No pain no gain
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3 EVALUATING MULTIMODAL EXPERIMENTAL PAIN MEASUREMENTS IN ACUTE PAIN TRIALS FOR OPIOID EFFICACY

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(SUBMITTED)
ABSTRACT

BACKGROUND Experimental acute pain trials are essential for the development of efficacious novel analgesics. Traditionally a wide variety of multimodal pain measures are used as outcome measures for clinical trials. More standardization within clinical trials can improve the quality of novel analgesics. In the present study we evaluated several pain measurements. Sensory and pain thresholds (mechanical and thermal) as well as continuous subjective pain ratings were measured before and during remifentanil infusion. Our aims were to use an extensive battery of pain measurements to get more insight in the most applicable and sensitive measurements of acute pain in early clinical development and clinical trials for opioid efficacy.

METHODS Twelve healthy volunteers were tested in a double blind, placebo controlled, cross-over study with 0.10 μg/kg/min intravenous remifentanil infusion for 45 minutes.

RESULTS All pain thresholds except the cold pain showed a significant increase in pain threshold during the remifentanil infusion. The cold pressor test (CPT) and the continuous heat pain stimulation (CHP) showed a clear analgesic effect of the remifentanil. Furthermore, the results indicated that both test period and treatment order influenced the pain thresholds. All baseline pain thresholds were higher during the second visit independent of treatment. Additionally, when the placebo infusion took place in the second period there was a lack of placebo analgesia.

CONCLUSIONS These results indicate that both pain thresholds as well as continuous pain ratings can be useful outcome measures of acute pain. It can be worthwhile to use a multimodal pain testing battery in early clinical development of novel analgesic compounds. Within our test battery, the heat pain threshold and the pressure pain threshold were the most robust pain threshold measures and the cold pressure test was the most effective continuous pain rating measure.
3.1 INTRODUCTION

Pain is possibly one of the most common symptoms in modern day medicine, making effective pain treatment one of the main goals for drug development. Experimental pain models in healthy volunteers can play a vital role studying the efficacy of novel analgesic drugs (Drewes et al., 2003). In a review discussing the applicability of human pain models for opioid efficacy, Staahl and colleagues conclude that “there is still a need for basic investigations of opioids in well-designed human experimental pain models” (Staahl et al., 2009a). Furthermore several reviews recommend there should be more standardized trial designs and outcome measures to achieve a better comparison between pain studies and clinical trials (Handwerker & Kobal, 1993; Woolf & Max, 2001; Staahl et al., 2009b).

Experimental pain models provide the investigator with strict control over the stimulus intensity, duration and location and result in a clear outcome measure for drug efficacy (Drewes et al., 2003; Staahl & Drewes, 2004). In contrast small patient trials in an early development phase often suffer from confounding psychological and social illness characteristics (Staahl et al., 2009a). Human models for acute pain have always used a wide variety of external stimuli to achieve a pain experience (Gracely, 2006). The most common used stimuli are heat, cold, mechanical stimulation, pressure, chemical stimulation or electrical stimulation (Staahl & Drewes, 2004). Using different types of measurements in a multi model approach can mimic chronic pain more accurately and gain advanced and differentiated information on the anti-nociceptive mechanism of the studied compound (Drewes et al., 2003).

Acute pain is commonly measured as a psychophysical measure of the individuals subjective experience of the pain expressed on standardized pain rating scales or as pain thresholds (Gracely, 1999; Staahl & Drewes, 2004). Pain detection thresholds attempt to identify the minimal intensity of a physical stimulus that elicits a painful experience (Gracely, 1999). Pain detection thresholds have been found to be sensitive to a number of different analgesic compounds and are a fast and reliable indicator of analgesic efficacy (Gustorff et al., 2001; Petersen et al., 2001; Gustorff et al., 2003; Rolke et al., 2006). In addition, a visual analog scale (VAS) or Numeric Rating Scale (NRS) consisting of a vertical bar on a screen anchored with the descriptors ‘no pain’ (numeric value = 0) and ‘worst possible pain’ (numeric value = 100) is often used to rate the subjective intensity of the pain. Both methods are easy and quick outcome measures for pain sensitivity and are often used in early clinical trials to test the efficacy of novel analgesics (Handwerker & Kobal, 1993; Gracely, 1999).

