Evolution of Endogenous Analgesia

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Chapter 1

Introduction

1

Pain modulation

Pain is a complex sensation influenced by biological, emotional, cognitive and behavioral factors. Early important evidence for this is described in a study by Beecher in 1946.[1] Beecher was an surgeon who worked for the US Army during World War II and during this time treated many wounded soldiers suffering from acute and severe pain. He observed that only a quarter of severely injured soldiers with penetrating traumas and long bone fractures (while mentally healthy) reported severe pain and requested analgesics. This indicated that strong emotions as experienced in the battlefield could block pain perception. Another interesting observation made by Beecher was the effect of placebo in severely injured soldiers. Due to shortages of medical supplies including strong analgesics like morphine, Beecher was forced to treat his patients with placebo substances. In several studies he performed, involving over 1,000 patients, he observed an average analgesic effect of placebo of about 35%.[2] These studies indicated that the human body is capable of modifying painful sensations and underlie the development of theories regarding endogenous control of pain.

The first clearly articulated concept of a pain modulatory system was described in 1965 by Melzack and Wall in the gate control theory.[3] In this theory a gating mechanism within the dorsal horn of the spinal cord of rodents was proposed which determined whether signals were sent to the brain based on the type of activated nerve fibers. Supraspinal influences on this system were suggested, although no clear evidence was present at that time for descending pathways (from the brain to the spinal cord) that could influence pain perception. Evidence for this concept was provided by Wall in 1967 who demonstrated that the blockade of descending impulses from the brain stem by spinal cord lesions spontaneously activated dorsal horn neurons.[4] This indicated that projections from the brain stem were able to inhibit neurons at the level of the dorsal horn in the spinal cord which was the basis for the current understanding of descending control of pain. In the beginning of the 1970s several regions of the brain stem in animals such as the periaquaductal gray (PAG) and rostral ventromedial medulla (RVM) were shown to be involved in the initiation of descending inhibitory pathways as electrical stimulation of these regions induced analgesia, inhibition of withdrawal reflexes and inhibition of dorsal horn neurons sensitive to noxious stimulation. The administration of morphine in these regions provided similar observations and currently we know that these pain modulatory pathways are the central substrate for the analgesic actions of opioids and endorphins.[5,6] In 1979 Le Bars et al. demonstrated in rats that afferent noxious information from various parts of the body was able to inhibit activity of nociceptive neurons in the dorsal horn which simultaneously received afferent noxious information from a different part of the body. This phenomenon was called diffuse noxious inhibitory controls (DNIC). In animals DNIC involves a spinal-bulbo-spinal feedback loop where afferent noxious pathways are able to activate descending inhibitory pathways originating in the brain stem to inhibit nociceptive neuronal activity at the level of the dorsal horn.[7,8] In the late 1980s, DNIC was demonstrated to also

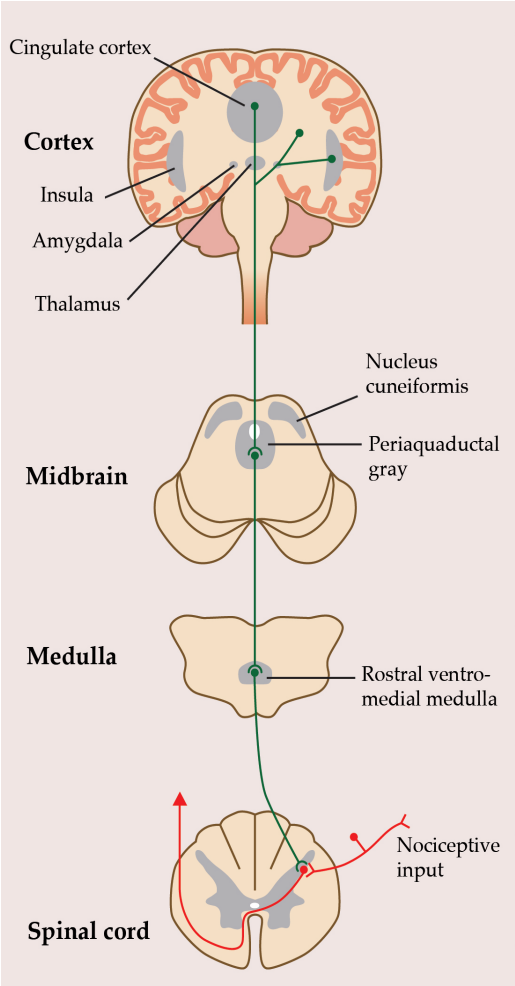

Figure 1. *Schematic illustration of the pain modulatory pathways. Nociceptive input reaches the brain via an afferent pathway (red). Next, the descending pain modulatory pathway is activated by several higher cortical sites that project to the brainstem to modulate nociceptive input at the level of the dorsal horn. This descending pathway can be either facilitatory or inhibitory. (adapted from: Dahan A, Niesters M, Sarton E. Endogenous modulation of pain is visible in the brain. Clin Neurophysiol. 2012; 123: 642-3).*

be present in humans.[9] However, imaging studies demonstrate that in humans descending control of pain also involves higher cortical areas, such as the amygdala, the thalamus, the insula and the anterior cingulate cortex (ACC).[10,11] The current understanding of nociceptive modulatory pathways in humans involves an afferent pathway for nociceptive input to several areas of the cortex and brain stem for pain perception and interpretation. Descending pathways, either facilitatory or inhibitory, can modulate this afferent noxious information at the level of the dorsal horn of the spinal cord as illustrated in figure 1.

Conditioned pain modulation

The biology of the DNIC-like effect in humans is more complex compared to rodents, for instance due to the involvement of higher cortical centers. Therefore, new terminology has been proposed to refer to the DNIC-like effect in humans to discriminate between the brain stem mediated inhibitory effect in rodents and the complex facilitatory and inhibitory pain modulatory properties present in humans. Two noxious stimuli are required during psychophysical research to explore descending control of pain in humans, which are referred to as the test stimulus and the conditioning stimulus. The test stimulus is the stimulus on which the conditioning effect is evaluated; the conditioning stimulus is the stimulus that induces the change in pain perception. The effect of the conditioning stimulus on the test stimulus is called “Conditioned Pain Modulation” (CPM)

which is the net effect of the facilitatory and inhibitory mechanisms of pain processing.[12]

In the current thesis CPM was evaluated using heat pain as test stimulus and cold pain as conditioning stimulus (Fig. 2A). Heat pain was administered on the lower part of the dominant arm using a 30-second stimulus during which the test subject continuously rated pain intensity. The test stimulus was applied with and without the conditioning stimulus, which was administered on the lower leg During effective descending inhibitory control of pain, as observed in healthy volunteers, the conditioning stimulus will decrease the pain intensity of the test stimulus (Fig. 2B).[9]

Offset analgesia

More recently, a novel model of endogenous inhibitory control of pain has been proposed that produces temporal alterations in pain processing named offset analgesia (OA).[13] OA is the perception of profound analgesia during a slight decrease of a noxious heat stimulus, which is more pronounced than would be predicted by the rate of the temperature decrease. Although a peripheral origin of OA is not excluded (*e.g.* related to primary afferent neurons within the dorsal horn), OA is generally considered an example of central inhibitory modulation of pain probably induced by neuronal circuits similar to CPM. A schematic illustration of a normal OA response as observed in healthy volunteers is shown in figure 3.

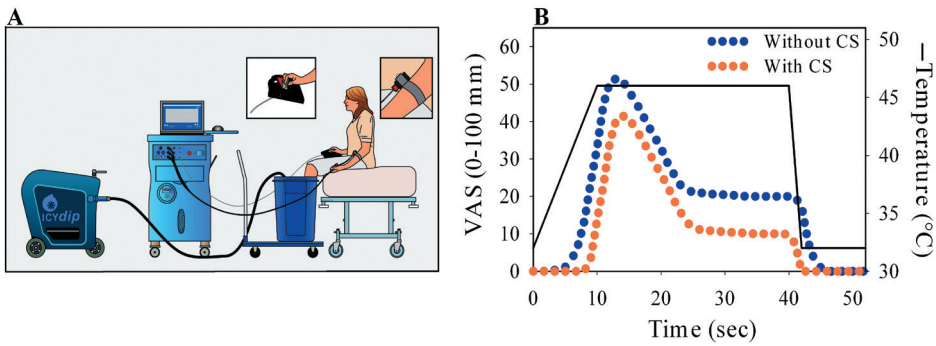

Figure 2. A. *Schematic presentation of the experimental set-up to evaluate conditioned pain modulation (CPM). Heat pain (test stimulus) was applied using a 3 x 3 cm peltier element on the lower part of the dominant arm while the subject rated pain intensity using a slide on a potentiometer using the other arm. Cold pain (conditioning stimulus) was applied using a cold water bath (6-12 °C) in which the lower leg and foot was immersed.* ***B.*** *Schematic illustration of CPM as observed in healthy volunteers. The dotted lines represent the pain intensity scores during the 30-second heat stimulus (straight black line) on the lower part of the arm without (blue line) and with (orange line) the conditioning stimulus. The difference between the two dotted lines represents the CPM effect. CS: conditioning stimulus; VAS: visual analogue scale.*

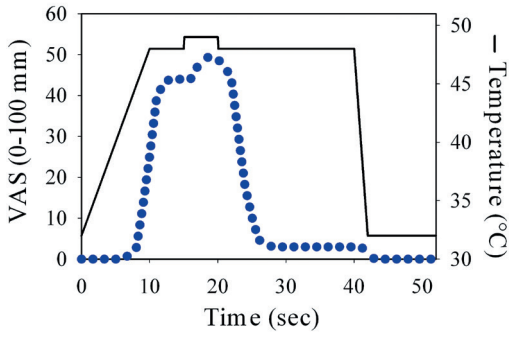

Figure 3. *Schematic illustration of offset analgesia (OA) as observed in healthy volunteers. The dotted line represents the pain intensity scores during a 30-second dynamic heat stimulus applied on the skin. Heat stimulation consists of 3 phases: 1) a ramp to the target temperature that is kept constant for 5 seconds; 2) a 1 °C temperature increase that is also kept constant for 5 seconds; 3) a 1 °C temperature decrease (back to the target temperature) that is kept constant for 20 seconds followed by a quick return towards the baseline temperature. OA is seen in response to the 1 °C temperature drop observed as a profound decrease in pain intensity.*

Outline of this thesis

The aim of the current thesis was to evaluate the effect of central-acting drugs on endogenous control of pain in healthy volunteers and patients with chronic neuropathic pain using psychophysical research and functional magnetic resonance imaging (fMRI).

In **chapter 2** the effect of short-term treatment with the analgesic ketamine on CPM and OA is evaluated in healthy volunteers in a placebo-controlled cross-over study.

Chapter 3 describes the presence of OA in a large group of healthy volunteers in the age range 6-80 years and a group of chronic neuropathic pain patients. Furthermore, the effect of short-term treatment with the analgesics ketamine, morphine and placebo on OA responses in neuropathic pain patients is evaluated.

In **chapter 4** the effect of short-term treatment with ketamine, morphine and placebo on CPM responses in chronic neuropathic pain patients using a cross-over study is described.

Chapter 5 describes the effect of a 4-week treatment with the new analgesic tapentadol on CPM and OA in patients with painful diabetic neuropathy in a double-blind, placebo-controlled study.

In **chapter 6** the effect of ketamine and pain perception during ketamine infusion on large-scale network interaction in the brain measured by resting-state fMRI is evaluated. We aimed to identify changes in brain connectivity for (1) brain areas involved in ketamine's pharmacodynamic profile with respect to intended (analgesia) and side effects (most importantly psychedelic effects) and (2) areas involved in pain processing.

Chapter 7 describes the effect of deafferentation induced by spinal anesthesia on intrinsic brain connectivity measured by resting-state fMRI and on the pain perception of non-deafferented skin. Our aim was to investigate whether (1) pain perception above the level of the anesthetic was altered and (2) whether this co-

incided with changes in functional neuroimaging markers of cortical and thalamic networks in healthy volunteers.

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Section 1

Psychophysical studies

Chapter 2

Effect of Ketamine on Endogenous Pain Modulation in Healthy Volunteers

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R.B. Fillingim, L.P.H.J. Aarts, E.Y. Sarton
Pain 2011; 152: 656-63

Introduction

The number of patients affected by chronic pain is growing. Various mechanisms may underlie the process of chronification of pain. Important mechanisms include NMDAR activation and up-regulation and inflammatory responses in the spinal cord;[1-3] both cause central sensitization and are related to repeated afferent excitation.[1] Another mechanism involved in pain chronification is dysfunction of inhibitory pathways or a shift in the balance between pain inhibition and pain facilitation. In the last decades the role of central pain modulation in the control of nociception been investigated intensively.[4,5] Inhibitory and facilitatory descending pathways, originating at higher central nervous system sites, such as the cerebral cortex, nucleus raphe magnus, periaquaductal grey (PAG), locus coeruleus and rostraventral medulla (RVM), modulate activity of dorsal horn nociceptive neurons.[4,6] Alterations in endogenous pain modulation have been observed in chronic pain diseases like irritable bowel syndrome, fibromyalgia, chronic tension headache, temporomandibular disorder and complex regional pain syndrome type 1 (CRPS-1).[5,7-9] Seifert et al. showed a shift from inhibition towards facilitation of nociceptive input in CRPS patients.[9]

Recent studies indicate that treatment of chronic pain patients with the NMDAR antagonist ketamine has a prolonged beneficial effect on spontaneous pain reporting and is effective when used in combinations with opioids in the treatment of acute postoperative pain and cancer pain management.[10-15] Ketamine may produce prolonged analgesia through multiple mechanisms. Most important and most frequently studied is its desensitizing effect on sensitized nociceptive neurons in the spinal cord by blocking the NMDAR.[1] As a result, ketamine blocks the enhanced signal transmission in the pain circuitry. The effect of ketamine on endogenous inhibitory pain control remains unknown. Since ketamine ameliorates chronic pain (such as occurs in CRPS patients) an effect on endogenous pain modulation is plausible. In the current study we address this issue by examining the effect of low-dose ketamine (40 mg/h) on two expressions of endogenous control of pain: Diffuse Noxious Inhibitory Controls (DNIC) and Offset Analgesia (OA).[5,7,16-20]

DNIC has been investigated in both animals and humans, showing central inhibition of a focal pain stimulus by administering a noxious stimulus at a remote area, thereby reducing the perception of the focal pain stimulus.[5,21] OA has recently been proposed as a second endogenous analgesia mechanism. This mechanism demonstrates profound analgesia during slight incremental decreases of a noxious heat stimulus, which is more rapid than would be predicted by the rate of temperature decrease.[16,19,20] Recent studies indicate that OA coincides with activation of the PAG, RVM and locus coeruleus, areas with substantial roles in descending inhibition of pain.[19]

The main aim of the present study is to explore whether ketamine interacts with pathways involved in endogenous pain modulation and whether it enhances

inhibitory control. To that end we studied the effect of low-dose ketamine on DNIC and OA in healthy volunteers. We hypothesize that ketamine enhances both DNIC and OA and by that contributes to the prolonged analgesic effect of ketamine in chronic pain patients.

Methods

Subjects
Ten healthy volunteers (4 men/6 women) were recruited for participation in the study, after approval of the protocol by the local medical ethics committee (Commissie Medische Ethiek LUMC). Informed written consent was obtained according to the Declaration of Helsinki from all participants. The study was registered in the Dutch trial register (www.trialregister.nl) under number NTR2005. Before participation all subjects received a physical examination and their medical history was taken. Exclusion criteria were: age < 18 years or > 75; presence or history of a medical disease such as renal, liver, cardiac, vascular (incl. hypertension) or infectious disease; presence or history of a neurological and psychiatric disease (*e.g.* increased cranial pressure, epilepsy, psychosis); glaucoma; pregnancy; obesity (BMI > 30) and any use of pain medication.

Pain assessment, DNIC and offset analgesia
Heat pain was induced using the Pathway Neurosensory Analyzer (Medoc Ltd, Ramat Yishai, Israel). Using a 3 x 3 cm thermal probe, the skin on the volar side of the arm was stimulated with a preset and computer controlled temperature scheme. Baseline temperature was set at 32 °C. During heat pain stimulation, subjects quantified the pain intensity of the noxious stimulus using the slider on an electrical potentiometer connected to a computer, allowing continuous monitoring of the Visual Analogue Scale (eVAS), that ranged from 0 (no pain) to 100 (worst pain imaginable). To overcome adaptation, the volar side of the arm was divided into three zones. The thermode was moved from zone to zone between stimuli. Prior to the study, the test temperature was determined by applying a series of heat stimuli, ranging from 42 °C to 49 °C with increments of 1 °C; each stimulus was applied for 10 seconds, with 5-10 min intervals between stimuli. The temperature evoking an eVAS of at least 50 mm was used during the remainder of the study (this is the test temperature).

Cold pain was induced by cold water immersion of the subject's foot and lower leg into a reservoir with cold water. Water with a predetermined temperature was produced by a rapid water-cooling system (IcyDip, IcySolutions BV, Delft, The Netherlands). The cold water temperature could be set at any value ranging from 6 °C to 18 °C. Prior to the study various water temperatures were tested. The temperature that produced an eVAS of at least 30 mm was used in the remainder of the study. After each exposure to cold water, the subject's foot was warmed to room temperature using the warm water reservoir of the IcyDip.

DNIC was determined according to the protocol described by King et al.[7] In short, for the experimental stimulus focal heat pain was applied to the subject's volar side of the left arm as follows: the temperature gradually increased from baseline (32 °C) to the test temperature (at 1.5 °C/s) and was held constant for 30 seconds. Next, the temperature decreased rapidly (at 6 °C/s) to baseline. Each heat stimulus was repeated for a total of three times, after which the same stimulus was applied for another three times but now in combination with the conditioned stimulus (immersion of foot and lower leg in cold water). The conditioned stimulus was applied 25 seconds before the start of the experimental stimulus and ending simultaneously with the end of the experimental stimulus. Between each heat stimulus there was a 3 minute rest period. During the heat stimulation the subjects rated pain intensity using the eVAS slider.

