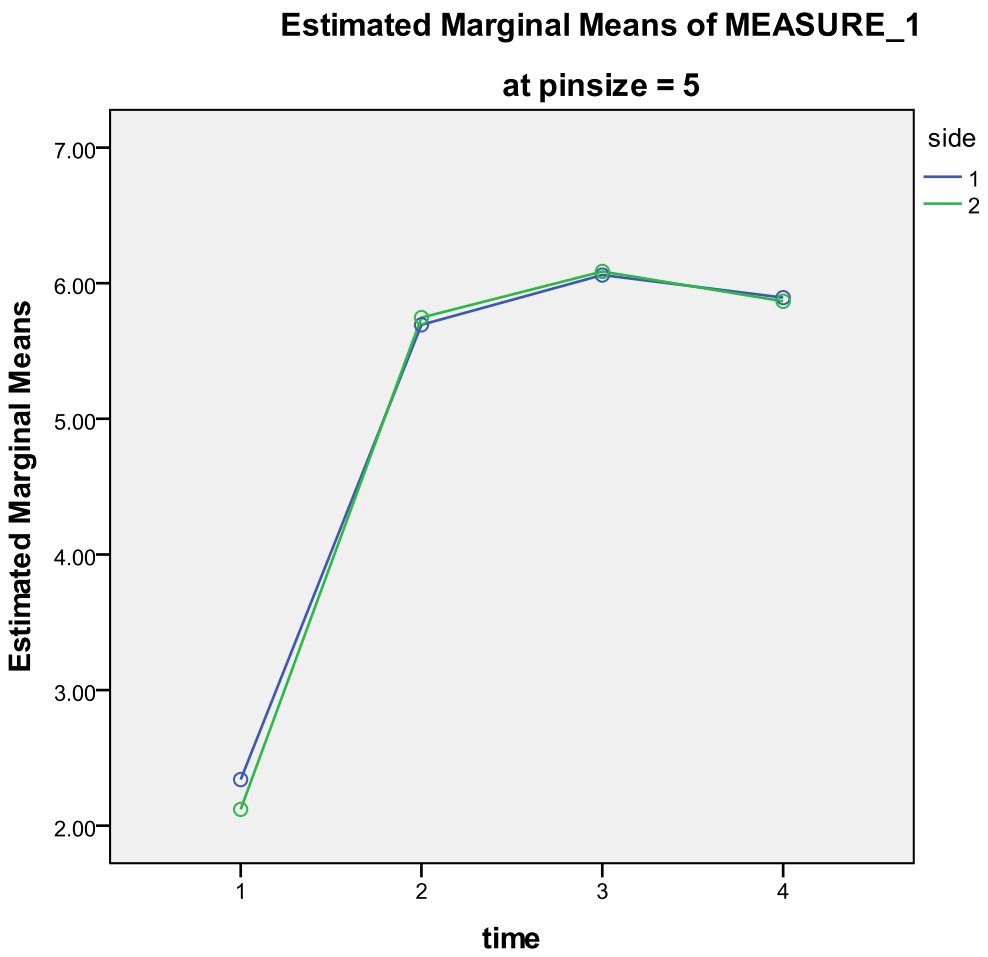
This figure illustrates the change in the primary hyperalgesia, induced by pin prick 1.6, from baseline until 72 h after the irradiation on both arms (1 = right arm, 2 = left arm). It is clear that the primary hyperalgesia at 24h, 48h and 72h are different from that at baseline, on both arms.



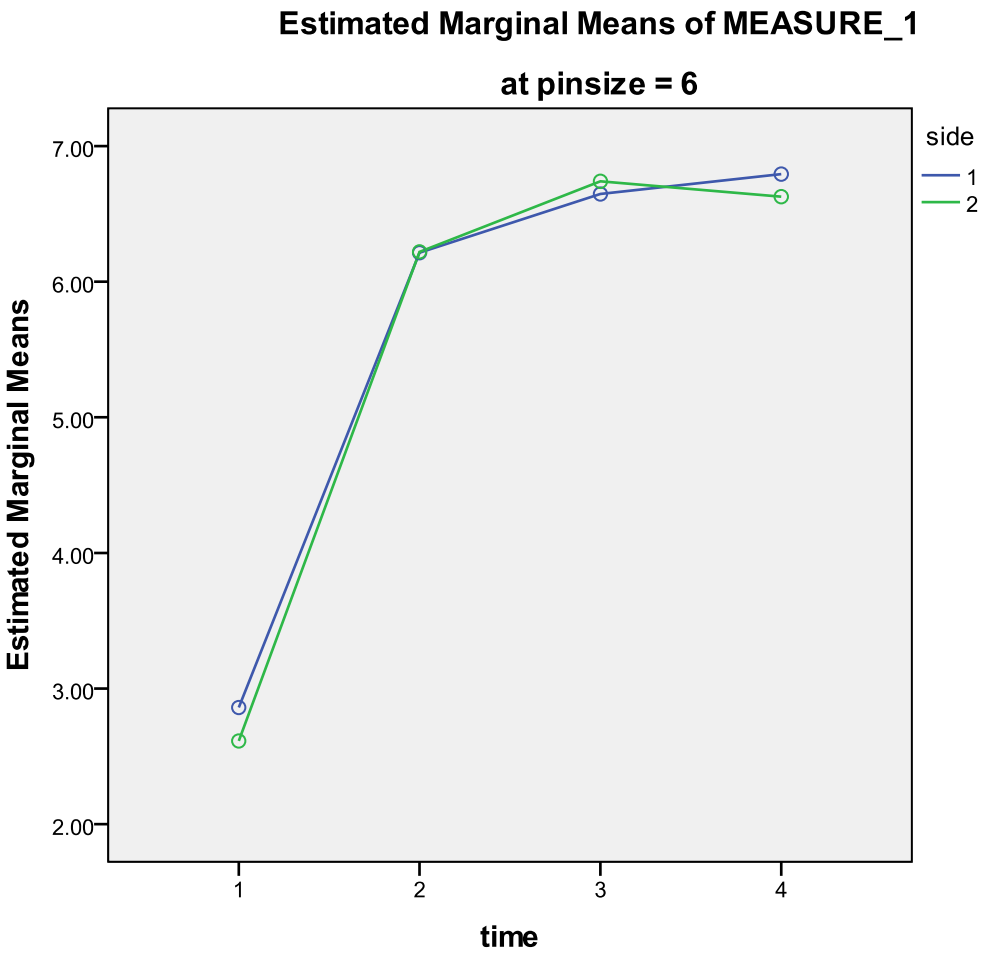
This figure illustrates the change in the primary hyperalgesia, induced by pin prick 3.2, from baseline until 72 h after the irradiation on both arms (1 = right arm, 2 = left arm). It is clear that the primary hyperalgesia at 24h, 48h and 72h are different from that at baseline, on both arms.