Opioids are the most common treatment option for moderate and severe pain (Staahl et al., 2009a). Remifentanil is a potent ultra-short-acting, fentanyl derivative, selective mu-opioid receptor agonist with a typical opioid pharmacodynamic profile (Scott & Perry, 2005). To achieve analgesia without anesthesia, remifentanil hydrochloride is administered
as an intravenous infusion ranging from 0.025 μg/kg/min to 0.15 μg/kg/min for analgesia without anesthesia (Gracely, 1999; Staahl & Drewes, 2004). Typical side-effects at this dosing range are nausea, vomiting, dizziness and cardio-respiratory effects (Servin & Billard, 2008). Remifentanil has a rapid onset of analgesic action (one minute) and a fast offset of action (3 – 10 minutes) irrespective of the duration of the infusion. Therefore remifentanil is considered a useful tool in evaluating experimental acute pain measurements for clinical trials (Scott & Perry, 2005).

In the present study we evaluate both sensory and pain thresholds as well as continuous subjective pain ratings as outcome measures for experimental acute pain trials. Remifentanil was administered as the analgesic intervention in a double blind placebo controlled two way crossover design

3.2 METHOD

3.2.1 SUBJECTS
This study adhered to the declaration of Helsinki and was approved by the local ethics committee.

Twelve healthy male volunteers participated within this study. Mean age of the participants was 22.8 (SD = 3.8) years. Exclusion criteria were: actual pain, history of neurological or psychiatric disorders, history of cardiovascular disease or bronchospastic respiratory disease and recent drug or alcohol abuse. Participants received a financial compensation for their time and travel expenses. This study was performed at the site of PRA International Zuidlaren, The Netherlands. All measurements were performed by a group of well-trained medical research assistants.

3.2.2 STUDY DESIGN
A double blind placebo controlled two way crossover design was applied to test the analgesic efficacy of remifentanil on several acute pain tests. Treatment consisted of either 0.10 μg/kg/min intravenous infusion of remifentanil hydrochloride or saline infusion, both for 45 minutes. Treatment order was randomized across all subjects. Heat detection and pain thresholds (HDT, HPT), cold detection and pain thresholds (CDT, CPT), mechanical detection and pain threshold (MDT, MPT) and pressure pain thresholds (PPT) and were tested combined with subjective pain ratings for continuous heat pain (CHP), continuous cold pain (CCP) and cold pressor pain (CPP).

All tests were administered prior to the infusion and repeated during the infusion period. A heat first (HDT-HPT-CDT-CPT-CHP-CCP-MDT-MPT-PPT-CPP) and a cold first
(CDT-CPT-HDT-HPT-CCP-CHP-MDT-MPT-PPT-CCP) sequence were randomized across subjects. In order to avoid cross-over effects from cold and heat stimulations all heat measurements were performed on the dominant arm while all cold measurements were performed on the non-dominant arm. The treatment infusion was administered between 08:00 and 11:00 AM, at least one hour after consumption of a standard breakfast. Each subject was studied at two separate visits 7 days apart.

3.2.3 EXPERIMENTAL PROCEDURES.

All thermal stimulations were performed with a computer-controlled thermode (PATHWAY Pain & Sensory Evaluation System, Medoc, Israel). All continuous pain ratings were recorded using an electronic visual analogue scale (COVAS, Medoc, Israel). The VAS scale consisted of a vertical bar, anchored by the descriptors ‘no pain’ (numeric value = 0) and ‘worst possible pain’ (numeric value = 100). The mechanical detection threshold was determined with a standardized set of modified von Frey filaments (Optihair2-Set, Marstock Nervtest, Germany). The mechanical pain stimulation was performed with in-house built pinprick probes (flat contact area of 0.2 mm in diameter) with fixed stimulus intensities that exerted forces of 8, 16, 32, 64, 128, 256 and 512 mN. Pressure pain threshold was measurements using a pressure gauge device or algometer (FDN200, Wagner Instruments, USA).