Offset analgesia was determined as described by Yelle et al.[20] In short, a focal heat stimulus was applied to the subject's volar side of the arm. The thermode positioned on the arm was ramped (1.5 °C/s) from baseline temperature to the individual's test temperature. The test temperature was kept constant for 5 seconds after which it was raised by 1 °C for 5 seconds and next decreased by 1 °C to the test temperature and kept constant for 20 seconds. Next, the temperature quickly returned (6 °C/s) to baseline. Subjects rated the intensity of the heat stimulus using the eVAS slider. Offset analgesia was determined three times with a 3 minute rest period between tests.

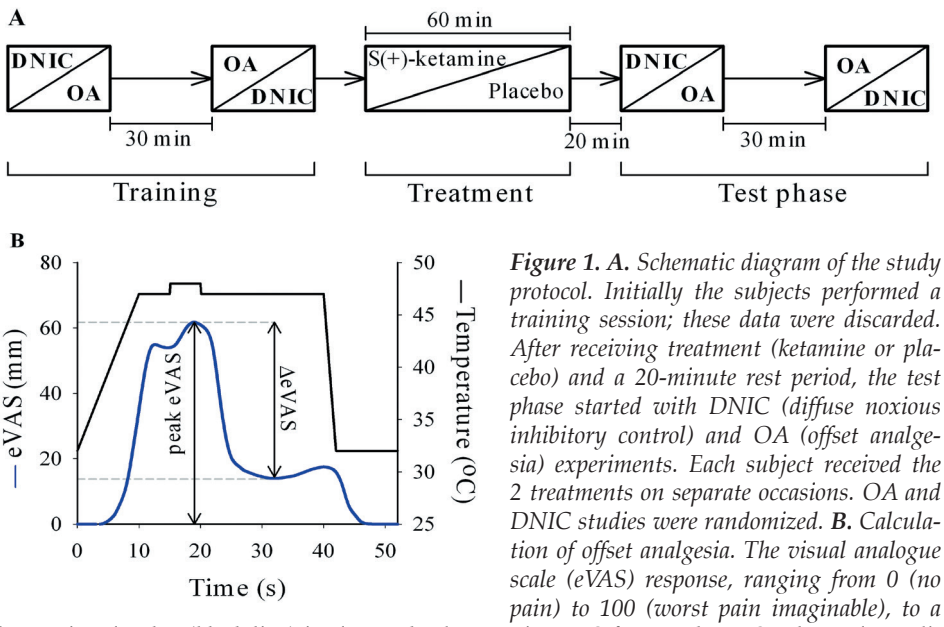

Figure 1. A. *Schematic diagram of the study protocol. Initially the subjects performed a training session; these data were discarded. After receiving treatment (ketamine or placebo) and a 20-minute rest period, the test phase started with DNIC (diffuse noxious inhibitory control) and OA (offset analgesia) experiments. Each subject received the 2 treatments on separate occasions. OA and DNIC studies were randomized.* ***B.*** *Calculation of offset analgesia. The visual analogue scale (eVAS) response, ranging from 0 (no pain) to 100 (worst pain imaginable), to a heat pain stimulus (black line) is given. The decrease in eVAS from peak eVAS value to its nadir following the 1 °C decrease of the heat pain stimulus (at t = 20 seconds) is calculated (ΔeVAS). To correct for the value of the peak eVAS, the ΔeVAS is divided by the peak eVAS giving the 'corrected' ΔeVAS or ΔeVAScc (= ΔeVAS/[peak eVAS]).*

Study design
The study had a single-blinded, cross-over design. Subjects were randomized for treatments (intravenous S(+)-ketamine (Ketanest-S, Pfizer BV, Capelle a/d IJssel, The Netherlands) or placebo (NaCl 0.9%)) and pain test order (offset analgesia or DNIC, experiment with and without conditioning stimulus). Randomization for studies with and without water immersion was performed to avoid a distraction or learning effect.[22] There were at least two weeks in between the placebo and ketamine sessions. On both experiment days, individual test temperatures for the experimental and conditioned stimulus were determined first. Next, the subjects were trained by performing DNIC and offset analgesia studies, as described above. Subsequently, the subjects received a 1-h infusion of either S(+)-ketamine (40 mg per 70 kg) or placebo. After a 20-minute wash-out period the test phase began with studies to determine DNIC and offset analgesia after placebo or ketamine treatment (with a 30-minute interval between studies). See also figure 1A.

Side effects
During ketamine and placebo treatment the occurrence of nausea and vomiting was recorded (yes/no), and drug high and drowsiness were scored using an 11-point numerical rating scale ranging from 0 (= no effect) to 10 (= most severe effect).

Data and statistical analyses
The DNIC and offset analgesia data collected during training were discarded. The eVAS data were averaged over 1-second periods. To quantify the DNIC data, the area-under-the-curve (AUC) of each eVAS response curve was calculated. A linear mixed model was used to compare the AUCs without and with conditioning stimulus after ketamine and placebo infusions. Group differences were tested by a chi-square test. To quantify offset analgesia the decrease in eVAS from peak eVAS value to the eVAS nadir following the 1 °C decrease of the test stimulus was measured (ΔeVAS; Fig. 1B) corrected for the value of the peak eVAS (ΔeVASc = ΔeVAS/[peak eVAS]). ΔeVASc values observed after ketamine and placebo treatment were compared using a linear mixed model. p-values < 0.05 were considered significant. Data are presented as mean (SEM) unless otherwise stated.

Results

Baseline values
All subjects completed the protocol without unexpected or major side effects. Baseline subject characteristics are listed in table 1. Between treatment days no significant differences were observed in test temperatures of the conditioning and experimental stimuli. Mean testing temperatures were 46.1 ± 2.5 °C (mean ± SD) and 45.9 ± 2.9 °C for placebo and ketamine study days, respectively (t-test: p = 0.908). The corresponding mean baseline eVAS scores were 52.1 ± 9.8 mm (placebo) and 51.7 ± 12.9 mm (ketamine; p = 0.942). The temperatures of the con-

Table 1. *Subject characteristics*

Number of subjects (M/F)	10 (4/6)
Age (year)	24.1 ± 3.7
Weight (kg)	76.5 ± 13.8
Baseline heat temperature in placebo/ketamine studies (°C)	46.1 ± 2.5/45.9 ± 2.9 (ns)
Baseline water temperature in placebo/ketamine studies (°C)	10.3 ± 3.9/10.7 ± 3.8 (ns)

Values are mean ± SD; ns = not significantly different.

ditioning stimulus were 10.3 ± 3.9 °C (placebo) and 10.7 ± 3.8 °C (ketamine; p = 0.838) with mean baseline eVAS scores of 29.9 ± 16.7 mm (placebo) and 32.6 ± 20.6 mm (ketamine; p = 0.764). The baseline or training eVAS responses to the two heat stimuli paradigms, DNIC and OA obtained prior to placebo and ketamine treatment, are given in figure 2 (the DNIC response is given without conditioning stimulus). It shows that the baseline eVAS responses were similar for the two treatment sessions.

Ketamine-induced analgesia and side effects

Despite an average 1-hour interval between the end of the ketamine infusion and the eVAS responses, ketamine analgesia persisted: eVAS (obtained at identical temperatures) were significantly lower after ketamine treatment compared to placebo treatment by 10 to 12 mm ($p < 0.01$). In order to get an indication of the analgesic effect of ketamine, we plotted the first 13 seconds of the eVAS responses following ketamine and placebo treatment for the 2 heat stimuli paradigms in figure 3. We present only the initial part of the response in relation to

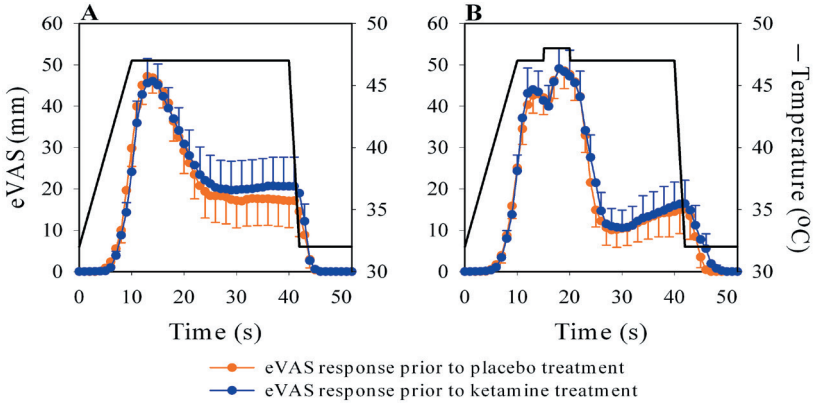

Figure 2. *Baseline eVAS (visual analogue scale ranging from 0 (no pain) to 100 (worst imaginable pain)) responses (obtained during training):* ***A.*** *responses obtained during the 30-second continuous heat stimulus and* ***B.*** *the varying heat stimulus used to induce offset analgesia. Orange circles are the eVAS response prior to placebo treatment; blue circles prior to ketamine treatment. Values are mean ± SEM. No differences in baseline responses were obtained between treatment sessions.*

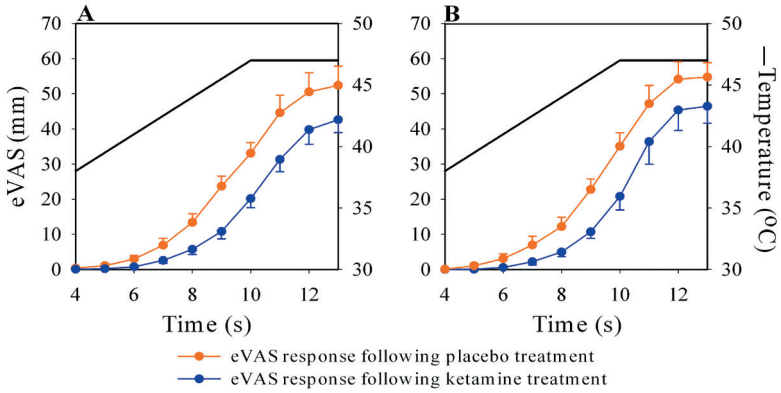

Figure 3. *Ketamine's analgesic effect. First 13 seconds of the eVAS (visual analogue scale ranging from 0 (no pain) to 100 (worst pain imaginable)) responses without conditioning stimulus after placebo (orange circles) and ketamine treatment (blue circles) for studies performed in the* ***A.*** *DNIC paradigm and* ***B.*** *offset analgesia paradigm. Ketamine produced a significant analgesic effect with a reduction in eVAS of 10-12 mm at t = 13 seconds.*

ketamine analgesia as it may be argued that other phenomena (*e.g.* adaptation, endogenous analgesic effects) influence the remainder of the response. Consequently, DNIC and OA eVAS responses (and their respective AUCs) in studies following ketamine treatment were smaller compared to responses obtained following placebo treatment. Ketamine produced nausea in six subjects and vomiting in three. Numerical rating scores for drug high were 6.0 ± 1.2 *versus* 1.2 ± 0.8 ($p < 0.05$) and for drowsiness 4.7 ± 0.8 *versus* 0.8 ± 0.6 ($p < 0.05$) during ketamine *versus* placebo treatment.

Diffuse noxious inhibitory control

eVAS responses (DNIC and OA) without conditioning stimulus were obtained 59 minutes (95% confidence interval 44 – 79 minutes) following the end of the ketamine or placebo infusion. eVAS responses (DNIC) with conditioning stimulus were obtained 56 minutes (41 – 76 minutes) following ketamine infusion. Mean DNIC responses are presented in figure 4. Heat pain stimulation for a period of 30 seconds gave an eVAS response with rapid temporal sensitization (0-10 seconds) followed by a phase of adaptation (10-30 seconds). After placebo and ketamine infusions, significant effects of the conditioning stimuli were observed ($p < 0.0001$). After placebo infusion significant inhibitory control (*i.e.* DNIC) was activated in all subjects. Experimental heat pain applied simultaneously with the conditioned stimulus resulted in eVAS values below those observed during testing just experimental heat pain (AUCs 764.3 ± 139.9 *versus* 1008.9 ± 178.2; $p < 0.001$). In contrast, after ketamine infusion, no DNIC was observed, but rather a significant facilitatory pain response occurred when heat pain was combined with the conditioning stimulus (AUCs 889.8 ± 201.5 *versus* 708.4 ± 155.9; $p < 0.01$).

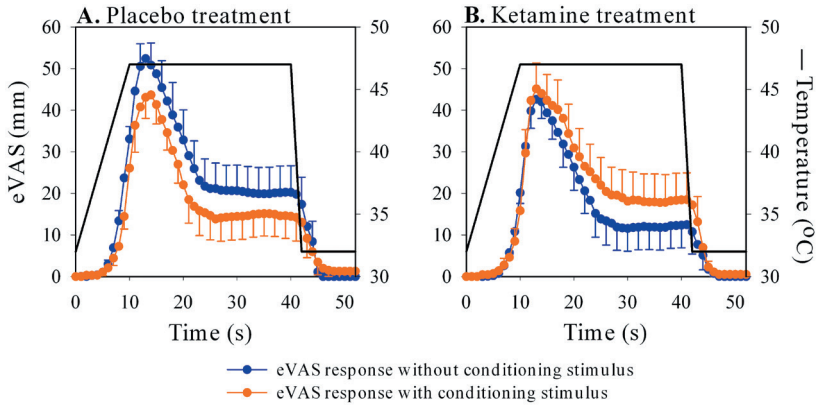

Figure 4. *Effect of* ***A.*** *placebo and* ***B.*** *ketamine treatment on endogenous pain modulation as determined by a diffuse noxious inhibitory control paradigm, that is, a heat pain stimulus (black line) applied without and with a conditioning stimulus (leg immersion in cold water). After placebo treatment the eVAS (visual analogue scale ranging from 0 (no pain) to 100 (worst pain imaginable)) response to experimental heat pain with the conditioning stimulus was significantly inhibited compared to the response without the conditioning stimulus ($p < 0.001$). After ketamine treatment the eVAS response to the noxious heat stimulus was increased when the conditioning stimulus was applied ($p < 0.01$). The data are the population mean of the subjects' mean eVAS values (SEM).*

In figure 5 the individual magnitudes of the eVAS responses without conditioning stimulus *versus* responses with stimulus are presented in a scatter plot. It shows that while the mean AUC without conditioning stimulus (x-axis) after ketamine treatment is reduced compared to placebo the individual data overlap. The plot further shows that magnitude of the eVAS response without con-

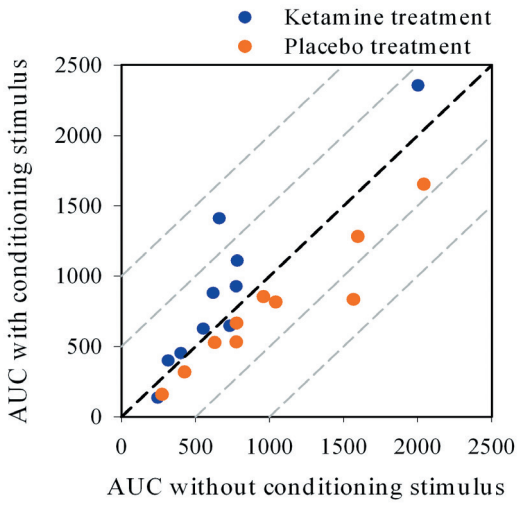

Figure 5. *Scatter plot of the effect of the conditioning stimulus on eVAS (visual analogue scale ranging from 0 (no pain) to 100 (worst pain imaginable)) responses after placebo treatment (orange circles) and ketamine treatment (blue circles). The dashed lines are lines of identity. On the x-axis the areas-under-the curve (AUC) of the eVAS responses without conditioning stimulus; on the y-axis the AUCs of the eVAS responses with conditioning stimulus.*

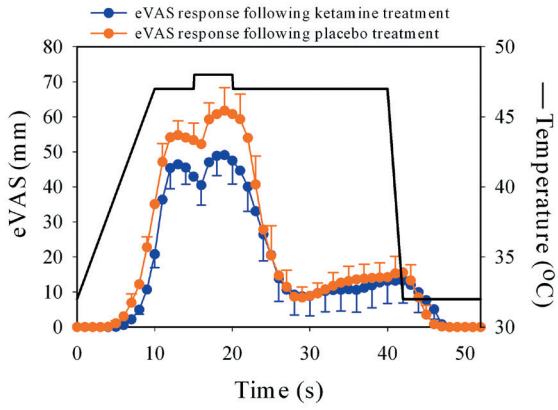

Figure 6. *Effect of placebo and ketamine treatment on endogenous pain modulation as determined by the offset analgesia paradigm. The ΔeVAS values (decrease in eVAS (visual analogue scale ranging from 0 (no pain) to 100 (worst pain imaginable)) from peak eVAS value to its nadir following the 1 °C decrease of the heat pain stimulus (at t = 20 seconds)) corrected for the difference in peak ΔeVAS did not differ between treatments. The data are the population mean of the subjects' mean eVAS values (SEM).*

ditioning stimulus had just limited effect on the magnitude of the response with stimulus (slope of linear regression on the ketamine data = 1.19; on the placebo data 0.75).