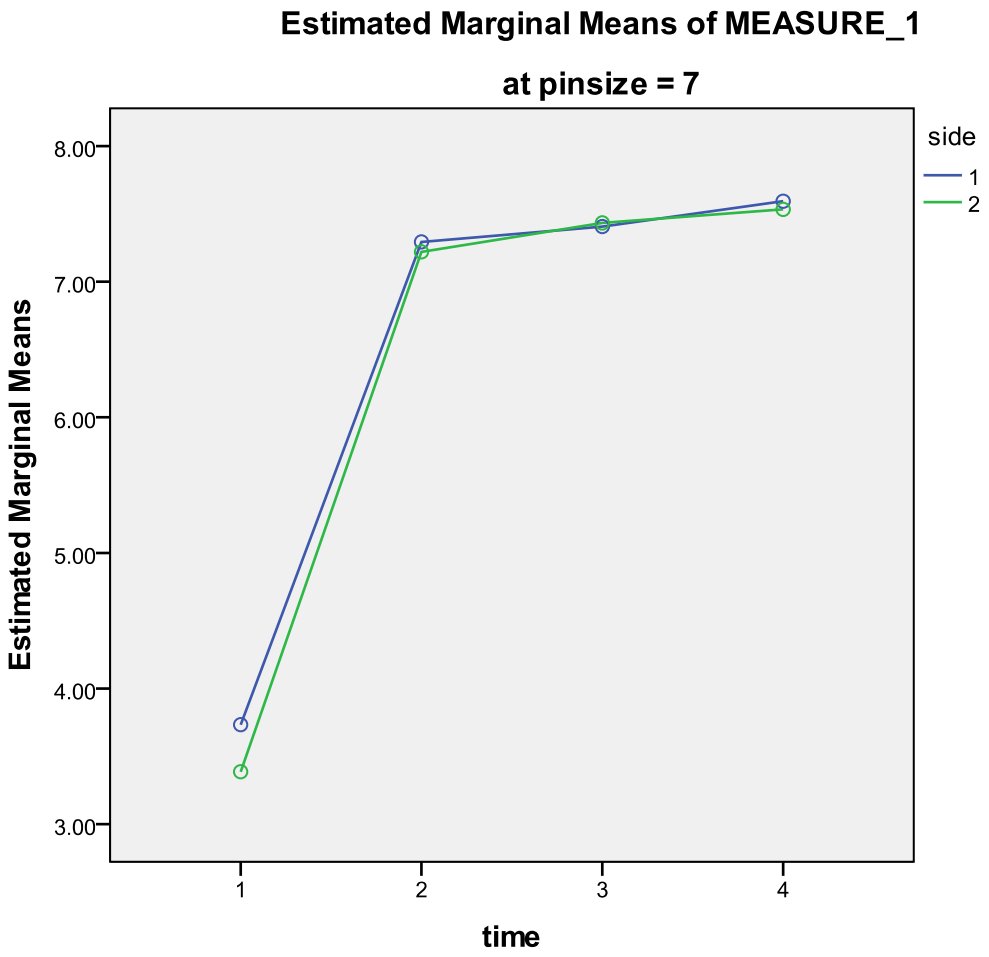
This figure illustrates the change in the primary hyperalgesia, induced by pin prick 6.4, from baseline until 72 h after the irradiation on both arms (1 = right arm, 2 = left arm). It is clear that the primary hyperalgesia at 24h, 48h and 72h are different from that at baseline, on both arms.



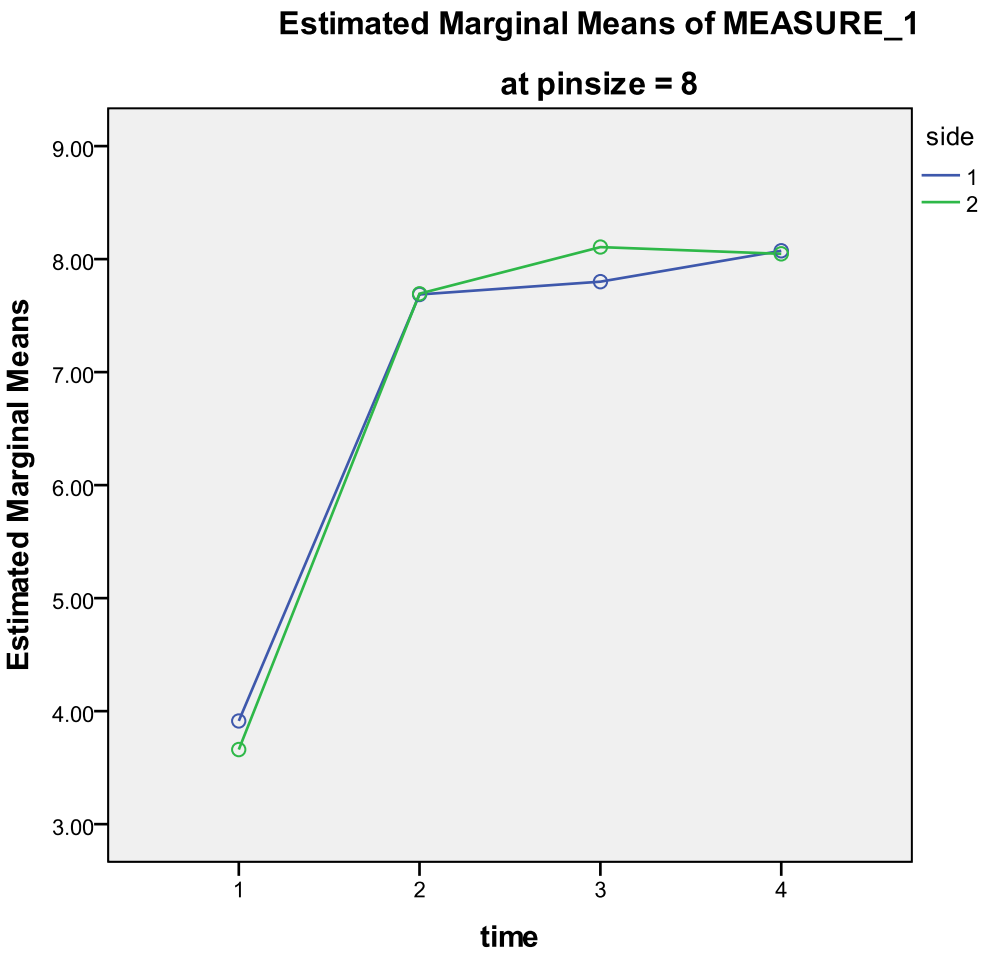
This figure illustrates the change in the primary hyperalgesia, induced by pin prick 12.8, from baseline until 72 h after the irradiation on both arms (1 = right arm, 2 = left arm). It is clear that the primary hyperalgesia at 24h, 48h and 72h are different from that at baseline, on both arms.