3.2.3.1 HEAT DETECTION THRESHOLD (HDT)

For the HDT procedure, the thermode temperature was increased from 32 °C with 1 °C/sec. HDT was defined as the lowest temperature perceived as perceptibly warm. Subjects were instructed to press a button as soon as they perceived a warm sensation. After the button press the thermode temperature would return to baseline with 1 °C/sec. The final threshold was calculated as the geometric mean of three stimulations. Inter stimulus interval ranged between 10 and 20 seconds.

3.2.3.2 HEAT PAIN THRESHOLD (HPT)

During the HPT procedure, the thermode temperature was increased from 32 °C with 1 °C/sec. HPT was defined as the lowest temperature that was perceived as heat pain. Subjects were instructed to press a button as soon as they perceived a painful heat sensation. After the button press the thermode temperature would return to baseline with 8 °C/sec. If the cut-off limit (52 °C) was reached before the subject gave a button press, the thermode would automatically return to the starting value of 32 °C. In this case 52 °C would be registered as the HPT. The final threshold was calculated as the geometric mean of three stimulations. Inter stimulus interval ranged between 30 and 60 seconds.
3.2.3.3 COLD DETECTION THRESHOLD (CDT)
For the CDT measurement the thermode temperature was decreased from 32 °C with 1 °C/sec. CDT was defined as the highest temperature perceived as cold. Subjects were instructed to press a button as soon as they perceived a cold sensation. After the button press the thermode temperature would return to baseline with 1 °C/sec. The final threshold was calculated as the geometric mean of three stimulations. Inter stimulus interval ranged between 10 and 20 seconds.

3.2.3.4 COLD PAIN THRESHOLD (CPT)
During the CPT procedure, the thermode temperature was decreased from 32 °C with 1 °C/sec. CPT was defined as the highest temperature perceived as cold pain. Subjects were instructed to press a button as soon as they perceived a painful cold sensation. After the button press the thermode temperature would return to baseline with 8 °C/sec. If the cut-off limit (0 °C) was reached before the subject gave a button press, the thermode would automatically return to the starting value and 32 °C. In this case 0 °C was registered as the CPT. The final threshold was calculated as the geometric mean of three stimulations. Inter stimulus interval ranged between 30 and 60 seconds.

3.2.3.5 MECHANICAL DETECTION THRESHOLD (MDT)
To measure the MDT subject were asked to indicate if they felt the stimulation with the filament or not. Filament forces varied between 2 and 512 mN on a 2log scale. Subjects were presented with five series of ascending and five series of descending stimulus intensities. The filaments were applied to the skin of the inner forearm with 2 seconds in between stimulations. Filament forces would be ascending until the subject reported that the stimulus was felt. Next, the filament forces were decreased until the subject would no longer report the stimulus to be felt. This procedure was repeated 5 times. The MDT was calculated as the geometrical mean of the ten registered filament forces.

3.2.3.6 MECHANICAL PAIN THRESHOLD (MPT)
During the MPT procedure subjects were asked to indicate if a pinprick stimulus felt ‘sharp’ or ‘blunt’. Pinpricks forces varied between 8 and 512 mN on a 2log scale. Subjects were presented with five series of ascending and five series of descending stimulus intensities. The pinprick stimuli were applied to the skin of the inner forearm with 2 seconds in between two pinprick stimulations. Pinprick forces would be ascending until the subjects reported the pinprick as ‘sharp’. Next, pinprick forces were decreased until the subject would report the pinprick to feel ‘blunt’. This procedure was repeated five times. The MPT was calculated as the geometric mean of the ten registered pinprick forces.
3.2.3.7 PRESSURE PAIN THRESHOLD (PPT)
To measure the PPT an algometer was applied on the thenar of the hand exerting a force up to 20kg/cm². The pressure was gradually increased with approximately 0.5 kg/cm² until the subject indicated the pressure to be painful. The PPT was calculated as the mean of three stimulations.