Offset analgesia

Mean OA responses are shown in figure 6. The response after ketamine treatment was smaller than after placebo treatment; a sign of persistent analgesic effect of ketamine in the hours following treatment. Offset analgesia was observed in all subjects after both placebo and ketamine infusion. The placebo ΔeVASc (0.91 ± 0.03) did not differ significantly from the ketamine ΔeVASc (0.86 ± 0.06), indicating no effect of ketamine on OA. No correlation was observed between placebo ΔeVASc and DNIC responses (inhibition determined by subtracting AUCs) ($p > 0.05$, correlation coefficient = 0.11).

Absence of sex differences

No sex differences in the response of ketamine relative to placebo were observed in the effects of ketamine on analgesia, DNIC and OA responses.

Discussion

Descending control of nociceptive spinal responses has both inhibitory and facilitatory components.[6] Diffuse noxious inhibitory control and offset analgesia are examples of dynamic inhibitory processes. DNIC is a spinal-medullary-spinal feedback loop that is activated when two painful stimuli are applied simultaneously.[5,17,18] A heterotopic noxious stimulus modifies the perception of another noxious stimulus, and, in case of DNIC, inhibits the perception of the primary (*i.e.* test) stimulus. It has been suggested that DNIC serves as a filter that helps to extract nociceptive signals from the background noise by inhibiting basal somatosensory activity of the population nociceptive neurons (*i.e.* increasing the signal-to-noise ratio).[17] In our current study we generated inhibition of a heat pain stimulus applied to the arm, by immersion of the foot and lower leg in cold

water (placebo data; Fig. 4A). An infusion with ketamine resulted in a blocked inhibitory pain response with more pain when a conditioning stimulus was combined with the test stimulus rather than an increase in DNIC (Fig. 4B; *i.e.* the null-hypothesis was rejected). Our data indicate that the balance between pain inhibition and pain facilitation (that normally has predominance for pain inhibition) was shifted by ketamine towards blocking DNIC, resulting in some form of pain facilitation. Similar alterations in endogenous pain modulation have been observed with acute and chronic morphine treatment.[23,24]

Ketamine analgesia *versus* anti-analgesia

The effect of ketamine on DNIC was not expected and is, at present, difficult to explain. Ketamine is usually associated with analgesia in chronic pain and in combination with opioids in cancer pain and perioperative pain.[10-15,25,26] Pain relief occurs due to blockade of excitatory NMDARs, which results in a reduced or even blocked signal transmission in the pain circuitry towards the thalamus and cerebral cortex. Still, there are several multiple, converging lines of evidence showing that ketamine and other NMDAR antagonists are associated with pain facilitation and antagonism of opioid-induced pain relief. We recently showed in healthy volunteers that while ketamine had a dose-dependent antinociceptive effect on static nociceptive pain (repetitive noxious heat pain stimuli), pain responses following infusion were perceived as more painful (by about 1 cm VAS) for more than 3 hours compared to pre-drug pain responses.[26,27] In agreement with these findings, Mitchell described a cancer patient that developed severe hyperalgesia and allodynia directly following treatment with ketamine.[28] During treatment, the noncompetitive NMDA receptor antagonist MK801 can cause an anti-analgesic effect in mice, where it significantly reduced morphine and stress-induced analgesia (an effect which displayed sex-dependency).[29-32] In another study, MK801 induced hyperalgesia in an acute pain model in the rat.[33] Interestingly, in the anesthetized rat, the NMDAR has been implicated in inhibition of chronic inflammatory pain. High dose (but not low dose) NMDA microinjected into the RVM inhibited primary hyperalgesia 3 hours following injection of complete Freund's adjuvant into a hind paw.[34] In total, these data indicate that apart from a potent analgesic effect, NMDAR antagonists, including ketamine, may, under specific circumstances, produce anti-analgesic and pain facilitatory effects.

Mechanisms of ketamine effect on DNIC

Multiple, non-exclusive mechanisms may cause anti-analgesic or pain facilitatory responses during and/or following ketamine treatment. (i) It has been argued that excitatory amino acids accumulate at spinal and supraspinal sites during effective blockade of the NMDAR.[30,33] These amino acids may activate non-NMDA excitatory receptors (metabotropic or non-NMDA ionotropic glutamate receptors) and consequently produce anti-analgesia and increased pain responses. (ii) Ketamine may recruit different nociceptive mechanisms (compare the mechanism proposed for the block of DNIC by morphine)[23] or additionally activate non-NMDA receptor systems, such as the opioid system causing a

net inhibitory effect on DNIC.[35] (iii) DNIC may require supraspinal processing, which is effectively disrupted by ketamine (*e.g.* a dissociative effect of ketamine between the limbic system and cerebral cortex).[1] And (iv), because we performed our experiments following ketamine infusion, a direct effect at the NMDAR is not excluded, for example by actions of accumulated excitatory amino acids (see "i"), which during the loss of NMDAR blockade from the decrease in ketamine concentration causes a rebound increase in NMDAR activity and consequently enhanced transmission of afferent nociceptive stimuli (*i.e.* pain facilitation).[26]

Because the link of excitatory receptor systems and the process of endogenous pain inhibition/facilitation (and its neurotransmitters such as serotonin and norepinephrine) remains understudied, further studies are needed to investigate the specific role of the excitatory receptors in the balance between pain inhibition and facilitation under physiological and chronic pain conditions.

Offset analgesia

Apart from DNIC, we tested the effect of ketamine on offset analgesia. OA is considered an inhibitory mechanism that increases the temporal contrast between stimuli (*i.e.* a temporal sharpening filter).[16,19,20] OA is defined as the decrease in pain intensity following a decrease in stimulus temperature that is disproportionate compared to a similar increase in temperature. After placebo infusion, we observed an increment in eVAS of 6 mm going from 46 to 47 °C while a subsequent similar decrease in temperature caused a drop in eVAS of 33 mm (a ratio of 5.5; Fig. 3). A similar ratio was observed after ketamine infusion. Furthermore, the drop in eVAS was twice as fast (average 6 mm/s) in OA experiments compared with the adaptation seen in DNIC experiments (3 mm/s). Ketamine had no effect on OA development, *i.e.* the enhanced and robust analgesia following the 1 °C decrease in noxious stimulus intensity remained unaffected by ketamine (drop in eVAS relative to peak eVAS = 0.91 ± 0.03 after placebo *versus* 0.86 ± 0.06 after ketamine treatment). These data contrast the observations in the DNIC experiments. As previously discussed, both in their generation and probably also in their neuroanatomic pathways the inhibitory processes OA and DNIC are distinct: OA relates inhibition to the offset of a noxious stimulus, while DNIC is related to the onset of a heterotopic stimulus.[16,19,20] In agreement with this is the observation that there was no correlation between ΔeVASc and DNIC. We further show that OA and DNIC are dissimilar in their interaction with the glutamatergic receptor system.

Critique of methods

We tested the effect of ketamine and placebo using a single-blind study design as we expected to become unblinded during the treatment of the subjects. Also the subjects noted more severe side effects during ketamine treatment but were not made aware that this was specific to the test treatment. We cannot exclude that specific a priori expectations may have influenced the study outcome at some level. For example, it was shown that the subject's expectation of hyperalgesia completely blocked the analgesic effect of descending inhibition on spinal no-

ciceptive reflexes.[36] To our advantage is that our subjects remained uninformed regarding a possible effect of ketamine on DNIC or OA and the a priori expectations of the investigators were towards an increase in inhibitory control. Furthermore, we observed that treatment sequence had no effect on outcome parameters (DNIC and OA, experiment with and without water immersion) and therefore we do not think that our approach influenced the study outcome significantly.

Finally, it may be argued that the ketamine effect on DNIC is related to the smaller magnitude of the eVAS responses following ketamine treatment and that, for example, the increase in eVAS responses during water immersion is due to a drift towards pre-ketamine baseline values (due to the loss of ketamine analgesic effect). There are several arguments opposing this assumption. DNIC eVAS responses without and with conditioning stimulus were randomized and on average occurred both about 60 min following the end of the ketamine infusion. Hence ketamine's analgesic effect was of similar magnitude in the 2 experiments. Furthermore, the magnitude of the eVAS responses with conditioning stimulus is minimally related to the magnitude of the responses without stimulus (Fig. 5). A small effect (ranging from 0 to 20%) cannot be excluded with larger eVAS responses with conditioning stimulus associated with larger responses without water stimulation. The reverse would be expected when the response would have drifted back to pre-ketamine baseline values.

Conclusions

In conclusion, our data suggest that ketamine treatment effects endogenous modulation of nociceptive stimuli as examined by the DNIC paradigm. DNIC responses following a 1-hour low-dose ketamine treatment displayed a modulated (blocked or even facilitated) DNIC. These findings suggest a modulatory involvement of the NMDA and/or other glutamatergic receptors at some level within the endogenous pain system. No effect of ketamine treatment was observed on the inhibitory effects of subsequent OA responses. This suggests that OA and DNIC differ in their susceptibility for glutamatergic influences. The enduring pain relief of chronic pain during and following ketamine treatment[10-15] cannot be explained by our current findings and suggests the absence between DNIC alterations and relief of spontaneous chronic pain from ketamine. However, it may well be that the effect of ketamine on descending pain modulation is different in chronic pain patients and cannot be addressed by performing ketamine studies in volunteers.

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Chapter 3

Offset Analgesia in Neuropathic Pain Patients and Effect of Treatment with Morphine and Ketamine

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Introduction

Offset analgesia (OA) is the perception of profound analgesia during a slight incremental decrease of a noxious heat stimulus, which is more pronounced than would be predicted by the rate of the temperature decrease.[1-4] In 2002, Grill and Coghill were the first to describe this analgesic phenomenon and argued that OA "may serve as a temporal contrast enhancement mechanism".[1] Although a peripheral origin of OA is not excluded (*e.g.* related to primary afferent neurons within the dorsal horn), OA is generally considered an example of central inhibitory modulation of pain probably induced by neuronal circuits within the periaqueductal gray, rostral ventromedial medulla and locus coeruleus, areas with substantial roles in descending inhibition of pain.[5,6] Other examples of central (inhibitory) modulation of pain include diffuse noxious inhibitory controls (DNIC), stress-induced analgesia and placebo analgesia, all of which share pain-related supraspinal and spinal pathways.[4,7,8] There are indication that central inhibitory modulation of pain is affected in various chronic pain states such as fibromyalgia, irritable bowel syndrome, and complex regional pain syndrome.[8-11] Thus far, OA has not been evaluated in chronic pain.

In the current study we measured OA responses in a population of patients with neuropathic pain (NP) due to small-fiber neuropathy (SFN) and compared their responses with an age- and sex-matched control group and a large group of healthy volunteers with an age range of 6 to 80 years of either sex. The description of OA in a large population allows a clear discrimination between OA in health and disease (NP); we studied volunteers of either sex from age 6 years on, which will give an indication of the developmental aspects of OA and possible sex differences. In addition, we assessed the effect of analgesic treatment on OA in NP patients. The effect of morphine and ketamine was tested using a randomized placebo-controlled design. Although morphine and ketamine are considered strong analgesics and frequently used to relief severe chronic pain (albeit through different pathways), various studies indicate that both agents have a facilitatory rather than an inhibitory effect on central modulation of pain.[4,12,13] For example, Niesters et al.[4] recently showed that ketamine treatment causes the shift of pain inhibition towards pain facilitation when testing DNIC with two heterotopic stimuli (heat pain as test stimulus and cold water pain as conditioning stimulus). However, the effect of morphine and ketamine on the central modulation of pain was assessed only in healthy volunteers. No knowledge is available on the effect of these agents on central modulation of pain in NP patients.

The main aims of our study are to (1) describe and compare OA in healthy volunteers and patients with chronic NP, and (2) assess whether age and sex differences exist in OA. The null hypotheses were that (1) there are no differences in OA in patients and healthy controls, and (2) there are no age and sex differences in OA.

Methods

Participants: volunteers, patients and controls
Three groups of subjects were recruited to participate in the study: volunteers, NP patients and control subjects who were age- and sex-matched to the pain patients. The study was approved by the Human Ethics Committee of the Leiden University Medical Center (Leiden, The Netherlands), and oral or written informed consent, as outlined by the Declaration of Helsinki, was obtained from all participants. For participants who were minors, consent was obtained from participants and their parents.

One hundred ten male and female volunteers were enrolled in the study after being selected from a convenience population (*i.e.* a convenience sample) and were in the age-range of 6 to 80 years. Ten patients with chronic NP were recruited. The patients had the diagnosis of isolated small-fiber neuropathy (SFN) and a pain score of at least 5 on an 11-point scale (0-10). Diagnosis was made when at least two of the following symptoms were present in legs and/or arms (in a stocking-glove distribution): (i) symmetrical dysesthesias or paresthesias; (ii) burning or painful feet with nighttime worsening of burning or pain; or (iii) tactile allodynia.[14,15] In addition, SFN had to be confirmed by neurological examination with normal tendon reflexes and absence of muscle weakness, and abnormal temperature thresholds had to be confirmed according to previously published criteria.[14] Exclusion criteria (for patients and controls) were: age younger than 18 years; presence or history of a severe medical disease (*e.g.* renal, liver, cardiac, vascular (incl. hypertension) or infectious disease); presence or history of a neurological and psychiatric disease (*e.g.* increased cranial pressure, epilepsy, psychosis); glaucoma; pregnancy; obesity (body mass index more than 30 kg/m^2), and use of strong opioid medication. Patients were allowed to continue the following pain medication: acetaminophen, non-steroidal anti-inflammatory drugs, tramadol, amitriptyline, gabapentin and pregabalin. Pain medication dosages were kept constant during the whole study period. Ten healthy male or female subjects who were not taking medication were enrolled in the study to serve as age and sex-matched controls to the patients. The control subjects were not recruited from the volunteer sample. A total of 130 subjects participated in the study.

Pain assessment and offset analgesia
The heat stimulus was applied on skin of the forearm where no painful sensations were present and the heat pain threshold was unaffected. Heat pain was induced with a 3 x 3 cm thermal probe positioned on the skin of the volar side of the non-dominant arm of the subject, using the Pathway Neurosensory Analyzer (Medoc Ltd., Ramat Yishai, Israel). A preset and computer-controlled temperature paradigm was used to generate a specific temperature pattern (Fig. 1). The subjects quantified the pain intensity of the heat pain stimulation using a slider on an electrical potentiometer connected to a computer. This allows continuous electronic monitoring of the visual analogue score (eVAS), which ranges from 0

(no pain) to 100 (worst pain imaginable). To overcome adaptation or sensitization, the volar side of the arm was divided into three zones. The thermode was moved from zone to zone between stimuli. The baseline temperature was set at 32 °C. Before testing, the thermode was tested and calibrated using a surface thermometer (K-Thermocouple thermometer, Hanna Instruments, Woonsocket, RI).

Offset analgesia was studied by applying a three-temperature paradigm as described by Grill and Coghill.[1] In NP patients and their matched controls, the study temperatures were individualized. To that end, a series of heat stimuli was applied; the stimulus duration was 10 seconds and there was 5-10 minutes between stimuli. The temperature of the first test stimulus was set at 42 °C. At 1 °C increments the lowest temperature that evoked an eVAS of 50 mm was identified and used in the study (*i.e.* the individual's test temperature). To test OA the temperature was ramped at 1.5 °C/s from baseline temperature to the individual's test temperature. The test temperature was kept constant for 5 seconds after which it was increased by 1 °C for 5 seconds and next decreased by 1 °C to the test temperature and kept constant for 20 seconds. Next, the temperature quickly returned (6 °C/s) to baseline. This temperature paradigm was applied three times with a 3-minute rest period between tests. For the volunteers from the convenience sample, the three-temperature paradigm was preset at 45 °C (for 5 seconds) – 46 °C (for 5 seconds) – 45 °C (for 20 seconds).

Study design

The study was registered in the Dutch trial register (www.trialregister.nl) as number NTR2005. In patients, the effect of S-ketamine and morphine on OA was tested using a single blind, placebo-controlled, randomized, crossover study design. Patients were randomized to receive a 1-hour placebo infusion (0.9% NaCl), a 1-hour infusion with S(+)-ketamine (total dose = 0.57 mg/kg; Ketanest-S, Pfizer BV, Capelle a/d IJssel, The Netherlands) or a 1-hour infusion with morphine (bolus of 0.05 mg/kg followed by 0.015 mg/kg for 1 hour; Morphine HCl, Pharmachemie BV, Haarlem, The Netherlands) on three separate occasions with 2-4 weeks between sessions. Each patient participated in all three sessions. Before treatment, the test temperature was determined after which three OA tests were performed (pretreatment or baseline studies). Then, treatment was given. After a 20-minute washout period the OA tests were repeated. Spontaneous pain scores were assessed using an 11-point numerical scale ranging from 0 (no pain) to 10 (most severe pain) before and after the infusion period. Controls and volunteers were studied on one occasion. In controls, after determination of the individual test temperature, three OA tests were obtained. In volunteers, after a brief explanation of the test, one OA test was performed, although in some this was preceded by a test experiment to familiarize the subject with the test procedure. In patients, during ketamine, morphine and placebo treatment, the occurrence of nausea and vomiting (yes/no) and the occurrence of drug high was scored on an 11-point numerical scale ranging from 0 (no effect) to 10 (most severe effect).