This figure illustrates the change in the primary hyperalgesia, induced by pin prick 25.6, from baseline until 72 h after the irradiation on both arms (1 = right arm, 2 = left arm). It is clear that the primary hyperalgesia at 24h, 48h and 72h are different from that at baseline, on both arms.



This figure illustrates the change in the primary hyperalgesia, induced by pin prick 50.1, from baseline until 72 h after the irradiation on both arms (1 = right arm, 2 = left arm). It is clear that the primary hyperalgesia at 24h, 48h and 72h are different from that at baseline, on both arms.



This figure illustrates the change in the primary hyperalgesia, induced by pin prick 60.0, from baseline until 72 h after the irradiation on both arms (1 = right arm, 2 = left arm). It is clear that the primary hyperalgesia at 24h, 48h and 72h are different from that at baseline, on both arms.

Analysis of the measurement for the assessment of secondary hyperalgesia

Mean blood flow in the area of secondary hyperalgesia

Within-Subjects Factors		
arm	time	Dependent Variable
rigth	BL	HSfluxBL
	24h	HSflux24
	48h	HSflux48
	72h	HSflux72
left	BL	VSfluxBL
	24h	VSflux24t
	48h	VSflux48t
	72h	VSflux72t

This table illustrates the analyzed data. The mean blood flow was measured on both arms at baseline and 24h, 48h and 72h after the irradiation.

Mauchly's Test of Sphericity

Within Subjects Effect	Sig.
arm	
time	.101
arm * time	.820

This table illustrates the sphericity of the data. There is a significant different sphericity in this data.

Tests of Within-Subjects Effects

Source		Sig.
arm	Greenhouse-Geisser	.514
time	Greenhouse-Geisser	.664
arm*time	Greenhouse-Geisser	.347

This table illustrates the test for any significant difference between the arms and between the times. There is no significant difference between the arms, which means that the degree of blood flow in the secondary area was similar on both arms. There is not a significant difference between the times, which means that the degrees of blood flow in the secondary area was similar the timeslots (BL, 24h, 48h and 72h). This table also shows that there is no interaction between the arm and time, which means that a similar degree of blood flow can be achieved at a given time point regardless the arm.

Pairwise Comparisons

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
BL	24h	-7.250	5.500	1.000	-24.129	9.629
	48h	-5.890	8.283	1.000	-31.308	19.528
	72h	-3.853	5.546	1.000	-20.872	13.166
24h	BL	7.250	5.500	1.000	-9.629	24.129
	48h	1.360	8.038	1.000	-23.306	26.026
	72h	3.397	7.013	1.000	-18.126	24.919
48h	BL	5.890	8.283	1.000	-19.528	31.308
	24h	-1.360	8.038	1.000	-26.026	23.306
	72h	2.037	5.459	1.000	-14.714	18.788
72h	BL	3.853	5.546	1.000	-13.166	20.872
	24h	-3.397	7.013	1.000	-24.919	18.126
	48h	-2.037	5.459	1.000	-18.788	14.714

Based on estimated marginal means

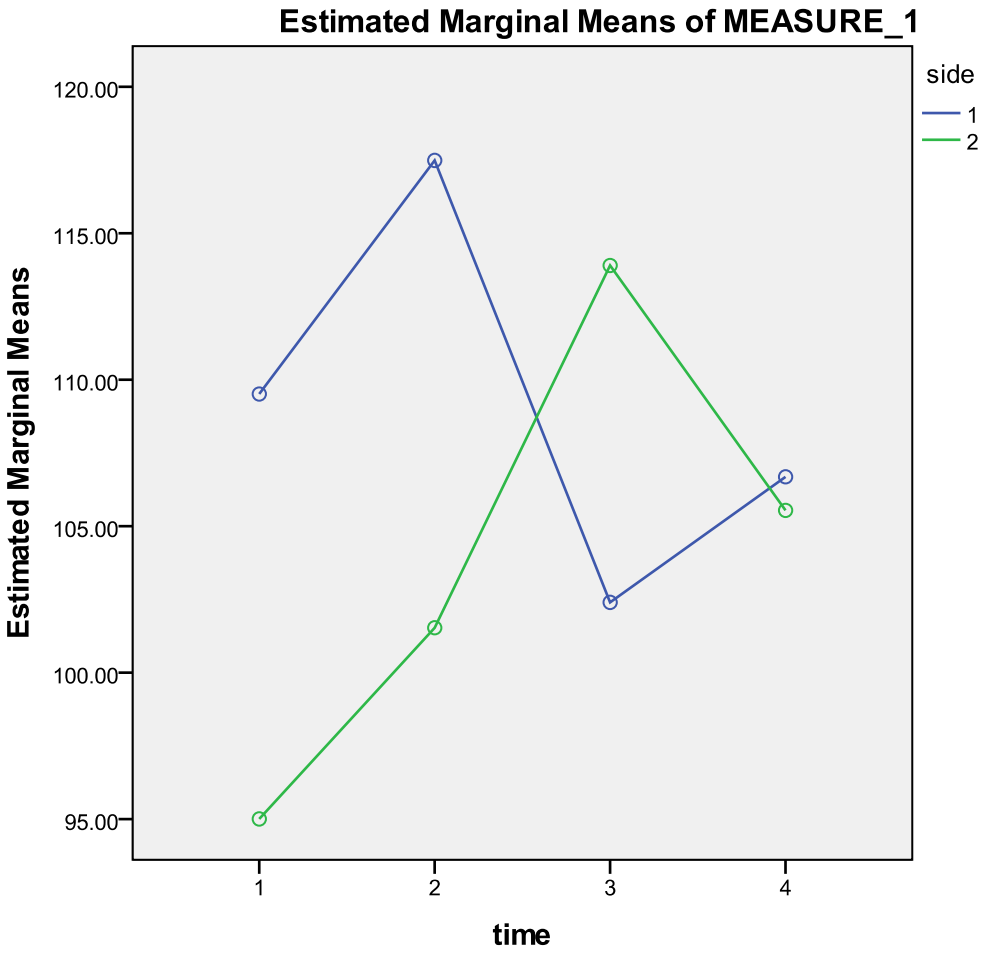
a. Adjustment for multiple comparisons: Bonferroni.

This table illustrates that there is no significant difference between the measurements from BL, 24h, 48h and 72h. This means that the degree of blood flow in the secondary area is similar between BL and 72 hours.

arm * time

arm	time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
right	BL	109.513	8.812	90.613	128.414
	24h	117.487	9.672	96.743	138.231
	48h	102.400	8.199	84.814	119.986
	72h	106.687	9.329	86.678	126.695
left	BL	95.007	5.640	82.911	107.102
	24h	101.533	8.824	82.608	120.459
	48h	113.900	12.553	86.977	140.823
	72h	105.540	9.913	84.278	126.802

This table shows the mean values of mean blood flow measurements on both arms during the different timeslots. The mean values for BL, 24h, 48h and 72h have similar confidence intervels, which further indicates a similar degree of blood flow during these timeslots.