3.2.3.8 CONTINUOUS HEAT PAIN (CHP)
Subjects were presented with a heat stimulus of 46 °C for 2 minutes on the skin of the inner forearm of the dominant arm. Subjects were asked to continuously rate the intensity of the pain.

3.2.3.9 CONTINUOUS COLD PAIN (CCP)
Subjects were presented with a cold stimulus of 2 °C for 2 minutes on the skin of the inner forearm of the non-dominant arm. Subjects were asked to continuously rate the intensity of the pain.

3.2.3.10 COLD PRESSOR PAIN (CPP)
For the CPP the non-dominant hand of the subject was submersed in a cold water bath of 2 °C for two minutes. Subjects were asked to continuously rate the intensity of the pain.

3.2.4 SAFETY MONITORING
Safety and tolerability assessments during the study consisted of continuous monitoring of oxygen saturation, heart rate and heart rhythm, blood pressure and respiratory rate. During the infusion of the study medication a pulse oximeter probe was placed on one of the fingers for continuous monitoring of peripheral oxygen saturation. Furthermore, heart rate and heart rhythm were recorded, blood pressure was recorded continuously and respiratory rate was recorded every 5 minutes for 30 seconds. In addition, all adverse events reported spontaneously by the subject during the course of the study were recorded.

3.2.5 DATA ANALYSIS
Detection and pain thresholds (HDT, HPT, CDT, CPT, MDT, MPT and PPT) are presented as group mean scores with the Standard Error of the Mean (SEM). Continuous pain ratings were resampled to one rating per second and presented as continuous group mean NRS scores ± SEM. For the continuous pain ratings the Area Under the Curve (AUC) was calculated as an outcome measurement for statistical analysis. If subjects had withdrawn their arm from the cold water bath before the end of the 2 minute period, the last NRS rating would be substituted for the remaining time in assuring an AUC could be calculated.
All analyses were performed using SPSS 18.0 (SPSS Inc., Chicago (IL), USA). As a primary analysis all measurements were analysed using a paired t-test between the placebo and the remifentanil conditions for the difference score of the during infusion measure minus the pre-infusion measure.

A second analysis was applied to test for order effects. A repeated measures ANOVA was performed that modelled ‘treatment’ (placebo/remifentanil) and ‘time of measurement’ (pre infusion/during infusion) as within subject factors and ‘treatment order’ as a between subject factor.

### 3.3 RESULTS

#### 3.3.1 TOLERABILITY AND SIDE-EFFECTS

All subjects completed the study and reported the study to be well tolerated. Most common side effects during the remifentanil infusion were: sleepiness (7), nausea (7), hot flushes (7), itch (7) and dizziness (6) and light headedness (4) and sweating (4). No relevant cardiovascular or respiratory side-effects were observed throughout the study period.