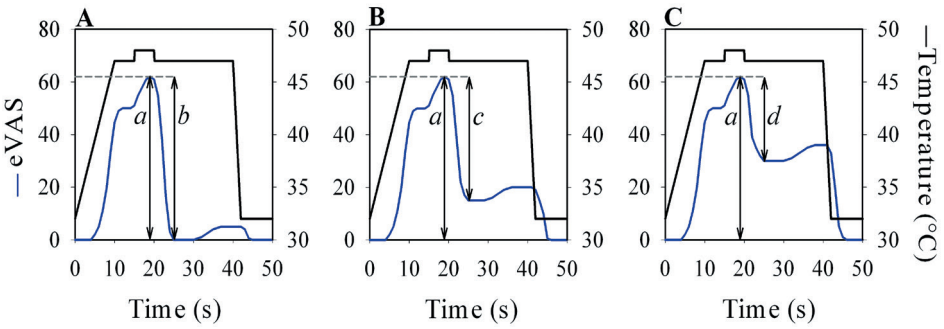

Figure 1. *Calculation of the magnitude of offset analgesia (OA) relative to peak effect (ΔeVASc), where eVAS = electronic visual analogue score, ΔeVAS = the decrease in eVAS from peak eVAS value to the eVAS nadir after the 1 °C decrease of the test stimulus, and ΔeVASc = ΔeVAS corrected for the value of the peak eVAS (ΔeVASc = (ΔeVAS/[peak eVAS])*100).* ***A.*** *Peak eVAS = a; reduction in eVAS after the 1 °C decrease in noxious heat stimulation (ΔeVAS) = b; ΔeVASc = (b/a)*100% = 100% because a = b.* ***B.*** *Peak eVAS = a; ΔeVAS = c; ΔeVASc = (c/a)*100% = 75% because c = 0.75a.* ***C.*** *Peak eVAS = a; ΔeVAS = d; ΔeVASc = (d/a)*100% = 50% because d = 0.5a.*

Data and statistical analyses

The eVAS data were averaged over 1-second periods. To quantify OA, the decrease in eVAS from peak eVAS value to the eVAS nadir after the 1 °C decrease of the test stimulus was measured (ΔeVAS), corrected for the value of the peak eVAS (ΔeVASc = (ΔeVAS/[peak eVAS])*100) (*i.e.* correction for the variation in the peak response among participants as explained in figure 1).[4]

In volunteers, five age cohorts were created: 6-12, 13-19, 20-39, 40-59 and 60-80 years. The effect of age (by cohort) and sex on ΔeVASc (ΔeVAS corrected for peak effect) was tested by one-way analysis of variance (ANOVA) and unpaired two-tailed t-test, respectively. To compare the ΔeVASc of patients with the responses of their age matched controls, the predrug patient data were compared with the control data using an unpaired two-tailed t-test. Treatment effect (placebo, ketamine and morphine) on ΔeVASc and spontaneous pain reporting was tested using a one-way ANOVA and post-hoc Bonferroni or t-tests. A receiver operating characteristic curve was calculated to get an indication of the cutoff value for a healthy value of ΔeVASc *versus* a value observed in NP patients. Statistical analysis was performed in SigmaPlot version 11 for Windows (Systat Software Inc., Chicago ILL). *p*-values < 0.05 were considered significant. Data are presented as mean ± SEM and 95% confidence intervals (CI) unless otherwise stated.

Results

OA in volunteers

The eVAS responses varied among the participants irrespective of age and sex. Using the preset temperature paradigm, eVAS-responses greater than 0 were present in 78 of 110 (70%) healthy volunteers. Eighteen of the 65 men (28%) and

14 of the women (31%) had no pain response to the fixed heat stimulus train. These individuals were distributed equally among the different age cohorts, and their data were not included in the analysis. For presentation purposes only, the data relative to peak eVAS responses (eVAS/[peak eVAS]*100%) are presented per age cohort in figure 2. To get an impression of the variability in the data, ΔeVAS (not corrected for peak value) per age cohort are plotted in figure 3A. It shows a trend towards a decrease in the ΔeVAS with increasing age and noticeably large variability in the response in the oldest cohort: 6-12 years: ΔeVAS = 66.1 ± 6.9 mm (95% CI: 51.6 – 80.7 mm); 13-19 years: 47.6 ± 7.7 mm (31.2 – 64.0 mm); 20-39 years: 45.3 ± 7.1 mm (29.9 – 60.8 mm); 40-59 years: 51.8 ± 4.5 mm (42.6 – 61.0 mm); and 60-80 years: 34.1 ± 9.0 mm (12.0 – 56.2 mm) (ANOVA main effect $p = 0.054$). The mean ΔeVASc of the total population that displayed a pain response greater than zero (n = 78) was 97 ± 1% (95% CI: 95-99%). No difference was observed in ΔeVASc scores between the age cohorts (Fig. 3B): 6-12 years: 92 ± 4% (85 – 100%; n = 17), 13-19 years: 98 ± 1% (96 – 100%; n = 17), 20-39 years: 96 ± 2% (92 – 100%; n = 14), 40-59 years: 99 ± 1% (96 – 100%; n = 23) and 60-80 years: 97 ± 3% (89 – 100%; n = 7) (ANOVA main effect $p = 0.54$). The larger variability observed in the age cohort 60-80 years is related to the small number of participants in this group rather than to an age effect. Male (n = 47) and female volunteers (n = 31) showed similar eVAS responses, with no difference in peak eVAS values: men 51.5 ± 4.0 mm (43.3 – 59.6 mm) and women 55.8 ± 5.2 mm (45.1 – 66.6

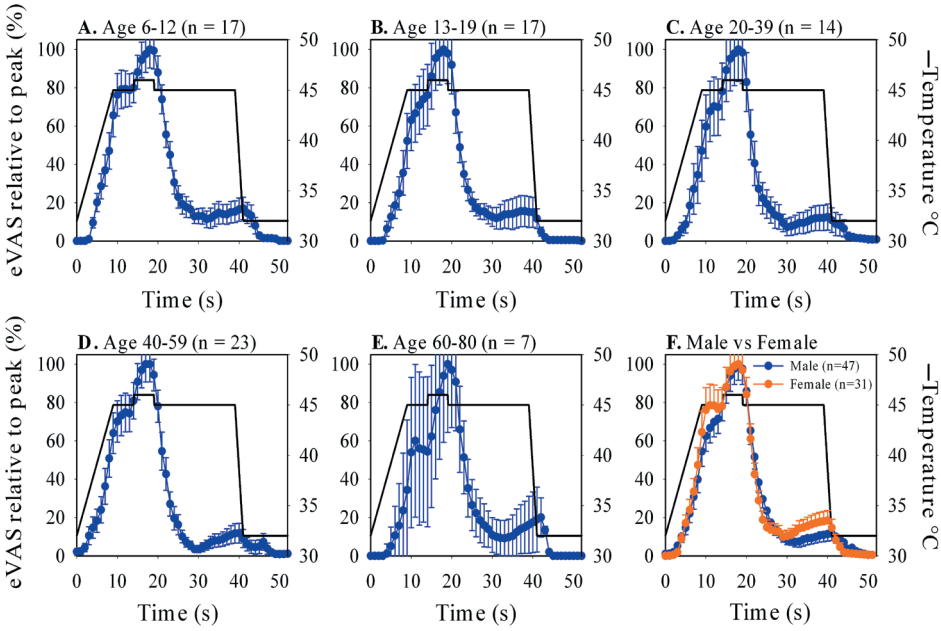

Figure 2. *Offset analgesia (OA) responses to a heat stimulus paradigm (black line) in a random study population of healthy volunteers of different age categories* ***(A-E).*** *In panel* ***F*** *the effect of the offset analgesia responses in men (blue) and women (orange) are compared. Values are mean ± SEM.*

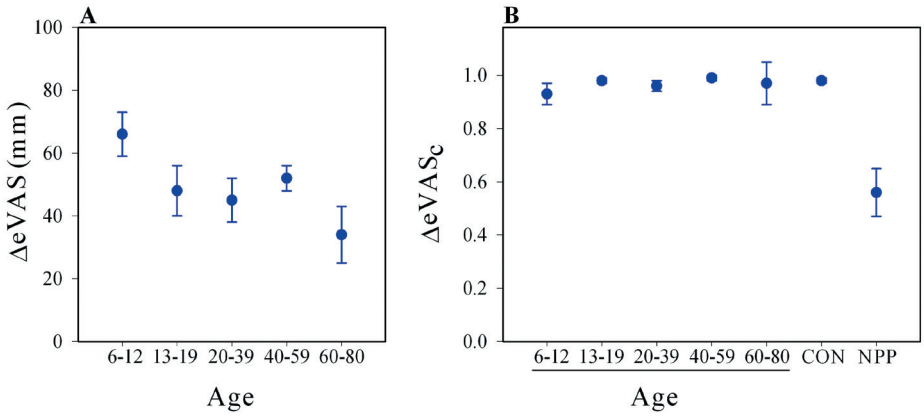

Figure 3. A. *Absolute magnitude of offset analgesia (OA) in mm (ΔeVAS) of the different age categories. The age effect was not significant (p = 0.054).* ***B.*** *OA response relative to peak effect (ΔeVASc) of the different age categories. ΔeVASc scores range from 92 to 99% among age cohorts (not significant). In addition, the data from healthy controls (CON) and neuropathic pain patients (NPP) are added. Values are mean ± SEM. eVAS = electronic visual analogue score; ΔeVAS = the decrease in eVAS from peak eVAS value to the eVAS nadir after the 1 °C decrease of the test stimulus; ΔeVASc = ΔeVAS corrected for the value of the peak eVAS (ΔeVASc = (ΔeVAS/[peak eVAS])*100).*

mm; $p = 0.57$; Fig. 2). However, a small but significant difference in difference in ΔeVASc was observed: male 98% ± 1 (97 – 100%) and female 94 ± 2% (90 – 98%; $p = 0.007$). This sex effect was age-dependent with absence of a difference in young volunteers (age group 6-19 years: male ΔeVASc 98 ± 1% *versus* female 93 ± 2%; $p = 0.185$) but persistent differences in the 20+ cohorts (20-80 years: male ΔeVASc 99 ± 1% *versus* female 95 ± 2%; $p = 0.002$; Fig. 4).

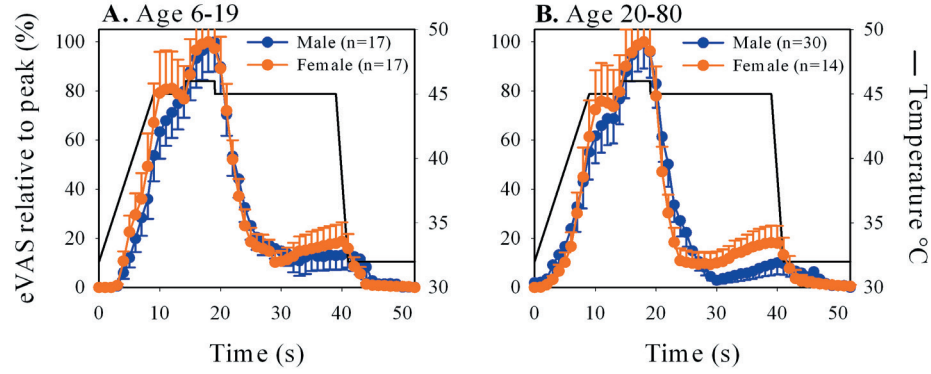

Figure 4. A. *Offset analgesia (OA) responses in male versus female volunteers in the age category of 6-19 years.* ***B.*** *OA responses in male versus female volunteers in the age category of 20-80 years. Responses are percentage of peak response. All value are mean ± SEM. eVAS = electronic visual analogue score.*

Table 1. *Characteristics of healthy controls and neuropathic pain patients*

	Healthy controls	Neuropathic pain patients	*p*-value
n (M/F)	10 (2/8)	10 (2/8)	0.957
Age (year)	48.3 ± 3.3	54.4 ± 4.2	0.268
Underlying disease		Diabetes mellitus *n* = 4	
		Sarcoidosis n = 2	
		Sjögren's disease *n* = 1	
		Unknown cause *n* = 3	
Test temperature (°C)	44.9 ± 0.7	45.0 ± 0.5	0.908
Ketamine		45.2 ± 0.5	
Morphine		45.1 ± 0.4	0.888
Placebo		44.8 ± 0.6	

Values are mean ± SEM.

OA in neuropathic pain patients *versus* age-matched healthy controls

Baseline characteristics of NP patients and age- and sex-matched controls are listed in table 1. The underlying disease causing NP varied, with four patients having neuropathic pain related to diabetes mellitus type 2, two related to sarcoidosis, one to Sjögren disease and three with NP of unknown origin. The extremities affected by the SFN were the two lower extremities in four patients and feet or legs together with hands in six patients. The patients used the following co-medication during the study: acetominophen, non-steroidal anti-inflammatory drugs, tramadol, gabapentin, pregabalin and amitriptyline. All participants

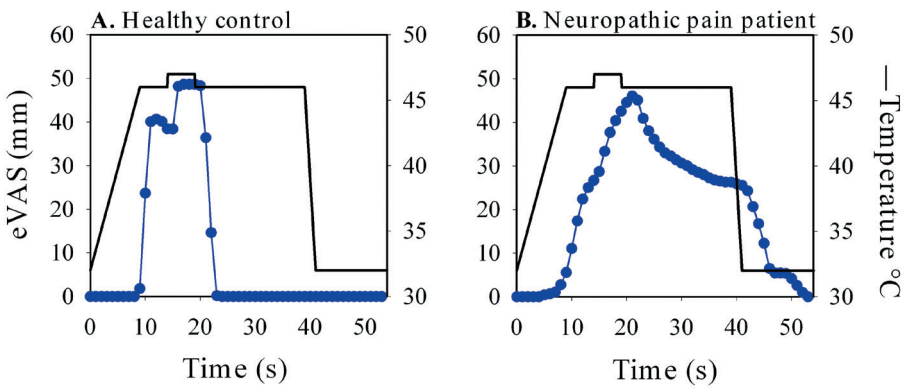

Figure 5. *Example of the offset analgesia (OA) response in a* ***A.*** *healthy control and a* ***B.*** *neuropathic pain (NP) patient. The healthy control shows OA with a ΔeVASc of 100%. The NP patient clearly shows an aberrant response to the heat stimulus paradigm (black line) with a delayed onset and offset in eVAS response. ΔeVASc was approximately 25%. eVAS = electronic visual analogue score; ΔeVAS = the decrease in eVAS from peak eVAS value to the eVAS nadir after the 1 °C decrease of the test stimulus; ΔeVASc = ΔeVAS corrected for the value of the peak eVAS (ΔeVASc = (ΔeVAS/[peak eVAS])*100).*

Table 2. *ΔeVASc values for the individual neuropathic pain patients and age- and sex-matched controls*

Neuropathic pain patients	ΔeVASc (%)	Age- and sex-matched controls	ΔeVASc (%)
id P001	33	id C011	93
id P002	85	id C012	93
id P003	26	id C013	92
id P004	42	id C014	100
id P005	0	id C015	100
id P006	76	id C016	100
id P007	88	id C017	100
id P008	98	id C018	100
id P009	34	id C019	100
id P010	75	id C020	100
Mean (95% CI)	56 (38-73)	Mean (95% CI)	98 (96-100)

CI = confidence interval; ΔeVASc = the corrected decrease in electronic visual analogue score from peak pain score; eVAS = electronic visual analogue score; id = identification code.

(patients and controls) completed the protocol without unexpected side effects with no measurements lost to observation or analysis. Between groups no significant differences were observed in test temperatures on the volar side of the arm to reach eVAS values of 50 mm: 45.0 ± 0.5 °C (43.7 – 46.3 °C) and 44.9 ± 0.7 °C (43.0 – 46.8 °C) for NP patients and age-matched healthy controls, respectively ($p = 0.91$).

Examples of eVAS responses to the OA temperature paradigm are given in figure 5. It shows that a healthy control displays a rapid increase in eVAS in response to increasing heat stimulation, followed by a rapid decline to an eVAS of zero when the temperature is decreased by 1 °C from 48 to 47 °C. In contrast, a 'typical' NP patient shows a delayed increase in eVAS with increasing heat stimulation and a delayed and relatively small decline in eVAS when the temperature is decreased by 1 °C from 48 to 47 °C. The eVAS response remains approximately 50% of peak eVAS at the end of the 30-second heat stimulation period. The mean eVAS responses for the two groups are given in figure 6, showing the distinct differences in response to the three-temperature paradigm. No difference was observed in mean peak eVAS: patients 47.9 ± 4.5 mm (95% CI: 37.7 – 58.2 mm) and controls 53.6 ± 5.4 mm (41.3 – 65.8 mm; $p = 0.44$). Most striking is the delayed and smaller decrease in eVAS upon the 1 °C decrease in test temperature in patients compared to controls. In control subjects ΔeVASc was significantly greater than in patients with pain; the ΔeVASc averaged to 98 ± 1% (96 – 100%) in controls *versus* 56 ± 9% (38 – 73%) in NP patients (Fig. 6, $p < 0.001$). Individual values of the eVASc of patients and controls are given in table 2.

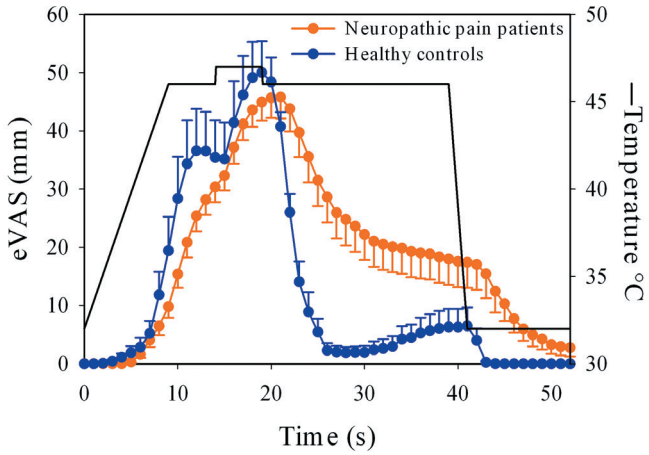

Figure 6. *eVAS responses to the three-temperature paradigm (black line) in neuropathic pain patients (n = 10, orange line) and age-matched healthy controls (n = 10, blue line). Values are mean ± SEM. eVAS = electronic visual analogue score.*

A receiver-operating characteristic curve was constructed to determine a ΔeVASc cutoff value between healthy subjects (volunteers and controls, n = 88) and patients with SFN (Fig. 7). A cutoff value of 0.88 (88%) yields a sensitivity of 90% (95% CI: 56 – 99%) and specificity of 91% (83 – 96%). The area-under-the-receiver-operating characteristic curve is 0.96 ± 0.02 (± SE; 95% CI: 0.91 – 0.99, $p < 0.001$).