This figure illustrates the change in the mean blood flow from baseline until 72 h after the irradiation on both arms (1 = right arm, 2 = left arm). It is clear that the mean blood flow at BL, 24h, 48h and 72h are similar, on both arms.

Secondary hyperalgesia to von Frey Filaments

Within-Subjects Factors		
side	time	Dependent Variable
right	24h	HvonFreyarea24h
	48h	HvonFreyarea48h
	72h	HvonFreyarea72h
left	24h	Vvonfreyarea24h
	48h	VvonFreyarea48h
	72h	VvonFreyarea72h

This table illustrates the analyzed data. The area of secondary hyperalgesia to von Frey filaments was measured on both arms at baseline and 24h, 48h and 72h after the irradiation.

Mauchly's Test of Sphericity

Within Subjects Effect	Sig.
arm	
time	.140
side * time	.354

This table illustrates the sphericity of the data. There is significant sphericity in this data.

Tests of Within-Subjects Effects

Source		Sig.
arm	Greenhouse-Geisser	.003
time (BL,24h,48h,72h)	Greenhouse-Geisser	.070
side * time	Greenhouse-Geisser	.037

This table illustrates the test for any significant difference between the arms and between the times. There is a significant difference between the arms, which means that the area of secondary hyperalgesia to von Frey filament was different on both arms. There is no significant difference between the times, which means that the degree of secondary hyperalgesia was similar within the timeslots. This table also shows that there is interaction between the arm and time, which means that the area of secondary hyperalgesia will be different in both arms at a given time.

Pairwise Comparisons

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
24h	48h	5.760	4.730	.763	-8.116	19.636
	72h	15.415	7.438	.204	-6.404	37.234
48h	24h	-5.760	4.730	.763	-19.636	8.116
	72h	9.655	4.964	.251	-4.907	24.217
72h	24h	-15.415	7.438	.204	-37.234	6.404
	48h	-9.655	4.964	.251	-24.217	4.907

Based on estimated marginal means

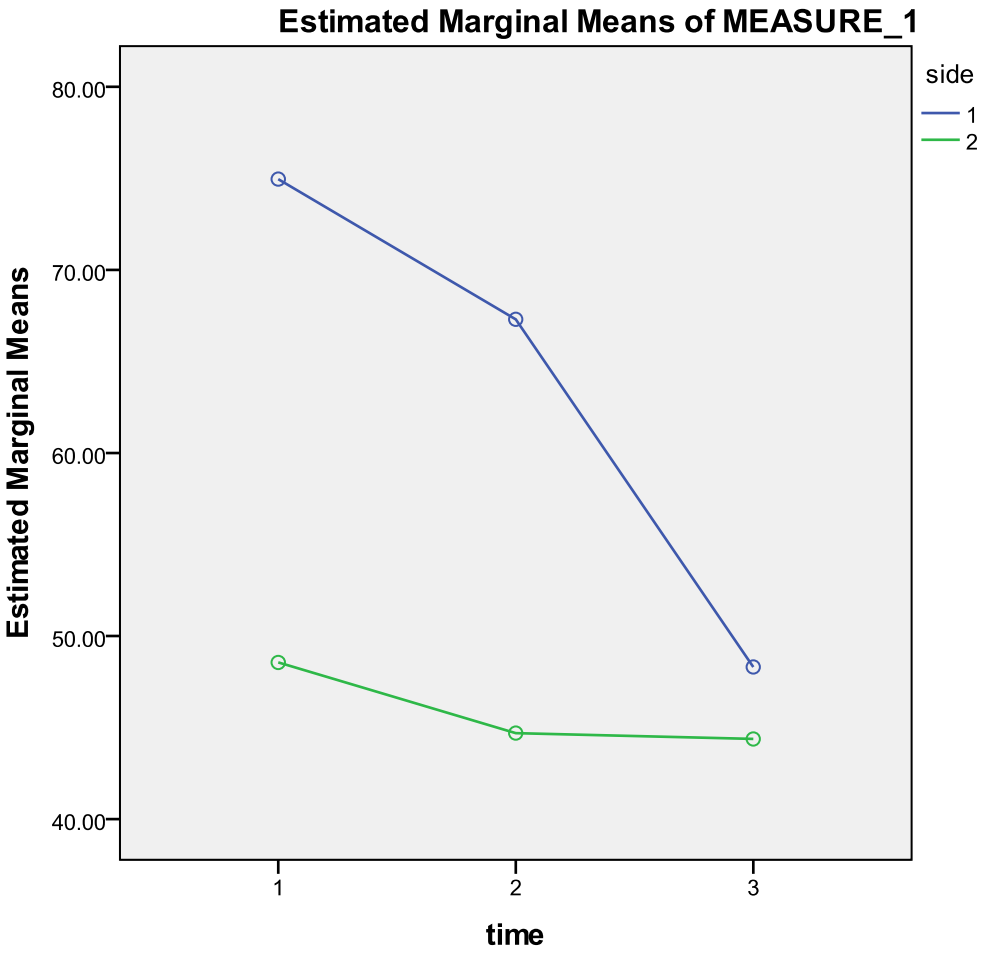
a. Adjustment for multiple comparisons: Bonferroni.

This table illustrates that there is no significant difference between the measurements at 24h, 48h and 72h after the irradiation. This means that the area of secondary hyperalgesia to von Frey filament was similar within the timeslots.

arm * time

arm	time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
right	24h	74.960	14.947	41.148	108.772
	48h	67.300	12.038	40.069	94.531
	72h	48.310	12.000	21.163	75.457
left	24h	48.560	12.399	20.512	76.608
	48h	44.700	10.277	21.451	67.949
	72h	44.380	13.144	14.647	74.113

This table shows the mean values of the degree of secondary hyperalgesia to von Frey filament on both arms during the different timeslots. The mean values for 24h, 48h and 72h have similar confidence intervels, which further indicates a similar area of secondary hyperalgesia during these timeslots.



This figure illustrates the change in the area of secondary hyperalgesia to von Frey filament from 24h until 72 h after the irradiation on both arms (1 = right arm, 2 = left arm).

Secondary hyperalgesia to pin Prick

Within-Subjects Factors

side	time	Dependent Variable
right	24h	HpinParea24h
	48h	HpinParea48h
	72h	HpniParea72h
left	24h	VpinPrear24h
	48h	VpinParea48h
	72h	VpinParea72h

This table illustrates the analyzed data. The area of secondary hyperalgesia to pin Prick was measured on both arms at 24h, 48h and 72h after the irradiation.

Mauchly's Test of Sphericity

Within Subjects Effect	Sig.
arm	
time	.529
side * time	.700

This table illustrates the sphericity of the data. There is significant sphericity in this data.