| TABLE 3.1 | Group means for the heat detection threshold (HDT), cold detection threshold (CDT), mechanical detection threshold (MDT), heat pain threshold (HPT), cold pain threshold (CPT), mechanical pain threshold (MPT) and the pressure pain threshold (PPT). Means are presented for the placebo and the remifentanil condition at baseline and during infusion. Delta (Δ) values are the difference between infusion and baseline. Data are mean (SE). *P<0.05, **P<0.01) |
|---|---|---|---|---|---|---|
| THRESHOLD | UNITS | PLACEBO | REMIFENTANIL |
| | baseline | infusion | Δ | baseline | infusion | Δ |
| HDT | °C | 35.5(0.5) | 37.5(0.9) | 2 | 35.2(0.2) | 39.0(1.1) | 3.8 |
| CDT | °C | 28.4(0.4) | 26.8(0.7) | 1.6 | 28.6(0.8) | 20.4(2.5) | 8.2 * |
| MDT | mN | 4.4(1.0) | 2.5(0.4) | -1.9 | 5.0(1.6) | 22.6(11.4) | 17.6 * |
| HPT | °C | 45.3(1.0) | 46.2(0.7) | 0.9 | 44.9(0.7) | 48.2(0.7) | 3.3 * |
| CPT | °C | 5.3(2.1) | 2.7(1.9) | 2.6 | 7.1(2.6) | 3.15(1.9) | 3.99 |
| MPT | mN | 158.8(18.4) | 158.2(14.7) | -1 | 139.4(16.1) | 182.7(26.3) | 44 * |
| PPT | kg/cm² | 7.6(0.5) | 7.9(0.5) | 0.3 | 7.4(0.5) | 9.5(0.4) | 2.1 ** |

#### 3.3.2 DETECTION THRESHOLDS

The results for the sensory thresholds are presented in figure 3.1 and table 3.1. Both thermal detection thresholds showed a slight desensitization during the remifentanil infusion. Heat detection threshold (HDT) was increased with 3.8 °C (SE=0.98, t(11)=1.68, p=0.06) and the cold detection threshold (CDT) was decreased with 8.2 °C (SE=2.7, t(11)=2.36, p=0.019). The mechanical detection threshold (MDT) was increased with 17.6 mN (SE=10.6) during remifentanil infusion compared to saline infusion, indicating a significant mechanical desensitization (t(11)=1.85, p=0.04). Looking at the graph for the MDT in figure 3.1, it is
clear that the standard error in the MDT during the remifentanil infusion was very large. Therefore an outlier analysis was performed indicating that only two cases had extreme desensitization during the remifentanil infusion. To be sure these cases were not biasing the result a second analysis without these two outliers was performed. Results from the second analysis confirmed that even without the two extreme cases a small but significant mechanical desensitization was present ($t(9)=3.05$, $p=0.007$).

### 3.3.3 Pain Thresholds

Results for the different pain thresholds are presented in figure 3.1. Pain thresholds for heat (3.4 °C, SE=0.71), mechanical pinprick (43.3 mN, SE= 18.7) and pressure (2.1 kg/cm², SE=0.37) were significantly increased during the remifentanil infusion compared to placebo (HPT: $t(11)=2.57$, $p=0.013$, MPT: $t(11)=2.21$, $p=0.025$ and PPT: $t(11)=4.48$, $p=0.001$). The pain threshold for cold was reduced (4.0 °C, SE=2.1) during the remifentanil infusion but this effect was not statistically significant due to a large placebo effects and large inter subject variability (CPT: $t(11)=0.56$, $p=0.59$).

**FIGURE 3.1** Each graph represents the group means for the heat detection threshold (HDT), heat pain threshold (HPT), cold detection threshold (CDT), cold pain threshold (CPT), mechanical detection threshold (MDT), mechanical pain threshold (MPT) and the pressure pain threshold (PPT). The red lines show the remifentanil infusion, black lines the saline placebo infusion. The two time points are the baseline measurement (BL) and the measurement during the infusion (INF). Error bars represent the standard error of the mean. Significant effect of remifentanil is indicated with * ($p<0.05$) or ** ($p<0.01$).
### 3.3.4 CONTINUOUS PAIN RATINGS