Treatment effects in NP patients

All neuropathic pain patients received a 1-hour intravenous infusion with ketamine, morphine and placebo. Nausea occurred in four patients receiving ketamine, two of whom vomited. After morphine nausea occurred in seven patients,

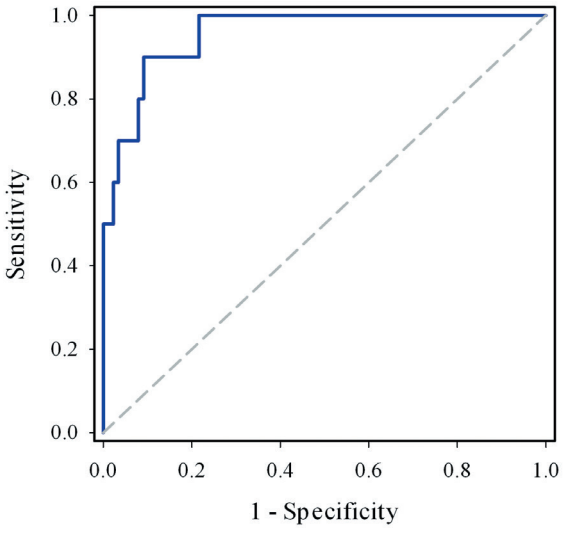

Figure 7. *Receiver operating characteristic (ROC) curve constructed to determine a ΔeVASc cutoff value between healthy subjects (volunteers and controls, n = 88) and patients with neuropathic pain. eVAS = electronic visual analogue scale.*

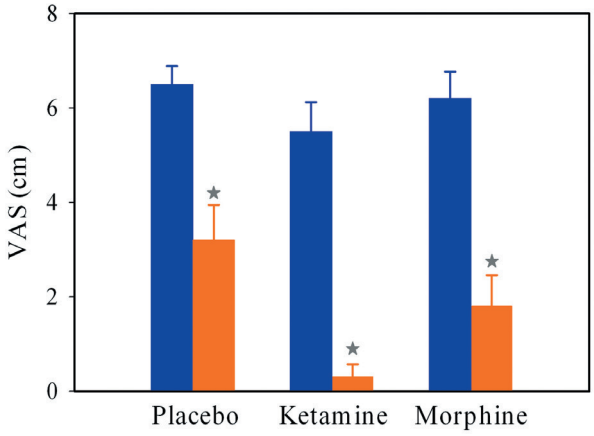

Figure 8. *Effect of placebo, ketamine and morphine treatment on spontaneous pain scores in neuropathic pain patients. The blue bars represent the pain scores before treatment, the orange bars represent the scores directly after treatment. All three treatments produced significant pain relief ($p < 0.01$). Values are mean ± SEM. VAS = visual analogue scale.*

of whom four vomited. No nausea or vomiting was seen in patients receiving placebo. At the end of infusion, the mean drug high scores were 7.2 ± 0.6 (6.0 – 8.4), 2.4 ± 0.5 (1.4 – 3.4) and 0.4 ± 0.2 (0 – 0.8) for ketamine, morphine and placebo, respectively. The NP spontaneous pain scores were 5.5 ± 0.6 (4.3 – 6.8) before and 0.3 ± 0.3 (-0.3 – 0.8) after ketamine treatment ($p < 0.001$), 6.2 ± 0.6 (5.0 – 7.4) before and 1.8 ± 0.66 (0.5 – 3.1) after morphine treatment ($p = 0.002$) and 6.5 ± 0.4 (5.7 – 7.3) before and 3.2 ± 0.75 (1.7 – 4.7) after placebo treatment ($p = 0.004$). All spontaneous pain scores were significantly reduced after the infusion, irrespective of the treatment; however, the greatest pain relief was seen after ketamine treatment (Fig. 8). None of the treatments influenced the eVAS responses to the three-temperature paradigm (Fig. 9). Mean ΔeVASc scores were 51 ± 1% (49 – 53%), 55 ± 1% (53 – 57%) and 34 ± 1% (32 – 36%) for placebo, ketamine and morphine treatment, respectively ($p = 0.51$).

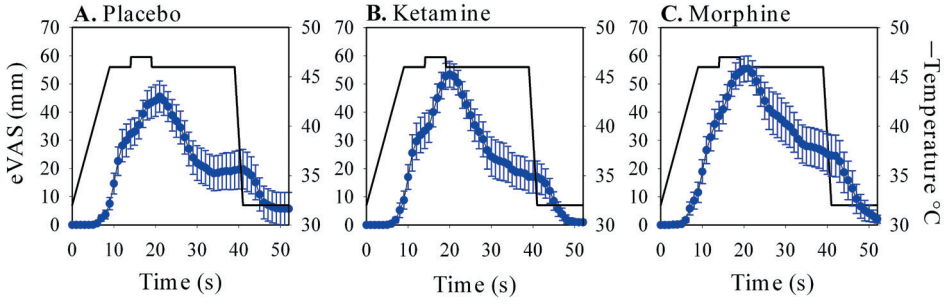

Figure 9. *Effect of **A.** placebo, **B.** ketamine and morphine **C.** treatment on the eVAS responses in neuropathic pain patients. ΔeVASc scores were not different among treatments ($p = 0.51$). Values are mean ± SEM. eVAS = electronic visual analogue score; ΔeVAS = the decrease in eVAS from peak eVAS value to the eVAS nadir after the 1 °C decrease of the test stimulus; ΔeVASc = ΔeVAS corrected for the value of the peak eVAS (ΔeVASc = (ΔeVAS/[peak eVAS])*100).*

Discussion

Offset analgesia

Offset analgesia, first described in 2002, is the phenomenon where a disproportionally large amount of analgesia is demonstrated during a slight decrease in noxious heat stimulation.[1-3] The large reduction in pain experience and short duration of effect distinguishes OA from simple stimulus adaptation. OA is considered a mechanism of endogenous pain modulation, akin DNIC (which is characterized by central inhibition of a focal pain stimulus by administering a second noxious stimulus at a remote area).[4,7] The phenomenon of OA was recently related to supraspinal modulatory mechanisms. Functional magnetic resonance imaging studies in healthy volunteers showed activation of periaqueductal gray and rostral ventromedial medulla during offset analgesia.[5,6] Spinal mechanisms also may be involved (*e.g.* a process related to the intrinsic response properties of primary afferent neurons within the dorsal horn). For example, Darian-Smith et al.[16] measured the response of warm fibers during a 39 °C stimulus to cooling pulses of graded intensity. They observed that cooling pulses greater than 1 °C suppressed discharge of the warmth-sensitive fibers. A similar mechanism may be involved in the OA experiments in addition to the central mechanisms involved. Additional studies examining the behavior of the primary afferent neurons are needed to increase the understanding of the mechanisms of OA.

Healthy volunteers

Offset analgesia was tested in volunteers from age 6 years on. The youngest age cohort (6-12 years) showed robust OA, with ΔeVASc mean scores of 92%, which is not different from values observed in other age cohorts. This suggests that OA is fully developed at the age of 6 years and does not undergo further maturation. Testing OA at an even younger age is not possible because the full cooperation of the subject is required. Absolute changes in VAS score were variable (Fig. 3A). We relate this to the well-known large variability in VAS responses to a standardized heat stimulus that we observed in our population of volunteers. In fact, about 29% of participants felt the stimulus train but experienced no pain at any point of the test (VAS remained 0 during the 30-second test period). An approach to reduce variability would have been to assess individual test temperatures (as was performed in controls and SFN patients). This was considered but rejected due to the time burden and consequently the possibly reduced compliance of the participants.

A trend in decreasing ΔeVAS scores was observed with increasing age (Fig. 3A). This was related predominantly to a smaller peak eVAS score in the oldest age cohort. In contrast, no effect of age was observed on ΔeVASc (Fig. 3B). Age effects have been described for DNIC with a decrease in inhibition with increasing age, an effect that starts at middle age.[17,18] It seems from our data that OA is more robust than DNIC over the years; however, one needs to consider that although a large number of volunteers was tested, some age cohorts were relatively small and we cannot exclude that this small sample size influenced the outcome of the

study at some level.

We observed no sex differences in peak eVAS in response to the fixed temperature stimuli (VAS: men 51.5, women 55.8 mm, $p = 0.57$) but did observe a small but significant greater OA in men (ΔeVASc: men 98%, women 94%, $p < 0.01$). This small difference cannot be explained by difference in peak eVAS and seems to be of limited clinical or mechanistic relevance. A systemic review on sex differences in DNIC describes a significantly more efficient DNIC in men than in women with a mean female-to-male ratio of 0.54, much smaller than observed here (OA female/male ratio = 0.96).

NP patients

Our patients were affected by SFN, which affects myelinated Aδ and unmyelinated C-fibers that innervate the skin and mediate pain and thermal sensations.[14,15] Patients experience NP in the limbs in a distal-to-proximal gradient. SFN occurs in a variety of conditions including diabetes, sarcoidosis and Sjögren disease,[15] as was diagnosed in seven patients in the current study. Three patients had SFN without an underlying diagnosis. We were careful not to test the OA responses on 'diseased' skin areas. Indeed, the observation that eVAS responses of 50 mm were obtained at temperatures not different from those in age and sex-matched controls (table 1) is an indication that nociceptive perception was not affected in the test areas chosen. Still, we cannot exclude that we may have overlooked some 'preclinical' changes of the nociceptive fibers in the skin of the test areas. OA responses were reduced or absent with delayed offset and relatively small decreases in VAS scores after the minor decrease in temperature (Figs. 5 and 6). Pain was scored as would be expected from the decrease in temperature, instead of a disproportionally large decrease in pain scores, as was observed in healthy controls. On average, the ΔeVASc was 56% in patients *versus* 98% in controls. The receiver-operating characteristic analysis yielded a ΔeVASc cutoff of 0.88 (88%, Fig. 7) with a sensitivity and specificity of 90%, indicating that OA is reliably discernable between NP patients and healthy volunteers. The alterations in the OA responses observed in the patients indicate the inability to modulate changes in pain stimulation with perseverance of pain perception where healthy subjects display strong analgesia.

The study included a rather small number of patients, so we cannot exclude a type I statistical error. However, of the 90 OA control tests performed in the patients, OA was reduced or absent in 90%. In addition, in a distinct set of patients with complex regional pain syndrome type 1, similar reduced OA responses were observed (oral communication June 6, 2011; Marieke Niesters MD MSc, Department of Anesthesiology, Leiden University Medical Center, Leiden, The Netherlands), suggestive of a common defect in OA responses in patients with chronic pain. We cannot exclude the possibility that this OA response was an effect of the medications used by our patients. Although no systematic effect of any medication was observed (data not shown), the numbers are small and no definite conclusions may be drawn. We are not aware of any studies showing an

effect of medication (including those used in the current study) on the magnitude and development of OA responses. Moreover, in line with our study, most published data on quantitative sensory testing in NP patients is with patients receiving medication. Additional studies are required to assess the effect of drugs such as pregabalin, gabapentin, antidepressants and anticonvulsants on OA responses.

The mechanism of the differences in OA responses between NP patients and healthy controls was not addressed in our study. OA in our study may have been affected at peripheral (due to 'preclinical' peripheral nerve damage) and/or central sites (*e.g.* spinal cord and supraspinal sites). There is evidence that various chronic pain states are linked to dysfunctional endogenous pain control, as tested by DNIC (such as complex regional pain syndrome type 1, irritable bowel syndrome, fibromyalgia, temperomandibular disorder, rheumatoid arthritis and chronic pancreatitis).[8-11] It is currently unknown whether DNIC and OA are both dysfunctional in NP patients. The large placebo effect that we observed is of interest here (Fig. 7), since top-down modulatory pathways underlie the phenomenon of placebo-induced analgesia.[8] Neuroimaging techniques established that the placebo response is mediated via cortical and subcortical regions also involved in endogenous pain control.[20] This suggests that central pain pathways common to OA and placebo responses remained intact in our set of SFN patients. This then implies a peripheral rather than a central mechanism involved in the altered OA responses in chronic pain and SFN. In contrast, the altered OA responses were obtained at (clinically) normal skin with normal nociceptive sensations, suggesting a more generalized and central origin of the altered OA responses in our patients. Additional studies are required to assess the location of the altered OA responses in NP patients.

Treatment effects

All treatments caused an analgesic effect on spontaneous pain scores with the largest effect observed for ketamine followed by morphine and placebo (Fig. 8). The analgesic effect from ketamine persisted for at least 24 hours, whereas those of morphine and ketamine effect lasted approximately 2 hours (data not shown). A prolonged analgesic effect of ketamine in NP states has been described before and is related to blockade of sensitized *N*-methyl-D-aspartate-receptors by ketamine.[21,22] In contrast to spontaneous pain, no effect was observed on OA responses from treatment with ketamine, morphine or placebo (Fig. 9). In healthy volunteers OA is similarly unaffected by ketamine.[4] These data indicate that the *N*-methyl-D-aspartate and μ-opioid receptors are less likely to be involved in OA mechanisms at central or peripheral sites. Alternatively, OA restoration may require long-term drug treatment.

Conclusions

In conclusion, we showed the presence of OA in a large healthy study population. OA was reduced or absent in patients with chronic NP with SFN that remained unaffected by treatment with ketamine, morphine or placebo. The abnormal

OA responses in patients with chronic pain indicate their inability to modulate changes in pain stimulation with perseverance of pain perception where healthy subjects display strong analgesia. Whether the altered OA responses contribute to the pain being chronic or are a consequence of the chronic pain process remains unknown and requires additional study.

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Chapter 4

Influence of Ketamine and Morphine on Descending Pain Modulation in Chronic Pain Patients

A randomized placebo-controlled proof-of-concept study

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Introduction

Normal pain processing involves the modulation of pain signals in the central nervous system by activation of endogenous inhibitory (analgesic) or facilitatory (algesic) mechanisms.[1-3] These modulatory mechanisms allow optimal functionality in response to an acute painful insult.[4] For example, activation of endogenous inhibition of pain allows for an evolutionary well-preserved fight or flight response;[5,6] facilitation of pain responses puts the emphasis on tissue damage and forces an individual to seek rest and/or medical attention.[6] In recent years, various experimental (surrogate) expressions of endogenous modulation of pain gained increasing interest in chronic pain research. Conditioned pain modulation (CPM, formerly known as diffuse noxious inhibitory controls or DNIC) has been investigated most intensively and induces central inhibition of a focal pain stimulus by administering a second noxious stimulus at a remote area.[7,8] In contrast to animals, where endogenous inhibition involves activation of a spinal-medullary-spinal feedback loops (*i.e.* DNIC),[9] in humans more complex supraspinal mechanisms also plays an important role (*i.e.* CPM).[7,10] Absent or impaired CPM responses have been observed in several chronic pain states.[8,11-13] Defects in CPM possibly reflect the inability to engage descending inhibition, either causing the perseverance of pain symptoms or possibly even leading to the development of chronic pain. For example, recent animal data show that less efficient descending inhibition is associated with a high probability of chronic pain development following peripheral nerve injury.[14,15]

Few studies address the effect of analgesic medication on CPM responses in chronic pain patients. It can be hypothesized that chronic pain patients may benefit from analgesics that enhance descending inhibition as measured by CPM.[14,16] A recent study showed that duloxetine-induced improvement of CPM responses correlated with drug efficacy in patients with painful diabetic neuropathy.[16] Hence, the positive effect of analgesics on CPM might have a predictive effect on their ability to cause (long-term) analgesia. In the current study, we assessed the effect of morphine and ketamine on CPM responses in a group of patients with chronic painful peripheral neuropathy. Both treatments are effective in chronic pain patients, but their effects on CPM responses have only been tested in volunteers but not in chronic pain patients. We hypothesized that both drugs enhance CPM responses and that the magnitude of these responses correlates positively with the magnitude and duration of spontaneous pain relief.

Methods

Approval of the study was obtained from the local human ethics committee, and written informed consent was obtained from all subjects. The study was registered in the Dutch trial register (www.trialregister.nl) under trial number NTR2005.

Subjects
Ten patients with chronic pain were recruited to participate in the study. They were diagnosed with chronic peripheral neuropathic pain and included on the basis of their symptoms, the results of quantitative sensory testing (QST) and a neurological examination.[17-19] Subjects were required to have at least two of the following symptoms in legs, arms or both (in a stocking-glove distribution): (i) symmetrical dysesthesias or paresthesias; (ii) burning or painful feet with night-time worsening; or (iii) peripheral tactile allodynia. With respect to the QST the patients were included if they had an abnormal warm and cold detection threshold, an abnormal warm and cold pain threshold, or allodynia.

Before participation, all subjects underwent a physical examination. Exclusion criteria for the study were: age < 18 years or > 80 years; presence or history of a medical disease such as renal, cardiac, vascular (including hypertension) or infectious disease; presence or history of a neurological and psychiatric disease such as increased cranial pressure, epilepsy or psychosis; glaucoma; pregnancy; obesity (body mass index > 30 kg/m^2); or the use of strong opioid medication. Subjects were allowed to continue the following pain medications as long as they used a constant dose for at least 3 months before the start of the study and could be kept constant during the whole study period: acetaminophen, non-steroidal anti-inflammatory drugs, amitriptyline, gabapentin and pregabalin.