Tests of Within-Subjects Effects

Source		Sig.
arm	Greenhouse-Geisser	.009
time (BL,24h,48h,72h)	Greenhouse-Geisser	.223
side * time	Greenhouse-Geisser	.035

This table illustrates the test for any significant difference between the arms and between the times. There is a significant difference between the arms, which means that the area of secondary hyperalgesia to pin Prick was different on the arms. There is no significant difference between the times, which means that the degree of secondary hyperalgesia was similar within the timeslots. This table also shows that there is interaction between the arm and time, which means that the area of secondary hyperalgesia will be different in both arms at a given time.

Pairwise Comparisons

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
24h	48h	3.255	3.826	1.000	-7.725	14.234
	72h	7.339	4.729	.455	-6.234	20.911
48h	24h	-3.255	3.826	1.000	-14.234	7.725
	72h	4.084	3.536	.825	-6.064	14.232
72h	24h	-7.339	4.729	.455	-20.911	6.234
	48h	-4.084	3.536	.825	-14.232	6.064

Based on estimated marginal means

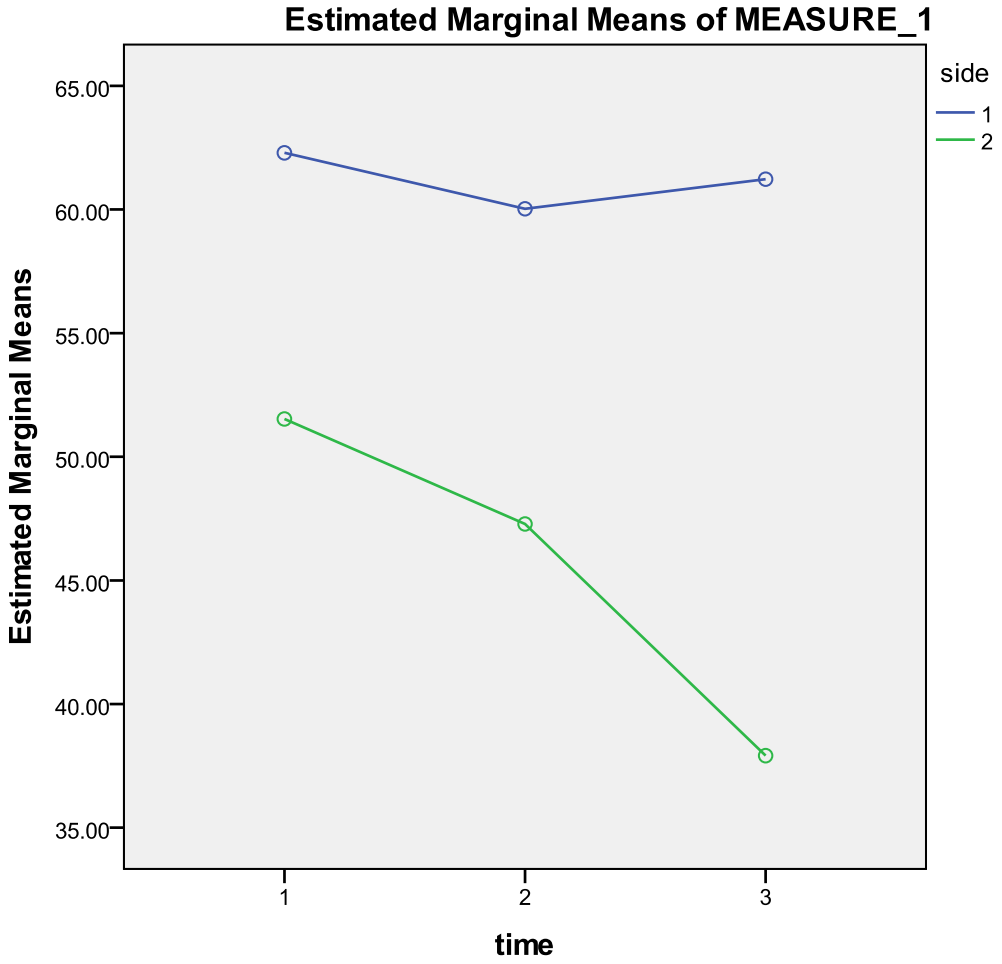
a. Adjustment for multiple comparisons: Bonferroni.

This table illustrates that there is no significant difference between the measurements at 24h, 48h and 72h after the irradiation. This means that the area of secondary hyperalgesia to pin Prick was similar within the timeslots.

arm* time

side	time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
right	24h	62.291	13.474	32.269	92.312
	48h	60.027	10.687	36.215	83.840
	72h	61.227	13.188	31.843	90.611
left	24h	51.527	12.179	24.390	78.665
	48h	47.282	9.603	25.886	68.678
	72h	37.914	10.273	15.023	60.804

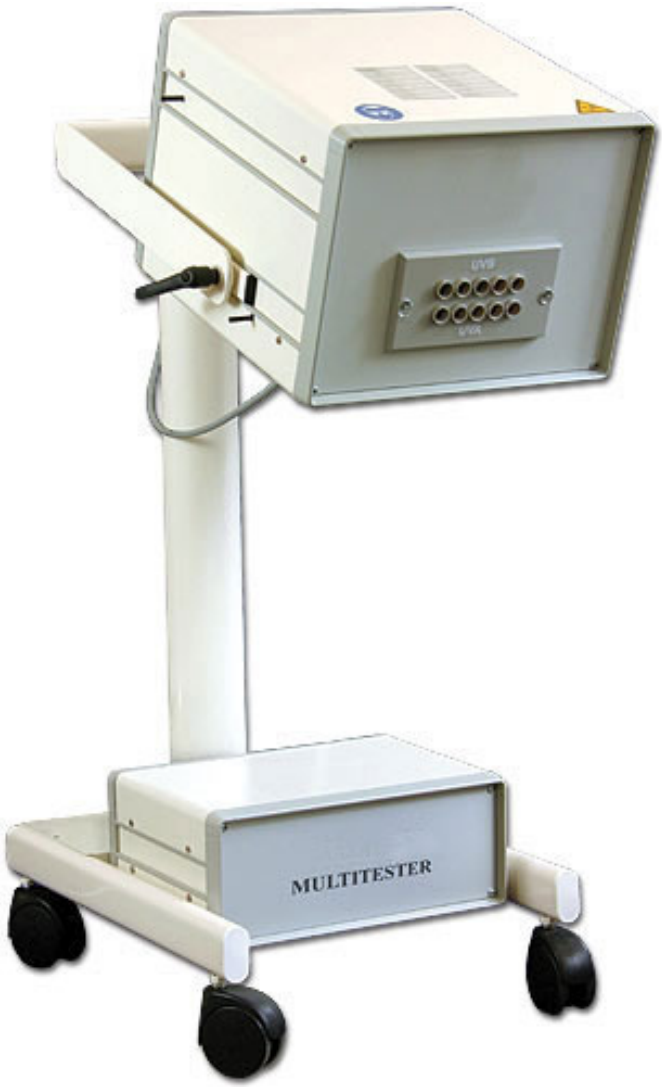
This table shows the mean values of the degree of secondary hyperalgesia to pin Prick on both arms during the different timeslots. The mean values for 24h, 48h and 72h have similar confidence intervels, which further indicates a similar area of secondary hyperalgesia during these timeslots.