The results for the continuous pain ratings are presented in figure 3.2. During the remifentanil infusion pain ratings for the cold pressure pain (CPP) and the continuous heat pain (CHP) were reduced compared to placebo. At baseline the CPP reached a mean VAS rating of 85.55 (SE=9.0) within the first 60 sec. and was stable until the end. During the remifentanil infusion the mean VAS rating at 60 sec. was 26.8 (SE=7.2) and slowly increased to 43.9 (SE=12.0) at 120 sec. For the CHP the pain ratings at baseline were 55.4 (SE=6.8) and 68.1 (SE=8.8) at 60 and 120 sec., respectively. During remifentanil the ratings were reduced to 32.6 (SE=5.8) at 60 sec. and slowly increased to 40.2 (SE=9.2) at 120 sec. VAS ratings for the continuous cold pain (CCP) with the thermode were for the baseline 43.4 (SE=8.3) at 60 sec. and 53.11 (SE=9.45) at 120 sec. compared to 26.0 (SE=5.6) and 31.77 (SE=7.22) during the remifentanil infusion. The area under the curve (AUC) was used for the statistical analysis. The results confirmed a significant difference for the CPP and the CHP (t(10)=4.7, p=0.001; t(11)=2.53, p=0.28, respectively) but not for the cold pain with the thermode (CCP: t(10)=1.23, p=0.25).

**Figure 3.2** Each graph represents the group means for the continuous pain ratings for 2 minutes (120 sec.) on a numeric rating scale for the cold pressor test (Cold Water), the continuous cold pain (cold thermode) and the continuous heat pain (heat thermode). The top row shows the data for the placebo condition, the bottom row for the remifentanil conditioning. Black lines indicate the baseline measurement and red lines the measurements during the infusion. Error bars represent the standard error of the mean. Significant effects of remifentanil on the area under the curve (AUC) are indicated with * (p<0.05) and ** (p<0.01).
**3.3.5 PERIOD AND ORDER EFFECTS**

To test for any effect of the measurement period independent of treatment (1st of 2nd week) we compared the baseline measurements before the treatment for both measurement days. Figure 3.3 shows that the starting points of the arrows are generally higher (or lower in case of the CDT and CPT) in the second period independent of the treatment subjects would receive after baseline. A depended sample t-test between testing days resulted in significant differences of 1.4 °C for the CDT (SE= 0.56, t(11)=2.43, p=0.033), 1.7 °C for the HPT (SE=0.40, t(11)=4.3, p=0.001), 38.2 mN for the MPT (SE=16.8, t(11)=2.34, p=0.039) and 0.61 kg/cm² for the PPT (SE=0.24, t(11)=2.60, p=0.025). No effect of the day of measurement was found for the continuous pain measurements.

Figure 3.3 also indicates that there is a difference in the placebo condition for the pain thresholds if the remifentanil infusion took place on the first visit. For the HPT, the CPT and the PPT there is very small or lack of placebo analgesia as there was no increase of the pain threshold during the saline infusion. For the MPT the effect is actually reversed if
the placebo infusion took place at the second visit. A repeated measures analysis of variance showed significant interaction effects between placebo and the treatment order for HPT \(F(1,10)=3.2, p=0.1\), CPT \(F=(1,10)=4.6, p=0.06\) and MPT \(F(1,10)=8.5, p=0.02\) but not for the PPT. Again no effects of treatment order were seen for the continuous pain rating measurements.

### 3.4 DISCUSSION

In the present study we evaluated several pain measurements for acute pain. Sensory and pain thresholds both mechanical and thermal as well as continuous subjective pain rating measurements for both heat and cold pain were applied before and during remifentanil infusion. Our aims were to use an extensive battery of pain measurements to get more insight in the most applicable and sensitive measurements of acute pain in early clinical development and clinical trials for opioid efficacy.

#### 3.4.1 SENSORY PERCEPTION THRESHOLDS

Remifentanil not only induces analgesia but general sensory perception also diminished reflected by a higher detection threshold for heat and mechanical stimulation. Measuring detection thresholds as well as pain threshold can be valuable in a clinical trial as it indicates if the effect of a drug is pain specific or that there is a general somato-sensory loss. An ideal drug will only affect the pain sensation and leave normal non-nociceptive perception intact.