Pain assessment and CPM
As examined by Pud and collegues,[7] noxious cold water is the most used pain modality as a conditioning stimulus combined with different pain modalities as test stimulus. We applied a heat pain stimulus as test stimulus and cold water as conditioning stimulus, in agreement with earlier studies from our laboratory and from King and colleagues.[8,20] The test stimulus was a noxious thermal stimulus applied to clinically normal skin of the volar side of the non-dominant forearm (with normal warm and cold thresholds). The skin was stimulated with a 3 × 3 cm thermal probe of the Pathway Neurosensory Analyzer (Medoc Ltd., Ramat Yishai, Israel). During the heat pain stimulus, subjects continuously quantified the pain intensity level of the stimulus using a slider on a computerized potentiometer that ranged from 0 (no pain) to 100 (worst pain imaginable), which allowed continuous, electronic monitoring of the visual analogue scale (eVAS). To overcome sensitization, a 3-minute interval was incorporated between tests and the volar side of the arm was divided into three zones.[8] The thermode was moved from zone to zone between stimuli. The test stimulus was obtained by gradually increasing the thermode temperature from baseline (32 °C) to the test temperature (at 1.5 °C/s). When the test temperature was reached it remained constant for 30 seconds. Next, the temperature rapidly decreased (at 6 °C/s) to baseline.

Before the test, individual test and conditioning temperatures were determined. For the test stimulus, a series of heat stimuli was applied. Baseline temperature was set at 32 °C after which the temperature increased by 1.5 °C/s to tempera-

tures ranging from 42 °C to 49 °C for 10 seconds. The temperature evoking an eVAS of at least 50 mm was set as test temperature and used during the remainder of the study for the experimental stimulus. Before testing, the thermode was calibrated using a surface thermometer (K-Thermocouple thermometer, Hanna Instruments, Woonsocket, RI).

The conditioning stimulus was cold water immersion in a cold-water bath which was filled and temperature adjusted using a rapid-water cooling system (IcyDip, IcySolutions BV, Delft, The Netherlands).[20] The subject's foot and lower leg was immersed into the cold water reservoir, which could be set at different temperatures ranging from 6 °C to 18 °C. The temperature that produced an eVAS of at least 30 mm was used in the study for the conditioning stimulus. After exposure to cold water, the subject's extremity was immediately warmed to normal temperature using the warm water reservoir of the IcyDip system.

To measure CPM, eVAS responses to the test stimulus were obtained without (n = 3) and with the conditioning stimulus (n = 3).[8,20] The conditioning stimulus was applied 25 seconds before the start of the test stimulus and ended simultaneously with the end of the test stimulus. The subject was instructed to only rate the pain intensity level of the test stimulus with the eVAS slider.

Study design
Each subject visited the laboratory on three days, at least 2 weeks apart, in which placebo, morphine and ketamine were tested using a double-blind, randomized cross-over study design. Initially, CPM was measured without treatment (baseline values). After a break, intravenous treatment was given (infusion duration 1 hour), and 20 minutes later, the CPM responses were repeated. Treatments were as follows: (A) a 1-hour intravenous infusion of 0.57 mg/kg S(+)-ketamine (Ketanest-S, Pfizer BV, Capelle a/d IJssel, The Netherlands); (B) morphine bolus of 0.05 mg/kg followed by 0.015 mg/kg per hour for 1 hour (Morphine HCl, Pharmachemie BV, Haarlem, The Netherlands); and (C) a 1-hour placebo (0.9% NaCl) infusion.

Disease-related pain
The effect of treatment on disease-related pain or spontaneous pain scores was measured after treatment on a 0 – 10 numerical rating scale (NRS). Subjects were contacted after their treatment to determine the duration of pain relief. An arbitrary distinction was made between pain relief lasting 0 – 6 hours post treatment, 6 – 12 hours post treatment and 12 – 24 hours post treatment.

Data and statistical analyses
The difference between the eVAS response to the test stimulus without and with conditioning stimulus is the generated CPM. The eVAS data were averaged over 1-second periods. To quantify CPM, the area under the curve (AUC) of each eVAS response was calculated. For analysis of the relative amount of CPM, the mean of the three AUC responses per condition was calculated (*i.e.* the mean of

the 3 AUCs without conditioning stimulus and the 3 AUCs with the conditioning stimulus). The percentage of CPM (CPM%) was calculated as: CPM% = [(mean AUC without CS stimulus – mean AUC with CS) / (mean AUC without CS)] × 100 (*i.e.* corrected for the variation in the magnitude of the peak response between sessions and between subjects). The drug study was powered to detect a significant difference between treatment effects on CPM%. Assuming a difference between groups of 20% (derived from previous data)[20] with SD 10%, $\alpha = 0.05$ and power = 0.9, we estimated a groups size of 10 (SigmaPlot v12, Systat Software Inc., Chicago IL, USA). A linear mixed model was used to compare the AUCs of the eVAS responses with and without conditioning stimulus within each experimental session. The effect of treatment on CPM% and spontaneous pain scores was tested by one-way analysis of variance with post-hoc Bonferroni correction. Statistical analysis was performed in SigmaPlot version 12.0 for Windows (Systat Software Inc., Chicago IL). *p*-values < 0.05 were considered significant. Data are presented as mean ± SEM unless otherwise stated.

Results

Subjects

All 10 subjects completed the protocol without major side effects. The study population included two men, eight women, and had a mean age of 54.4 ± 4.2 years

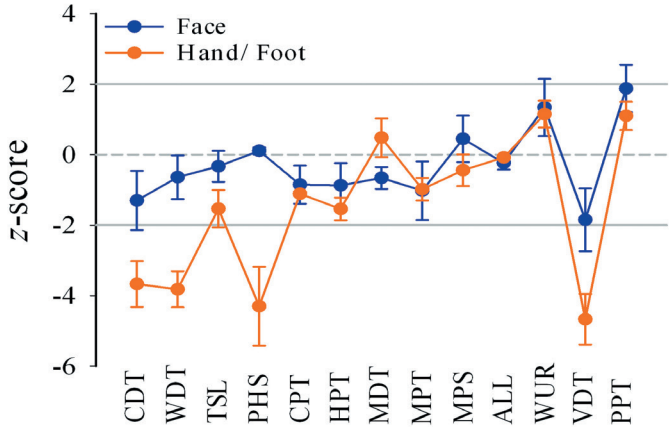

Figure 1. *Quantitative sensory testing. The test site was the site most affected by pain (either hand or foot; orange symbols), the control site was the face (blue symbols). Data are the population mean z-scores ± SEM. Z-scores were calculated in relation to a population of healthy subjects as determined by Rolke and collegues.[39] The horizontal broken lines indicate the +2 and -2 z-score boundaries. A specific QST test is considered abnormal if the test value lies above the upper or below the lower boundary. CDT: cold detection threshold; WDT: warm detection threshold; TSL: thermal sensory limen; PHS: paradoxal heat sensation; CPT: cold pain threshold; HPT: heat pain threshold; MDT: mechanical detection threshold; MPT: mechanical pain threshold; MPS: mechanical pain sensitivity; ALL: dynamic mechanical allodynia; WUR: windup ratio; VDT: vibration detection threshold; PPT: pressure pain threshold.*

and mean weight of 83.6 ± 7.6 kg. All suffered from chronic neuropathy with signs of mixed small and large fiber neuropathy on the QST (significant abnormalities in cold detection threshold, warm detection threshold, paradoxal heat sensation and vibration detection threshold (Fig. 1). The patients were diagnosed with diabetes mellitus (n = 4), sarcoidosis (n = 2) and Sjögren's syndrome (n = 1). In three subjects the origin of the pain was unknown. Feet were affected in all subjects; in four subjects the hands were affected as well. Subjects used the following medication during the study: acetaminophen, non-steroidal anti-inflammatory drugs, gabapentin, pregabalin and amitriptyline.

CPM responses

Average test and conditioning stimulus temperatures were 45.1 ± 0.1 °C and 9.8 ± 1.0 °C, respectively. At baseline, the average eVAS scores were 43.0 ± 2.4 mm and after treatment 49.0 ± 3.4 mm, 50.1 ± 2.9 mm and 51.1 ± 2.9 mm for ketamine, morphine and placebo, respectively. No significant CPM responses were detected before treatment: AUC without conditioning stimulus 1180 ± 71 mm·sec compared with AUC with conditioning stimulus 1080 ± 79 mm·sec ($p > 0.05$). After all three treatments, significant CPM was detected indicating an inhibitory effect of the cold water conditioning stimulus on the experimental heat pain stimulus. Placebo AUCs were reduced by the conditioning stimulus from 1241 ± 209 to 862 ± 135 mm·sec ($p = 0.001$); morphine AUCs were reduced from 1503 ± 224 to 1049 ± 185 mm·sec ($p < 0.001$); ketamine reduced the AUCs from 1352 ± 118 to 809 ± 159 mm·sec ($p = 0.000$) (Fig. 2A). Ketamine caused the largest increase in CPM: mean CPM% after placebo 22.1 ± 12.0% (95% confidence interval (95% CI): -5.1 –

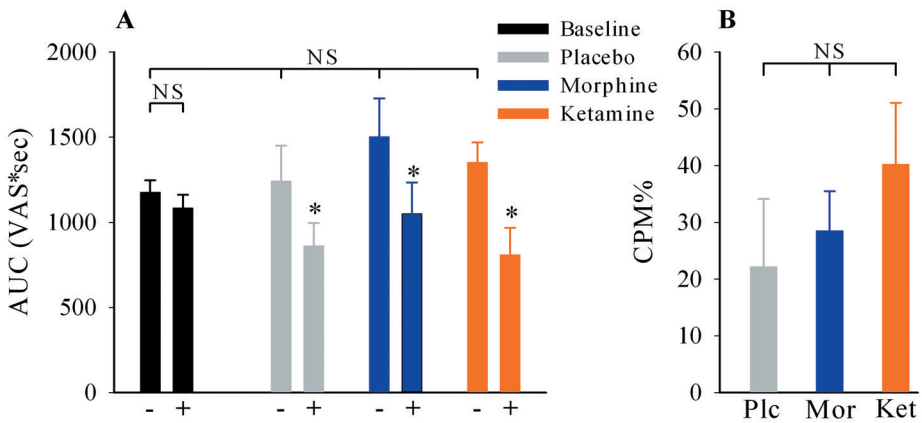

Figure 2. A. *AUC values of the eVAS-time responses without conditioning stimulus (–) and with conditioning stimulus (+). The conditioning stimulus had no effect on baseline responses, but decreased eVAS responses after treatment with placebo, morphine and ketamine. * $p < 0.001$ vs AUC of eVAS-time responses without conditioning stimulus. AUCs of responses without the conditioning stimulus were similar for baseline, placebo, morphine and ketamine. NS = not significant.* ***B.*** *Magnitude of conditioned pain modulation (CPM%) responses after treatment with placebo (Plc), morphine (Mor) and ketamine (Ket). The magnitude of CPM% responses did not differ among treatments.*

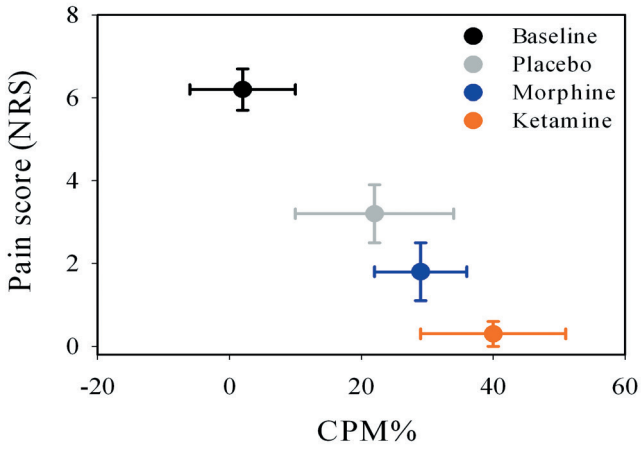

Figure 3. *Conditioned pain modulation (CPM% responses) versus spontaneous pain ratings in chronic pain patients. Data are mean ± SEM.*

49.3), after morphine 28.5 ± 7.0% (95% CI: 12.8 – 44.3) and after ketamine 40.2 ± 10.9% (95% CI: 15.6 – 64.6); however no difference in CPM enhancement could be detected between the three treatment groups ($p > 0.05$, see also Fig. 2B).

Pain relief and magnitude of CPM

The mean NRS at baseline was 6.2 ± 0.5. In terms of magnitude, pain relief was greatest after ketamine (mean NRS after treatment: 0.3 ± 0.3, $p < 0.01$ *vs.* baseline), followed by morphine (1.8 ± 0.7) and placebo (3.2 ± 0.7). In terms of duration of effect, ketamine had effects lasting > 12 hours in eight of 10 subjects and > 6 hours in the remaining two subjects. Morphine had effects lasting > 12 hours in zero of 10 subjects, > 6 hours in eight of 10 subjects and < 6 hours in the remaining two subjects. After placebo treatment all analgesic effects had dissipated within 6 hours of treatment. The magnitude of CPM correlated positively with the magnitude of pain relief (Fig. 3) and duration of spontaneous pain relief.

Side effects after analgesic treatment

Minor side effects occurred with nausea in four subjects (two of whom vomited) during ketamine infusion and in seven patients (four of whom vomited) during morphine infusion. No nausea or vomiting was observed during placebo infusion. At the end of the infusion, the mean drug high scores were 7.2 ± 0.6 for ketamine, 2.4 ± 0.5 for morphine and 0.4 ± 0.2 for placebo.

Discussion

We tested CPM responses in a relatively homogenous population (in terms of QST abnormalities) of subjects with chronic pain related to peripheral neuropathy. The main findings of our explorative studies are that CPM responses were not detectable in this patient population, but that treatment with ketamine, morphine and placebo caused activation of CPM responses ($p < 0.001$). The magni-

tude of CPM responses correlated positively with the magnitude and duration of spontaneous pain relief.

Descending inhibitory and facilitatory pathways involved in the modulation of pain originate at higher sites in the CNS including the prefrontal cortex, rostral anterior cingulate cortex (rACC) and insula, which project to the periaquaductal gray and rostral ventromedial medulla (RVM) and modulate nociceptive input at the level of the dorsal horn.[1-4,6] Activation of inhibitory pathways will reduce trafficking of nociceptive input to supraspinal sites involved in pain processing and perception. Activation of facilitatory pathways will have the reverse effect. A shift in the balance between inhibition and facilitation has been suggested as an underlying mechanism in the development or maintenance of pain.[20] There are various expressions of descending inhibitory pain modulation, including placebo and stress-induced analgesia and CPM.[3,21,22] CPM is considered to be a central mechanism with activation of specific brain regions involved in descending inhibition.[23,24]

Dysfunctional endogenous pain modulation (as tested by CPM or CPM-like paradigms) has been observed in several chronic pain states.[8,11-13,25] In our current study we included patients with chronic (poly)neuropathic pain (from mixed small and large fiber neuropathy who all displayed abnormal CPM responses. Previous studies in healthy volunteers showed that females have less efficient CPM responses compared to males and that CPM efficiency decreases with increasing age (starting at middle-age).[26-29] Indeed, in a separate set of healthy people of similar age and sex as our current study population, we did not observe significant CPM responses (M. Niesters, unpublished observation). Since the patient population in this study was predominantly middle-aged and female, CPM responses were a priori not expected or were at least assumed to be small. Our data and those of others indicate that patients of 40 years and older (especially females) have absent of less activated pain modulation mechanisms (compared to younger patients) and are therefore at a disadvantage in situations where a functional descending inhibitory mechanism is necessary for normal pain perception. Consequently, in response to a noxious insult, pain may be more severe and persistence of pain may occur, which possibly is one of the factors involved in the development of chronic pain. There is indeed evidence from animal studies for a link between chronic pain development and efficacy of descending inhibitory pain pathways. Animals with more efficient engagement of descending inhibition show a reduced probability of peripheral nerve injury-induced chronic allodynia compared with animals with less efficient descending inhibition.[14,15]

A novel observation in our study is that CPM responses in neuropathic pain patients could be re(activated) after pharmacological treatment and that ketamine, morphine and placebo were equally effective in this respect. The large effect of a 1-hour intravenous treatment with placebo was not unexpected. There is ample evidence that activation of descending pain control underlies placebo-analgesia, via central opioidergic mechanisms.[22,30] For example Levine and collegues[30]

showed that placebo analgesia is abolished by the opioid receptor antagonist naloxone. Furthermore, animal research demonstrated that remifentanil and placebo analgesia both activate brain areas involved in descending inhibition including the rACC.[31]

An important finding in our study is that there was a significant correlation between the magnitude of CPM responses and the magnitude (and duration) of spontaneous pain relief (Fig. 3). As stated by De Felice and collegues,[14] such findings provide a mechanistic explanation for medications that engage descending inhibitory control or mimic its consequences and cause efficient and long-term pain relief, such as we observed after treatment with ketamine. To the best of our knowledge our study is the first to show that morphine enhances CPM responses in chronic neuropathic pain patients. Recently, Arendt-Nielsen and collegues[32] tested the effect of two opioid analgesics (fentanyl and buprenorphine transdermal patches) on CPM in healthy volunteers and showed enhanced responses after treatment. In contrast to these and our data, others observed that morphine reduces rather than increases CPM responses in healthy volunteers (after an intravenous infusion of 0.05 mg/kg) and non-neuropathic chronic pain patients (after prolonged opioid treatment).[33,34] We have no conclusive explanation for these differences in the effect of opioid treatment on CPM engagement. Involvement of endogenous opioids in CPM engagement is inferred from studies showing that naloxone reduces CPM.[35] Furthermore, opioid receptors are expressed on neurons involved in pathways of descending pain modulation both at spinal and supraspinal sites.[1-3]

Similar to morphine, ketamine enhanced CPM responses in our patient population. Ketamine has gained a position in the treatment of chronic pain, especially of therapy-resistant neuropathic pain.[36] Ketamine treatment results in prolonged analgesia, with persistence of effect beyond the treatment period. For example, we showed previously that a 100-hour ketamine infusion (20-30 mg/h) results in pain relief up to 3 months after intravenous treatment in patients with complex regional pain syndrome type 1.[37] The mechanisms through which ketamine exerts these prolonged effects remain unknown. Possibly one of the factors that contribute to ketamine's prolonged analgesic effect is desensitization of upregulated *N*-methyl-D-aspartate receptors within the spinal cord.[37] Another mechanism might be that ketamine activates endogenous pain modulatory pathways. The observation that ketamine produced greater analgesia than morphine (or placebo) in our patient population correlated with a greater ability to engage descending inhibition as tested by CPM (Fig. 3). Recently, we assessed the effect of ketamine on brain function using the technique of resting-state functional magnetic resonance imaging. Ketamine altered connectivity in brain regions responsible for pain sensing and the affective processing of pain, and also in regions involved in activation of descending inhibitory pain pathways, including the rACC, insula, orbitofrontal cortex and brain stem.[38] These findings corroborate our observation of ketamine's effect on CPM in neuropathic pain patients.