This figure illustrates the change in the area of secondary hyperalgesia to pin Prick from 24h until 72 h after the irradiation on both arms (1 = right arm, 2 = left arm).

Appendix I - Standard operating procedure UVB model

Standard operating procedure UVB-model



Saalmann Multitester
SSB LT 400TM
MEDLight GmbH
Oststraße 36
32051 Herford
Germany

Phone: +49 5221 2044
Telefax: +49 5221 27235
E-Mail: info@medlight.eu
Internet: www.medlight.eu

17/05-2011
Asiah Rahi and Line Christensen

Device identification: UVB Stimulation

Version: 01	Approved by:
Prepared by:	
Asiah Rahi and Line Christensen	
Date: 17/05-2011	Date:

Scope

This standard operating procedure (SOP) contains useful information on the Saalmann Multitester, which is used during conduct of the UVB-pain model. This SOP gives information about the following:

- Different components of the Multitester.
- Procedures to run through before using the Multitester.
- Instructions for general use of the Multitester.
- Instructions for the use of the Multitester for UVB stimulation and for determination of the minimal erythema dose (MED).
- Risks, risk protection and risk management while using the Multitester.
- Maintenance and the calibration of the Multitester.
- Technical data of the Multitester.
- Data output from the Multitester.

Purpose

The purpose of the present SOP is to standardize and document the procedures for the use of Saalmann Multitester in the UVB-pain model. This SOP should be used as a training document in clinical pain research at Aalborg University.

References

- Saalmann Multitester SBC LT 400/Medtester
  User's Manual and Technical Description
- Human Experimental Pain Models 1: The Ultraviolet light UV-B Pain Model
  (James G. Modir and MarkS. Wallace 2010)
- http://www.medlight.eu/
- http://www.uv-groebel.com/
- "Calibration sheet" Excel file found in the CD at the back of the SOP.

The Saalmann Multitester

Components: (appendix A)

1. Saalmann-Multitester SBC LT 400
2. Graduated test attachment (UVA and UVB 10 circles d 15 mm)
3. Photopatch test attachments UVA or UVB
   (UVA/UVB square 62 x 105 mm or UVB circle with a diameter of 50 mm)
4. Template for adjusting (Multitester Folie)
5. Protective goggles

The Multitester is installed on a mobile console. The main unit “irradiation head” is vertically adjustable by an electric motor and it can also be turned and tilted around two axis, allowing for a variety of test positions, see fig. 1.

The irradiation unit is situated at the front of the head. Two light-sources are also situated at the front of the head close to the attachments. The light-sources can be used to align the Multitester to the test area. The control panel is situated on the opposite side of the irradiation head, on the back. The control panel has a small display, two signal lamps and operation buttons.

An integrated special liquid filter is installed in the irradiation head for absorbing the IR radiation fraction, assuring unaltered, reproducible test results.

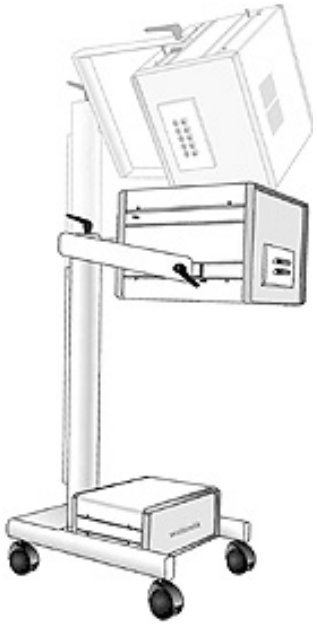


Figure 1
The head of the Multitester can be vertically adjusted by an electric motor and can also be turned and tilted around two axes providing a variety of test positions.

The graduated light attachment can be fixed on the front panel of the head. The graduated light attachment has 5 fields for each UVB and UVA testing. Each field area has a diameter of 15 mm, the energy is decreased gradually over the 5 fields. The energy graduation of the 5 different areas is fixed and is shown in the “dosage table” in appendix B.

The photopatch attachments for high intensity UVB or UVA surface irradiation (UVB emission 290-320 nm and UVA emission 321-400nm) can be fixed on the front panel of the head. The available irradiation area is 62 x 105 mm (UVA or UVB) or a circle with 5cm in diameter (UVB).

The template marked as “Multitester Folie” is used for exact alignment of the irradiation area to the test area.

Protective glasses are required prior to switching on the Multitester and should be worn both by the subjects and the experimenters throughout testing.

Check up before experiment

- Protective glasses are required prior to switching on the Multitester and should be worn throughout testing.
- Always attach a light test attachment prior to use. This is done by inserting the two threaded pins (bayonet-screws) of the light test attachment into the two provided holes next to the front of the irradiation head. The attachment should be locked in place by turning both pins clockwise.
- Never use the Multitester without a test attachment in the front, as it leads to incorrect dosages.
- The Multitester should always be calibrated with a radiometer before use.
- The Multitester "User's Manual" should be kept in an easily accessible place near the Multitester.
- Remember that the Multitester requires a warm up period for approximately two minutes.
- Subjects should be asked to avoid sun exposure to the test area prior and after the irradiation.
- The Multitester should only be operated with the use of original accessories.

Instruction

Buttons and lamps in the control panel on the back of the irradiation head: (appendix D)

1. Green switch "on/off"
2. White switch "adjusting photopatch"
3. Black +/- keys for UVB or UVA ranges
4. Black "start" button
5. Black "stop" button
6. Green indicator lamp "ready/ bereit"
7. Yellow indicator lamp "in operation /betrieb"

- Connect the Multitester to a power socket.
- Switch the Multitester on by using the green "on/off" button at the right hand side of the control panel.
- A warm-up period runs for approximately two minutes.
- The green indicator lamp "ready/ bereit" will light up and indicate that the Multitester has reached its maximum output capacity.
- The current intensities for UVB and/or UVA are shown in the display when the warm-up period has finished.
- The wanted UVB or UVA dosage can now be entered by using the black button "+ /- UVB" or "+/- UVA".
- The correct dosage is calculated by the Microprocessor Control System which is incorporated into the control panel. The dosages are manually entered and automatically corrected to different intensity levels.
- The Multitester is moved towards the skin surface until it touches the surface without any gaps or applying any pressure.
- The irradiation is started by pressing the black "start" button on the control panel. When the button is pressed, a "click" sound from the Multitester indicates opening of the UV shutter.
- The irradiation progress is indicated in the control panel by the lighting up of the yellow lamp "in operation /betrieb".
- The display in the control panel shows the calculated irradiation time, which runs off in decreasing order.