#### 3.4.2 PAIN THRESHOLDS

All pain thresholds except the cold pain showed a significant increase in pain threshold during the remifentanil infusion. The HPT is a well-known measure that has been validated to be sensitive to opioid analgesia (Brennum et al., 1993; Gustorff et al., 2003). Previous studies have shown that different ramping speeds for heat activate different nociceptors. Rates below 1 °C/sec activate c-fibres whereas faster increases activate aδ-fibers (Handwerker & Kobal, 1993). This could potentially influence the outcome of the drug efficacy depending on the mechanism of action of the specific compound. In the present study we used a temperature ramping speed of exactly 1 °C/sec activating c-fibres. Opioids are known to selectively attenuate nociceptive input from c-fibres in the dorsal horn supporting the use of a slow ramping speed for heat pain thresholds (Le Bars et al., 1976; Dickenson & Sullivan, 1986; Curatolo et al., 2000a).

There was a clear increase in the mechanical pain threshold (MPT) present during the remifentanil infusion. The MPT procedure that was applied was developed for quantitative sensory testing in neuropathic pain patients (Rolke et al., 2006). In experimental pain
models mechanical pinprick stimulation is often used to assess hyperalgesia but the present study suggests the MPT can also be applied for acute pain models (Koppert et al., 1999; Gustorff et al., 2004). As the MPT is thought to activate aδ-fibers instead of c-fibers it is a very useful measurement within a multimodal acute pain test battery (Le Bars et al., 2001).

The PPT was the most sensitive pain threshold measurement. The PPT is thought to measure deep tissue mediated nociceptive processing (Handwerker & Kobal, 1993; Graven-Nielsen et al., 2004). In line with our findings Curatolo and colleagues report that remifentanil has a stronger analgesic effect on electrical pain in the deep muscular tissue compared to the effect on electrical pain at the skin (Curatolo et al., 2000b). This congruent evidence underlines the effectiveness of the PPT as a measure for opioid efficacy.

The cold pain threshold (CPT) was decreased during the remifentanil infusion but this effect was not statistically significant. This was due to a strong placebo analgesia effect combined with a high inter subject variability. Davis reported that cold pain is less well understood than heat pain as cold pain is mediated by a highly complex interaction of primary afferent input involving both aδ- and c-fibers (1998). This complex interaction of nociceptive pathways involved in cold pain processing could explain the in larger between subject variability for the cold pain threshold. In line with our findings Gustorff et al. reported a lack of sensitivity for remifentanil in the cold pain measurements (Gustorff et al., 2003). The authors attribute this failure to detect opioid efficacy to the dominant role aδ-fiber in cold pain processing. Overall the CPT seems to be a less suitable outcome measure for opioid efficacy in acute pain trials.

### 3.4.3 Continuous Pain Stimulation

Three continuous pain measurements were applied while the subjects had to continuously rate the pain on a visual analogue scale. The cold pressor pain (CPP) was the most robust and effective measurement. This seems to be in contrast with the cold pain threshold measurement which did not result in a significant difference between placebo and remifentanil. However the CPP is mediated by pain due to vasodilatation which is different from pure cold pain (Handwerker & Kobal, 1993; Curatolo et al., 2000a). The CPP is mediated by the nociceptors on cutaneous veins involving both a-delta and c-fibers. This difference in pain mechanism between cold and cold pressor pain could cause the lack of analgesic effect with the continuous cold pain stimulation with the thermode and the cold pain threshold.

Furthermore previous research indicates that the CPT activates the endogenous analgesic DNIC mechanism ('Diffuse Noxious Inhibitory Controls') which is reinforced by opioids (Willer et al., 1990). It can be hypothesized that the robust reduction in painfulness of the CPT during remifentanil infusion is caused by an additive effect of endogenous and exogenous opioid mediated mechanisms. However, this would mean that continuous pain stimulation
paradigms might overestimate the efficacy of the compound due to endogenous analgesic mechanisms like the DNIC that are activated within the long stimulation time of the outcome measure.