The effects of ketamine on CPM responses in chronic patients differ from results in volunteers.[20] In a population of healthy young volunteers, ketamine shifted the balance between pain inhibition and pain facilitation towards pain facilitation. Major differences between study populations (*i.e.* age and underlying disease) could be responsible for the difference in study outcomes. For example, in healthy volunteers, CPM responses may be at maximum strength such that treatment leads to activation of interfering pathways (facilitatory pathways). This may also explain the effect of morphine on CPM in healthy volunteers and possibly also in chronic non-neuropathic pain patients.[33,34] We can also not exclude that treatment at maximum CPM activates noise sources resulting in inconsistencies in the data.

Critique of methods

One might contend that no population of healthy age- and sex-matched controls was included to make a comparison of treatment effects between groups possible. However, as discussed above, healthy volunteers lack underlying disease, that is a primary hit (*i.e.* peripheral nerve damage) and possibly also secondary damage (*i.e.* a defect in the descending inhibitory control system) of their pain pathways. Although we believe that knowledge on the effect of treatment on CPM responses in volunteers is valuable on its own, we argue that a direct comparison between populations is of limited value, as treatment induced changes in CPM responses in volunteers may differ mechanistically from those in patients.

Subjects were allowed to continue their pain medication as long as they had used these drugs for at least 3 months and dosages were constant during the study period. Subjects that used pain medication did not have a larger (or smaller) enhancement of CPM responses compared with those that did not. Therefore, we do not believe that continuation of analgesics during the study period affected our outcome.

Finally, we tested a small group of predominant middle-aged female pain patients. While this reflects the majority of chronic pain patients in clinical practice, our study needs replication in younger patients (including males) with neuropathic pain. This will further clarify the link between sex, age, defective CPM responses and chronic pain.

Conclusions

In chronic neuropathic pain patients with similar QST abnormalities, treatment with placebo, morphine and ketamine activated prior absent CPM responses, suggesting a role of engagement of descending pain inhibition in their analgesic efficacy. We suggest that in clinical practice, drugs that cause enlargement of re-engagement of CPM should be the first drugs of choice in the treatment of chronic (neuropathic) pain.

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Chapter 5

Tapentadol potentiates Descending Pain Modulation in Chronic Pain Patients with Diabetic Polyneuropathy

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Introduction

Endogenous pain modulatory pathways are important regulators of human pain perception. Both inhibitory and facilitatory descending pathways, originating at higher centers, modulate the activity of nociceptive neurons at the level of the spinal dorsal horn, enhancing or inhibiting noxious signal propagation to the brain.[1] A shift in the balance between pain inhibition and facilitation has been suggested to underlie the development or maintenance of many chronic pain syndromes, such as fibromyalgia, irritable bowel syndrome, chronic pancreatitis and neuropathic pain syndromes.[2-5] Animal studies show that effective engagement of descending inhibition protects against chronic neuropathic pain development.[9] Various neurotransmitter systems are involved in the descending pain pathways including endogenous opioid peptides, noradrenaline (NA) and serotonin. Release of endogenous opioids and noradrenaline underlie pain inhibition, whereas the serotonergic pathway has both pain inhibitory and facilitatory properties.[7,8] The new analgesic tapentadol is a centrally acting drug with a dual mechanism of action. Tapentadol is a weak μ-opioid receptor (MOR) agonist (its affinity for the MOR is 50 times less than that of morphine) and inhibits neuronal reuptake of noradrenaline.[9,10] Both mechanisms act synergistically to produce analgesia.[11] Animal studies indicate that the opioidergic component is more important in the treatment of acute pain, whereas the noradrenergic component is largely involved in the treatment of chronic neuropathic pain.[8]

As tapentadol modulates opioidergic and noradrenergic pathways simultaneously, the analgesic effect of tapentadol is thought to rely on the enhancement of descending pain inhibitory activity.[12] However, up to know, no studies have been conducted to confirm the presence of such an effect in humans. In the current study the effects of tapentadol on two experimental paradigms, conditioned pain modulation (CPM) and offset analgesia (OA) were tested in chronic pain patients with diabetic polyneuropathy (DPN). CPM is an experimental measure of endogenous pain modulation that gates incoming pain signaling as consequence of a preceding or simultaneous tonic painful stimulation.[13-18] OA is a test in which a disproportionally large amount of analgesia becomes apparent upon a slight decrease in noxious heat stimulation.[19,20] Both tests have been used previously to evaluate the engagement of pain modulatory pathways.[4,15,20]

We performed a randomized, parallel-design, placebo-controlled study in chronic pain patients with diabetic polyneuropathy on the effect of a 4-week tapentadol treatment on CPM, OA and pain relief. We hypothesize that tapentadol's analgesic efficacy relies, in part, on the engagement of endogenous pain inhibitory pathways.

Methods

Chronic pain patients were recruited to participate in the study performed at Leiden University Medical Center over the period January 2012 to October 2012, after approval of the protocol was obtained from the local Medical Ethics Committee and the Central Committee on Research involving Human Subjects (CCMO, The Hague, The Netherlands). The study was registered at the Dutch trialregister under number NTR2716 and has EudraCT number 2010-012175-26. The study was registered as an addendum to an earlier trial on the effects of a single dose of tapentadol and morphine on CPM. All participants gave written informed consent and underwent a physical examination before enrollment in the study.

Patients were recruited via an advertisement in the journal of the national diabetic society. All recruited patients had diabetes and chronic pain in hands and/or legs and feet. They were included in the study when they were 18-75 years, had a body mass index below ≤ 40 kg/m^2 and had: (1) presence of at least two of the following symptoms in legs and/or arms (in a stocking-glove distribution): (i) symmetrical dysesthesias or paresthesias, (ii) burning or painful feet with nighttime worsening or (iii) peripheral tactile allodynia; and (2) an abnormal warm or cold detection threshold, an abnormal warm or cold pain threshold, or allodynia observed with quantitative sensory testing. Exclusion criteria included: indication of the presence of severe medical diseases (*e.g.* liver function elevation); allergy to opioids; current use of benzodiazepines and/or other sedatives; present or past use of illicit/recreational substances; present or past alcohol abuse; history of mental illness or epilepsy; pregnancy and/or lactation; current use of strong opioids; and inability to understand the purpose and instructions of the study. The patients were allowed to continue the following pain medications as long as they used a constant dose for the 8 weeks prior to the study and the dosage could be kept constant during the whole study period: acetaminophen, non-steroidal anti-inflammatory drugs, amitriptyline, gabapentin and pregabalin. Patients that had been using opioids previously (and terminated treatment due to absence of efficacy or side effects) were eligible for inclusion.

Study design

This randomized, double blind, placebo-controlled study was performed in 24 DPN patients (see Consort flow chart, Fig. 1). Twelve patients were treated orally for 4 weeks with tapentadol slow release (SR), twelve others with placebo. The dose of tapentadol SR was titrated to effect starting with 100 mg twice daily in week 1, followed by 200 mg twice daily in week 2 and 250 mg twice daily in week 3 and 4. In case of the presence of side effects unacceptable to the patient, the tapentadol dose was decreased to a dose were side effects were absent or acceptable. All patients were tested twice, once 1 day before the treatment period and once on the last day of treatment. On each study day, the subjects were familiarized with the test procedures. Next the CPM and OA responses were obtained. Spontaneous pain scores (using a 11-point numerical rating scale (NRS) from 0

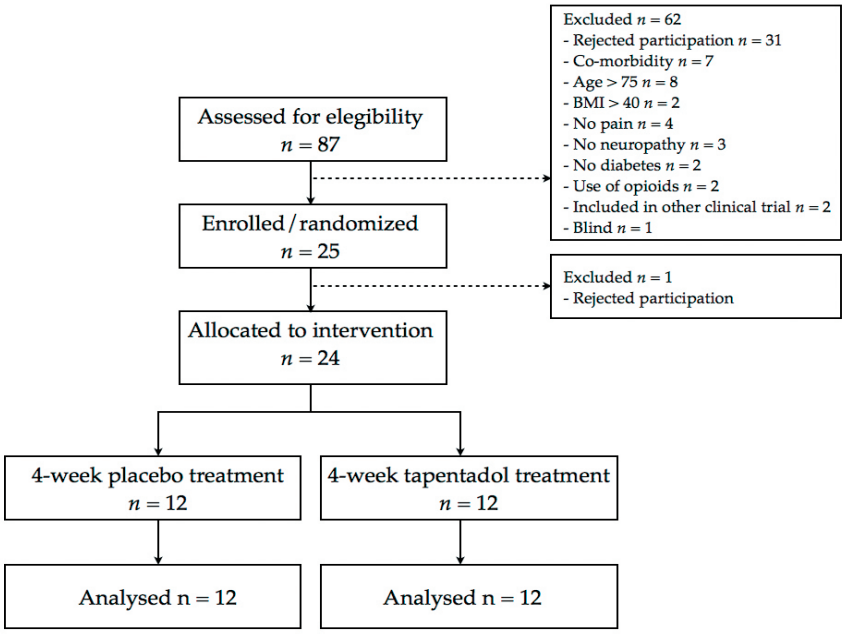

Figure 1. *Consort study flow chart.*

(corresponding with no pain) to 10 (corresponding with most imaginable pain)) and side effects (presence of nausea, vomiting, drowsiness, dizziness and dry mouth, using a dichotomous scale (yes/no)) were monitored on a weekly basis.

To get an indication of the nerve-fiber involvement in the patient population, quantitative sensory testing (QST) was performed according to the standardized protocol of the German Research Network on Neuropathic Pain.[21] In short, this protocol assesses cold, heat and mechanical detection and pain thresholds; paradoxical heat sensations; mechanical pain sensitivity; allodynia; wind-up and vibration and pressure pain thresholds. Sensory testing was performed on the hand and foot of all pain patients included in the study.

Application of nociceptive stimuli for CPM and OA testing

Heat pain was induced on the lower part of the non-dominant arm with a 3 x 3 cm thermal probe connected to the Pathway Neurosensory Analyzer (Medoc Ltd., Ramat Yishai, Israel). The probe was calibrated according to the specifications of the manufacturer. During the heat pain stimulation, subjects continuously quantified the pain intensity level of the stimulus using a slider on a computerized potentiometer that ranged from 0 (no pain) to 100 (worst pain imaginable). This allowed for continuous monitoring of the visual analogue scale (eVAS). To overcome sensitization, the thermode was moved between different zones on the forearm and ample time was incorporated between the different heat stimuli. On each of the two study days (that is before treatment and at 4-weeks of treatment), the individual test temperature was determined by applying a series

of heat stimuli. First the temperature was increased from 32 °C (baseline temperature) by 1.5 °C/s to a target temperature of 42 °C and kept constant for 10 seconds. If the eVAS was less then 50 mm a next test was performed increasing the target temperature in steps of 1 °C. The cut-off temperature for these series was 49 °C. The temperature evoking an eVAS of at least 50 mm was used during the remainder of the study.

Cold pain was induced using a cold-water reservoir produced by a rapid water-cooling system (IcyDip, IcySolutions BV, Delft, The Netherlands). The subject's foot and lower leg was immersed into the cold water reservoir, which could be set at different temperatures ranging from 6 °C to 18 °C. The temperature that produced an eVAS of at least 30 mm was used in the remainder of the study. After the exposure to cold water, the subject's extremity was warmed to normal temperature using warm water collected from the counter-current outlet of the IcyDip system.

Conditioned pain modulation and offset analgesia
The method to induce CPM has been published previously.[2,4,15] In short, to measure CPM two series of three pain tests were performed. One series included stimulation of the forearm with the experimental stimulus (heat pain). For this, the temperature of the heat probe gradually increased from baseline temperature (32 °C) to the earlier set test temperature (at 1.5 °C/s) and remained constant for 30 seconds. Next, the temperature rapidly returned (at 6 °C/s) to baseline. The second series included stimulation with both the experimental stimulus and the conditioning stimulus (cold pain). The conditioning stimulus was applied 25 seconds before the start of the experimental stimulus and ended simultaneously with the end of the experimental stimulus. In both sessions the subject's only rated the pain intensity level of the experimental stimulus (heat pain on the arm). There were 3-minute intervals between single tests.

OA was studied by applying a three-temperature paradigm as described by Grill et al.[19] The temperature was ramped at 1.5 °C/s from baseline temperature to the previously set test temperature. The test temperature was kept constant for 5 seconds after which it was raised by 1 °C for 5 seconds and next decreased by 1 °C for 20 seconds. At the end of the test the temperature quickly returned (6 °C/s) to baseline. This temperature paradigm was applied three times with a 3-min interval between tests.

Randomization and blinding
Randomization and allocation was performed by the local pharmacy using a computer-generated randomization list. Placebo tablets were fabricated by the pharmacy and were identical to the tapentadol tablets in form, size and taste. The tablets were repackaged into unmarked containers and delivered to the research team and subsequently by the research team to the patients. The research team remained blinded to treatment until all CPM and OA responses had been analyzed.

Table 1. *Patient characteristics*

	Tapentadol	Placebo
Men/Women (*n*)	7/5	7/5
Age (years; median (range))	63 (54 - 75)	62 (53 - 71)
Weight (kg; median (range))	95 (56 - 140)	97 (71 - 125)
Height (cm; median (range))	177 (169 - 196)	178 (168 - 194)
Duration of disease		
Diabetes mellitus (years; median (range))	12 (3 - 35)	11 (2 - 45)
Neuropathic pain (years; median (range))	6 (1 - 10)	6.5 (2 - 25)
Affected limbs		
Legs (*n*)	8	8
Legs + arms (*n*)	4	4
Medication		
Insulin	8	6
Metformin	11	7
Pregabalin	3	2
Duloxetin	2	0
Amitriptyline	1	1
Steroids	0	2
Paracetamol	1	1

Data analyses

To quantify the magnitude of CPM, peak eVAS scores were used in the analyses. For each subject, the average peak eVAS without and with conditioning stimulus (CS) was calculated. Next, relative CPM responses were calculated to correct for variations in peak response between sessions and subjects using the formula: [(mean eVAS without CS stimulus – mean eVAS with CS)/(mean eVAS without CS)] × 100%.[2,26,27]

OA responses were quantified as previously described.[15,20] In short, the decrease in eVAS from the peak eVAS value to the eVAS nadir following the 1 °C decrease of the test stimulus was measured (ΔeVAS) and corrected for the value of the peak eVAS: ΔeVASc = [ΔeVAS/(peak eVAS)] x 100%.

Sample size and statistical analysis

A sample size of 24 (12 per treatment level) was calculated by assuming an increase in CPM of 20% (15%) (mean (SD)) with $\alpha = 0.05$ and $\beta > 0.95$. An effect of 20% was chosen as this constitutes the "average" value of CPM in healthy volunteers and is probably the maximum magnitude of CPM attainable in humans.[15]

The effect of the conditioning stimulus on the relative eVAS responses was tested by two-tailed paired-t-test. Treatment effects were assessed by two-way repeated measures analysis of variance (factors: time and treatment). For all analyses, the software package SigmaPlot version 12.5 for Windows (Systat Software Inc., San Jose, CA) was used. Data are presented as mean ± SEM unless otherwise stated and p-values < 0.05 were considered significant.

Results

Eighty-seven patients responded to the advertisement (Fig. 1). Thirty-one decided not to participate after they were informed on the nature of the study. Thirty-one others were excluded because of absence of pain, diabetes or neuropathy (as assessed by QST), not meeting age- or body mass index-related inclusion criteria, the use of strong opioids or their inclusion in another trial. Twenty five subjects were enrolled in the study and randomized. One patient retracted her consent after randomization; she was replaced by another subject. The demographics of the participating patients are given in table 1.