- At the end of each irradiation application the control mechanism automatically cuts off the light-source.
- The UV generator will still be operational and the Multitester is still ready for use.
- The irradiation can be interrupted at any time by pressing the black “stop” button on the control panel.
- The irradiation can be resumed subsequently by pressing the black “start” button on the control panel. Then the irradiation will continue to run off the remaining time as shown on the display.
- Pressing the black “stop” button twice terminates the irradiation and the originally entered dosage will appear in the display.
- The light test attachments can easily be interchanged via the attached bayonet-screws.
- The program automatically reads which attachment is in position currently and it changes the display read-out accordingly.
- For the exact alignment of the irradiation area to the test area the white button “Adjusting photopatch” should be switched on at the right hand side of the control panel. Subsequently the alignment can be performed by using the enclosed template marked “Multitester Folie”. The Multitester should be aligned by placing the template “Multitester Folie” onto the test area and then moving the irradiation head until the two light spots exactly meet the designated circles on the template. Then the template is removed and the Multitester is carefully moved down to the test area until it has the maximum possible surface contact.
- If you should notice any unusual noises, smoke or sparking during the operation, remove the Multitester off the mains immediately! Do not continue use of the Multitester before proper repairs have been carried out by the company.
- To avoid overloading of the Multitester the maximum continuous time of use should not exceed two hours.

Skin preparation and MED determination for UVB stimulation

- The skin should be cleaned with hydrex solution to remove any cosmetics.
- MED is determined with the graduated test attachment attached to the front of the irradiation head.
    - The recommended starting dosage is 100-40 mJ/cm2 of UVB irradiation. The required dosage is entered by pressing the black button “+/- “UVB on the control panel. The possible dosage units increase by 10 mJ per set. The minimum and maximum dosage to be reached is 10 and 400 mJ/cm2 respectively.
    - The five sites are irradiated simultaneously and the light is graded out at different levels, with the highest dose being clear in the outermost left area. The following areas to the right are graduated down by 40%, 55%, 70% and 85% of the entered dosage as specified in the “dosage table” (appendix B).
    - The UVB stimulation last less than 60 s.
    - The MED is determined visually by a scale 24 hours after irradiation:
        0. No erythema
        1. Very slight erythema (barely perceptible)
        2. Well-defined erythema
        3. Moderate to severe erythema
        4. Severe erythema (beet redness) to eschar formation
            - A well-defined erythema is the target.

UVB stimulation

- The skin should be cleaned with hydrex solution to remove any cosmetics.
- The UVB irradiation is performed with the UVB photopatch test attachment. Two photopatches are available a square of 60x100mm or a circle with a diameter of 5mm.
- The recommended dosage is 3XMED.
  - The UVB stimulation last less than 60 s.

Maintenance and calibration

- The Multitester should always be calibrated with a radiometer before use.
  - E.g. radiometer “RM 12, Dr.Gröbel, UV-elektronik GmbH” (appendix C).
- The Multitester is recommended to be serviced annually by the company in order to maintain the working conditions.
- All electric leads, sockets, plugs, control panels and the casing must be in perfect working in order to gain a safe operation of the Multitester.
- Always take off the Multitester from the mains before any maintenance or cleaning is carried out.
- General cleaning of the surface of the Multitester should be carried out by using a slightly damp cloth.
- The test attachments should be detached from the irradiation head and disinfected by using a diluted alcoholic liquid i.e. surgical sprit and a soft cloth. This procedure should be carried out after each application.

Risk Management

There is a risk of UV overdosing when using the Saalmann Multitester for UV stimulation. This risk should be prevented by determination of the MED prior to irradiation. The starting dose for MED determination is recommended to be 100mJ/cm2 or less (appendix B). An overdose should be treated as 2nd degree burn injury.

A risk of eye damage exists for both the volunteers and the examiners. This risk should be prevented by always wearing the protective goggles (appendix A). An eye specialist should immediately be consulted in case of any eye damage. To reduce the risks, a trained staff member should always be present during the application of the irradiation. The stimulation can always be stopped by pressing the black “stop” button (appendix D).

Reporting

The Minimal Erythema Dose (MED) is documented as mJ/cm2 and used for determination of the desired dose of UVB stimulation (3XMED).

Data output for MED determination:

The MED is determined visually by this scale (well-definde erythema is the target):

0. No erythema
1. Very slight erythema (barely perceptible)
2. Well-defined erythema
3. Moderate to severe erythema
4. Severe erythema (beet redness) to eschar formation

MED is read after: _____hours
Doses applied:

UVB mJ/cm2		Erythema scale
	100%	
	85%	
	70%	
	55%	
	40%	

MED is determinate to: _____mJ/cm2 3xMED:_____mJ/cm2

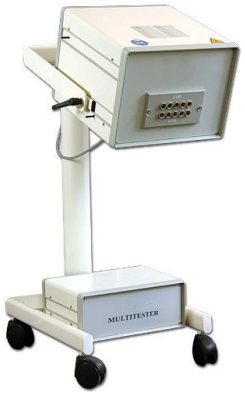
Technical data

- Power supply: 230V, 50Hz
- Fuse: 6,3 A/T
- Power consumption: 580 W
- Dimensions: 40 cm (width), 70 cm (depth) and 70 - 140 cm (height)
- Weight: 50 Kg
- Main united “irradiation head”: B 36 x H 28 x T39 cm

Appendix A

Components:

1. Saalmann-Multitester SBC LT 400
2. Graduated test attachment (UVA and UVB 10 circles d 15 mm)
3. Photopatch test attachments UVA or UVB
   (UVA/UVB square 62 x 105 mm or UVB circle d 50 mm)
4. Template for adjusting (Multitester Folie)
5. Protective goggles



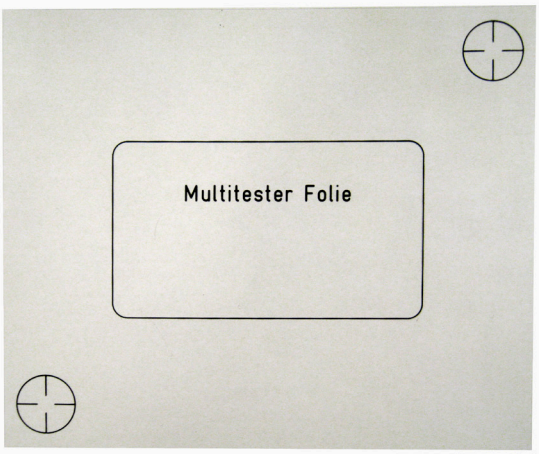
1

2

3

3

3



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5

Appendix B

3.3.1 Graduated Light in the UVB Range

Entered Dosage Area 1 [mJ/cm²]	Dosage Area 2 [mJ/cm²]	Dosage Area 3 [mJ/cm²]	Dosage Area 4 [mJ/cm²]	Dosage Area 5 [mJ/cm²]
10	9	7	6	4
20	17	14	11	8
30	26	21	17	12
40	34	28	22	16
50	43	35	28	20
60	51	42	33	24
70	60	49	39	28
80	68	56	44	32
90	77	63	50	36
100	85	70	55	40
110	94	77	61	44
120	102	84	66	48
130	111	91	72	52
140	119	98	77	56
150	128	105	83	60
160	136	112	88	64
170	145	119	94	68
180	153	126	99	72
190	162	133	105	76
200	170	140	110	80
210	179	147	116	84
220	187	154	121	88
230	196	161	127	92
240	204	168	132	96
250	213	175	138	100
260	221	182	143	104
270	230	189	149	108
280	238	196	154	112
290	247	203	160	116
300	255	210	165	120
310	264	217	171	124
320	272	224	176	128
330	281	231	182	132
340	289	238	187	136
350	298	245	193	140
360	306	252	198	144
370	315	259	204	148
380	323	266	209	152
390	332	273	215	156
400	340	280	220	160