The continuous heat pain stimulation (CHP) showed a clear analgesic effect of the remifentanil infusion similar to that of the heat pain threshold. This underlines the applicability of heat pain measures to test opioid efficacy either as a threshold or as a supra-threshold continuous stimulation.

### 3.4.4 PERIOD EFFECTS

A general increase in the sensory detection and pain thresholds was seen for the baseline measurement at the second visit independent of the treatment. We are not the first to report this effect, Sycha and colleagues also found an increase in heat pain threshold at the second period while investigating the efficacy of ibuprofen (Sycha et al., 2003). Yarnitsky et al. reported that pain thresholds can increase between the first and second visit (1996). We tried to overcome these period effects by familiarizing all subjects with the pain measurements during a screening visit, but clearly this was not sufficient to avoid these confounding effects completely. Another possibility would be that the subjects who received the remifentanil first, were exposed to higher heat temperatures (due to the analgesic nature of remifentanil) which could have resulted in peripheral nociceptors damage. It is known that capsaicin induced damage to the TRPV1 receptor can last up to 6 weeks (Simone et al., 1998). Heat nociception is also mediated by the TRV1 receptor. It is known that short stimulation of 53 degrees can result in burn injury associated with short term sensitization (Raja et al., 1984). Short term sensitization of TRPV1 receptors can result in long term desensitization for heat (Simone et al., 1998). If the possible heat induced nociceptor damage lasted for more than a week, a long lasting desensitization could explain the higher baseline thresholds reported at the second visit. As these effects were not only present for the heat pain threshold but also for the cold pain threshold and the pressure pain threshold this could be a general mechanism of nociceptor damage after normally painful stimulation under analgesia. This explanation warrants further investigations as it could have both clinical implication for analgesia as well as methodological implications in clinical trials.

### 3.4.5 REDUCED PLACEBO ANALGESIA

We observed a reduction or lack of placebo analgesia in the pain thresholds if the remifentanil infusion took place at the first visit. The expectation of a therapeutic effect is the most important factor for placebo analgesia. The expectation of pain reduction directly causes pain relief upon placebo administration (Price et al., 1999). Recently, Bingel et al. showed that the expectancy of the treatment also influences the efficacy of remifentanil analgesia.
Within the present study almost all subjects experienced side-effects like nausea, dizziness or sleepiness during the remifentanil infusion. The presence of these strong side-effects could induce a bias among the volunteer indicating that they received the active treatment at day one and this might lead to an expectancy-bias that they would receive the placebo infusion at day two. If this was the case the subjects would not expect any analgesia the second visit. As expectancy is the main cause of placebo analgesia, this could explain our reduced placebo analgesia.

Alternatively there could have been a ceiling effect for the pain threshold in the second week during the placebo infusion because the baseline pain threshold was increased in general for the second visit. In turn this could leave less room for the increase in the threshold during the infusion. However if this was the case the analgesic effect of remifentanil should also be smaller the second visit and this was not the case.

Efforts to overcome possible deblinding within the study have to be a high priority in clinical trials studying a compound with a strong side-effect profile. Several studies using remifentanil have already tried to overcome these problems by using an active placebo like diazepam (Dellemijn & Vanneste, 1997) or diphenhydramine (Wang et al., 2008). Another possibility would be to instruct the subject that they will receive a maximum of two drug interventions during the two visits. This would ensure the possibility of another treatment period therefore not changing the expectations of the subjects.

3.4.6 CONCLUSIONS
Within the present study the HPT the PPT are the most robust and sensitive pain threshold measures to use in clinical trials for opioid efficacy. The cold pressor test and the continuous heat pain stimulation are the most effective continuous pain rating measures. However one should keep in mind that these measures are most suitable to test with opioids. Different classes of drugs may exert their efficacy by means of interacting with different receptor (sub-) types and nociceptive pathways. Therefore it will always be worthwhile to use a multimodal pain testing battery in early clinical development of novel analgesic compounds.
REFERENCES