Table 1
Graduated Light in the UVB rangee

Appendix C

Dr. Gröbel UV-Elektronik GmbH
Goethestr. 17, 76275 Ettlingen, Germany
Phone: +49 7243/71839-0
Fax: Fax: +49 7243/71839-300
E-mail: info@uv-groebel.de
Internet: http://www.uv-groebel.com
The radiometer RM 12 is a hand held device used to measure UV intensity.
UVB or UVA sensors can be attached to the radiometer.

Technical data:

- Dimension: 160 x 85 x 35 mm
- Weight: 300 g
- Power supply: 9 V battery
- Battery lifetime: about 50 hours
- Operation temp.: 0 – 40 °C
- Storage temp.: -10 – 40 °C
- Humidity: < 80% (non condensing)
- Sensor connector: 5-pole plug

The radiometer should ideally measure the same intensity as showed in the Multiteser display “set dose”. An interactive excel sheet can be used for calculation of the radiometer output measured in watt (W).

The Multitester output is in millijoule (mJ). The calculation for the conversion of watt to mJ is: mJ = (W x s)/10. A difference of +/- 10 mJ/cm2 of the radiometer measurement compared with the set dose of the multitester is considered acceptable.

This sheet also calculates any factor between the graduated and photopatch test attachments, i.e. if the Multitester delivers the same intensity with the different test attachments on. This factor should ideally be 1 (100%).

Date[1]	Multitester[2] XXXXX				Measr after 8s		Measr at shutter end	
	Front	Area Factor	UVB Dose Set[5]	Time Applied (s)[6]	Intensity Measured RM12[7]	Dose Measured[8] (8s)	Intensity Measured RM12[7]	Dose Measured[8] (SE)
		%	mJ/cm²	s	W/m²	mJ/cm²	W/m²	mJ/cm²
	MED[3]	100%	100			0		
		100%	250			0		0
		100%	400			0		0
	Average				########		########	
	UVB[4]	100%	100			0		
		100%	250			0		0
		100%	400			0		0
	Average				########		########	
	xxx/xxx Factor[9]					**########**		########

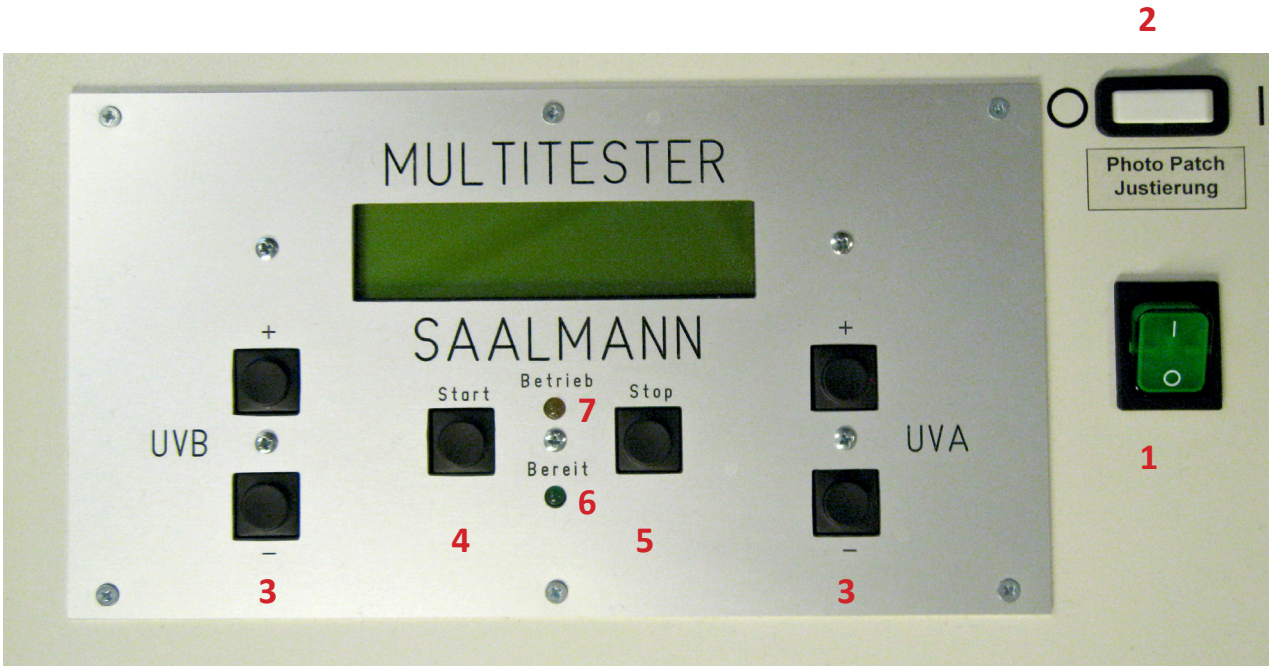
Table 2
Segment of an interactive Excel sheet used to calculate the conversion of watt to mJ.

1. The Date of calibration.
2. Identity code.
3. Graduated test attachment (UVA and UVB 10 circles d 15 mm).
4. Photopatch test attachments UVA or UVB.
5. The chosen dose –(millijoule) (see the display of the multitester).
6. Time applied (seconds) (seen the display of the multitester).
7. Reading from the radiometer (watt) after 8 seconds and at the shutter end (SE).
8. Excel calculates the measured intensity in mJ (mJ = (W x S)/10).
9. Excel calculates the factor between the graduated and photopatch test attachments.

Appendix D

Button and indicator lamps in the control panel:

1. Green switch “on/off”
2. White switch “adjusting photopatch”
3. Black +/- keys for UVB or UVA ranges
4. Black “start” button
5. Black “stop” button
6. Green indicator lamp “ready/ bereit”
7. Yellow indicator lamp “in operation /betrieb”



Signature

By signing below the instructor declares that each person who has received this training is qualified to perform UVB stimulation and handle UVB model technique.

Training Log Name, Date and Signature