All patients completed the study without major side effects. QST measurements obtained from affected hands and feet are presented in figure 2. The patients presented with a mixed small- and large fiber neuropathy as evidenced by re-

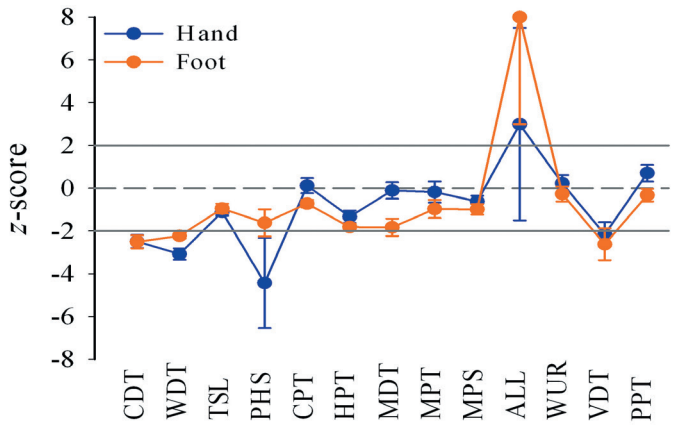

Figure 2. *Results of the quantitative sensory tests obtained on the affected skin areas (hand/feet). The data are the populations mean z-scores (SEM). Z-scores were calculated in relation to a population of healthy subjects as determined by Rolke et al.[21] Z-values above the broken line indicate a gain of function whereas values below this line are indicative for a loss of sensory function. CDT: cold detection threshold; WDT: warm detection threshold; TSL: thermal sensory limen; PHS: paradoxal heat sensations; CPT: cold pain threshold; HPT: heat pain threshold; MDT: mechanical detection threshold; MPT: mechanical pain threshold; MPS: mechanical pain sensitivity; ALL: dynamic mechanical allodynia; WUR: windup ratio; VDT: vibration detection threshold; PPT pressure pain threshold.*

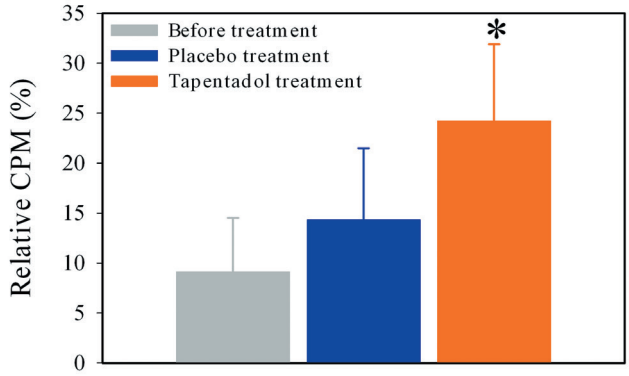

Figure 3. *Relative CPM responses at baseline (before treatment), in patients receiving a 4-week placebo treatment and in patients receiving a 4-week tapentadol treatment. At baseline the effect of the conditioning stimulus was not significant (p = 0.09). After placebo and tapentadol treatment the effect of the conditioning stimulus was significant (placebo p = 0.04, tapentadol p < 0.01). A treatment effect was present with greater increase in CPM responses during tapentadol treatment than during placebo treatment (* p < 0.001 vs. placebo).*

duced cold and warm detection thresholds and paradoxical heat sensation (signs of small fiber involvement) and a reduced vibration detection threshold (on the feet more than on the hands; a sign of large fiber involvement). Importantly, allodynia was observed in 7 (of 24) patients. During the study period the daily drug dose was titrated to a level with sufficient analgesic effect and acceptable side effects to the patients. In the placebo group the maximum daily dose of 500 mg per day was reached in all subjects compared to an average of 433 ± 31 mg per day in the tapentadol SR group. Reported side effects were nausea (placebo: n =

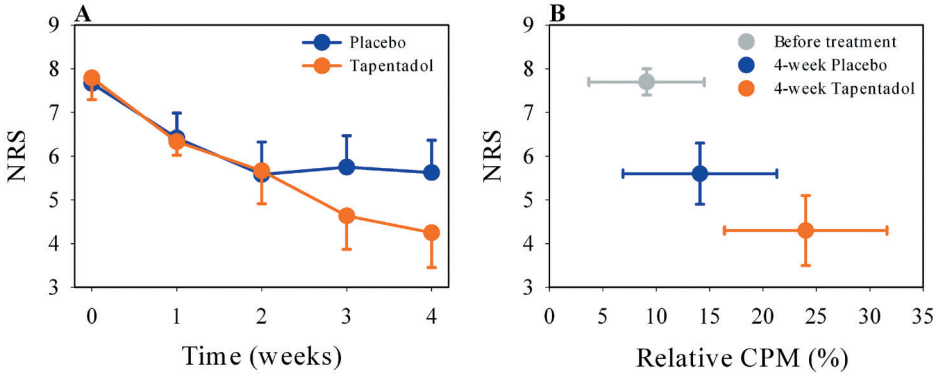

Figure 4. A. *Average spontaneous pain scores of patients with painful diabetic neuropathy during the 4-week treatment period. There was a significant treatment effect with greater pain relief during tapentadol treatment (p = 0.03).* ***B.*** *Relative CPM responses versus pain scores. Values are mean ± SEM.*

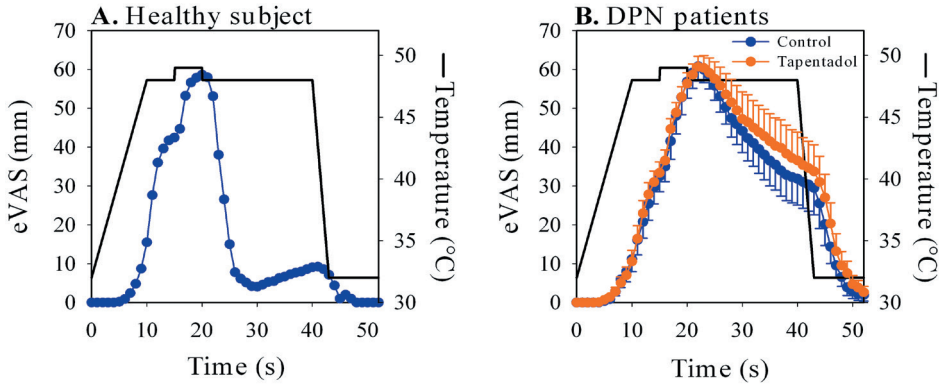

Figure 5. *Offset analgesia responses.* ***A.*** *An example of a healthy subject (female, 60 years). Data taken from ref. 20.* ***B.*** *Absence of tapentadol treatment on offset analgesia in painful diabetic neuropathy patients. DPN: diabetic polyneuropathy; eVAS: electronic visual analogue scale.*

4; tapentadol: n = 3), vomiting (placebo: n = 0; tapentadol: n = 2), sedation (placebo: n = 2; tapentadol: n = 6), dizziness (placebo: n = 2; tapentadol: n = 6) and dry mouth (placebo: n = 1; tapentadol: n = 5).

Prior to treatment significant CPM responses were not detectable as the effect of the conditioning stimulus was not significant (CPM = 9.1 ± 5.4%, $p = 0.09$, Fig. 3). Following both treatments CPM responses increased to significant levels (placebo: CPM = 14.3 ± 7.2%, $p = 0.04$; tapentadol SR: CPM = 24.2 ± 7.7%, $p < 0.01$). A clear treatment effect was present with tapentadol SR CPM responses being greater than placebo responses ($p < 0.001$, Fig. 3).

Weekly pain scores following tapentadol and placebo treatments are given in figure 4A. It shows a clear distinction in pain reduction in weeks 3 and 4 of treatment with greater analgesia in patients treated with tapentadol SR (pain scores at baseline 6.5 ± 0.6 reduced to 4.8 ± 0.7 following placebo and 3.9 ± 0.6 following tapentadol; 4-week treatment effect $p = 0.03$). Plotting pain relief *versus* CPM responses shows that greater pain relief from tapentadol SR coincided with enhanced CPM responses (Fig. 4B).

OA responses prior to tapentadol treatment and at week 4 of treatment are given in figure 5. As contrast, an example of an OA response in age and sex-matched healthy volunteer is added in figure 5A (data from ref. 20). ΔeVASc values in healthy volunteers in the age cohort 40-80 range between 90 and 100%, irrespective of sex.[20] Prior to treatment ΔeVASc was 40.7 ± 7.4%. Neither placebo (change from baseline +2.6 ± 11.6%) nor tapentadol SR treatment (change from baseline -0.8 ± 3.7%) had an effect in the magnitude of OA (treatment effect $p = 0.78$).

Discussion

Tapentadol is a new centrally acting analgesic agent for treatment of acute and chronic pain,[12,22-25] that acts through MOR agonism and neuronal noradrenaline reuptake inhibition (NRI).[8-10,26] Through this dual mechanism of action it is thought that tapentadol engages and potentiates descending pain inhibitory pathways,[12] although there are no human studies to substantiate this. We studied tapentadol's effect on two experimental paradigms of endogenous pain modulation (CPM and OA) in chronic pain patients with DPN. The main findings of our studies are that in DPN patients tapentadol SR caused significant pain relief that coincided with enhanced CPM responses. No effect of tapentadol was observed on OA responses. Taken these results we reason that relief of chronic pain in DPN patients by tapentadol is associated with engagement and potentiation of descending inhibitory pain pathways.

Conditioned pain modulation

Modulation of pain in humans involves activation of higher cortical centers (prefrontal cortex, anterior cingulate cortex, insula), brainstem (periaquaductal gray, rostral ventromedial medulla) and descending pathways projecting to the dorsal horn of the spinal cord.[1,27,28] These descending pathways may be inhibitory or excitatory. Consequently, nociceptive input that enters the spinal dorsal horn will undergo some form of modulation, either facilitation or inhibition, which results in an amplified or inhibited pain sensation at central sites. Various chronic pain syndromes show loss of descending pain inhibition, including fibromyalgia, irritable bowel syndrome, chronic tension headache, temperomandibular disorder, complex regional pain syndrome and chronic pancreatitis.[2-5] Of importance is the finding by De Felice *et al.* who showed in rodents that a genetic predisposition to activate descending inhibition protects against the development of chronic pain following peripheral nerve damage.[6] In humans, examples of efficacious engagement of descending inhibitory pain modulation include placebo analgesia, stress-induced analgesia and CPM.[16-18,29,30] CPM is an experimental and consequently surrogate tool used to quantify descending pain inhibition in humans. Central inhibition of a focal noxious stimulus is induced by the administration of a noxious stimulus at a remote area (conditioning stimulus), thereby reducing the perception of the focal or test pain stimulus ("pain inhibits pain").[13,16] The central nature of CPM has been ascertained by the observation that specific brain regions involved in descending inhibition are activated during CPM-tests in volunteers.[31,32]

Volunteer studies show that CPM engagement is less effective in women relative to men and that CPM efficacy is reduced in elderly people (starting at middle-age).[33,35] Indeed in our middle-aged DPN patient population (mean age 59 years) CPM was not present prior to the intake of study medication. Whether this is related to the underlying disease or an age-effect is unknown. Irrespective, individuals that are less able to activate CPM may have a higher probability of chronic pain development following a specific insult such as peripheral nerve

damage from diabetes (cf. De Felice *et al*.[6]) or surgery. Yarnitsky *et al.* showed that patients with less efficient CPM responses were at risk for development of chronic post-thoracotomy pain.[17] The method of induction of CPM has been validated previously by us in healthy volunteers and is applied and others in chronic pain patients.[15,17]

Taken its mechanisms of action, tapentadol will interact within the descending modulatory system by activation of MORs and inhibition of neuronal noradrenaline reuptake.[7,8] Both neurotransmitter systems play an important role in the activation of descending inhibitory pain pathways at supraspinal sites as well as in the spinal dorsal horn (at pre- and postsynaptic sites). See for an excellent review on this topic ref. 1. For example, animal studies show that activation of MORs on brainstem nociceptive "on-cells" will release the inhibition of brainstem nociceptive "off-cells" that project to the spinal dorsal horn where nociceptive signal propagation is subsequently inhibited.[1] Activation of spinal dorsal horn pre- and postsynaptic α_2-adrenergic receptors will cause potent analgesic responses by inhibiting nociceptive afferent input. Such analgesic effects are observed after the intrathecal administration of the postsynaptic α_2-adrenergic receptor agonist clonidine.[36] Although tapentadol displays weak MOP-receptor affinity, animal studies show that its synergistic effect at MOP- and adrenergic-receptor systems will cause potent analgesic responses.[9,10,26] Indeed, animal studies and clinical trials show that tapentadol is an effective analgesic in a variety of chronic pain syndromes (for example osteoarthritis pain, low back pain, neuropathic pain).[8,12,25,37,38]

We observed that the analgesic efficacy of analgesic treatment (tapentadol/placebo) was coupled to its effect on CPM (Fig. 4). A 4-week treatment with placebo caused small analgesic effects (ΔNRS = 1.7 cm) coupled to a modest increase in CPM (+14.3%), while tapentadol treatment caused a larger analgesic response (ΔNRS = 3.9) coupled to a large CPM response (+24.2%). This latter CPM value is similar to those observed in young healthy volunteers.[14] These findings support a mechanistic role for the endogenous analgesia system in producing effective pain relief by tapentadol, possibly by its synergistic effect at MOP and α_2-adrenergic receptors (see above). Yarnitsky *et al.*[18] showed a coupling between drug efficacy and magnitude of CPM responses for duloxetine, a serotonin-noradrenaline reuptake inhibitor (SNRI) in DPN patients with initially less effective CPM responses. While our small patient population, with initially minor or absent CPM responses benefited from the 4-week tapentadol SR treatment, we remain uninformed on the efficacy of tapentadol in chronic pain patients with "normal" CPM responses (*i.e.* responses of similar magnitude to those observed in young and healthy volunteers). Extrapolating the duloxetine data from Yarnitsky *et al.* would suggest that tapentadol is less effective in these patients. There is now ample evidence to argue that in painful neuropathy patients with absent or reduced CPM, CPM responses may be reactivated or potentiated by analgesic treatment that targets one or more components of the endogenous pain modulatory system.[4,18]

In chronic pain patients, the effect of tapentadol SR requires several weeks to develop (Fig. 3). Similar observations have been made for other S(N)RI-type of analgesics and tricyclic antidepressants.[39] Hence, it is recommended to evaluate the start of pain therapy with these agents not earlier than after 2 weeks of treatment.[40] Taken the similarities of mechanisms of action among these analgesics, we argue that the slow accumulation of noradrenaline at its putative effector sites may be held responsible for its slow onset of action. Our findings stress the importance of the noradrenergic component in inducing tapentadol analgesia in chronic pain as was earlier observed in animal studies.[8]

Two patients in the tapentadol group used duloxetine (duration of treatment > 1 year), a serotonin and noradrenaline reuptake inhibitor without opioidergic activity. Theoretically, the use of this drug may have enhanced the CPM responses induced by tapentadol. However, prior to tapentadol treatment these patients had no detectable CPM response and the magnitude of their CPM response after the 4-week tapentadol treatment was well within the range observed in patients not on duloxetine. We argue that these two patients did not confound the results of our study.

Offset analgesia

OA is a relatively novel model of endogenous analgesia that produces temporal alterations in pain processing. The phenomenon occurs when a small decrease (1 °C) in temperature during noxious stimulation evokes a disproportionately large decrease in pain perception.[19,20] We previously assessed OA responses in a large population of volunteers aged 6-88 years and observed response values ranging from 92-99%. It has been suggested that OA is of central origin as functional imaging studies show that OA activation coincides with activation of brain regions involved in the central modulation of pain.[41] However, it cannot be excluded that OA is initiated by dynamic responses of primary afferents or spinal processes. For example, Darian-Smith *et al.*[42] reported that in monkeys the discharge of heat-sensitive nerve fibers innervating the skin was nearly completely suppressed during a 10 second 1 °C cooling pulse from a baseline temperature of 39 °C. A similar mechanism may occur during OA activation. A peripheral origin of OA is further supported by the observation that central acting drugs such as opioids (tapentadol, morphine, remifentanil), opioid antagonists (naloxone) and NMDA receptor antagonists (ketamine) are unable to affect OA responses in volunteers and neuropathic pain patients.[15,20,43] Finally, a recent observation that while offset analgesia is present on the forearm of healthy volunteers, it is absent on the palm of the hand further suggests that peripheral mechanisms are important in the development of offset analgesia.[44]

We reproduce our earlier observation that OA responses are absent or reduced in patients with peripheral neuropathy.[20] The ΔeVASc values observed in the DPN patients were about 40% of those previously observed by us in healthy volunteers of the same age and sex.[20] No improvement or alteration of OA responses was observed after the 4-week tapentadol treatment, which indicates that this

phenomenon of endogenous analgesia is without opioidergic or noradrenergic involvement. However, it may well be that the large and small nerve fiber damage that was present in our current population prevented their ability to discern small changes in skin temperature and consequently prevented peripheral activation of OA.

Conclusions

In conclusion, our results show that patients with DPN that display absent CPM responses benefit from tapentadol causing pain relief coupled to (re)-activation of descending inhibitory pain pathways.

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Section 2

Resting-state fMRI studies

Chapter 6

Effect of Subanesthetic Ketamine on Intrinsic Functional Brain Connectivity

A placebo-controlled functional magnetic resonance imaging study in healthy male volunteers

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Introduction

The noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist ketamine has been used since the early 1960s as an anesthetic agent. It is the most potent NMDA receptor antagonist currently clinically available. At subanesthetic concentrations, ketamine is a potent analgesic and is used in the treatment of acute and chronic pain.[1] Ketamine use is hampered by central side effects, including serious psychedelic effects.[1] The influence of ketamine on central sites (*i.e.* within the central nervous system) has been investigated extensively with various imaging techniques, including positron emission tomography and task-related functional magnetic resonance imaging (fMRI).[2-8] Several brain regions display ketamine-dependent activity changes. For example, during a short-term ketamine infusion, frontal and temporal brain regions are affected (these regions may be involved in the psychedelic effects of ketamine), as are several regions involved in pain processing, such as the anterior cingulate cortex, the insular cortex and the thalamus.[3,4]

Positron emission tomography and task-related fMRI studies have several disadvantages, including limited temporal and spatial resolution, radiation dose restrictions in positron emission tomography and the need to postulate an a priori hypothesis about the site of drug action for task-related fMRI. Furthermore, large-scale network interactions of brain regions on which central nervous system functions depend cannot be adequately captured with these techniques.[9] A new approach in central nervous system drug research is resting-state fMRI (RS-fMRI), which measures these intrinsic interactions at baseline activity of the brain (*i.e.* not task-related).[10] This technique shows that spontaneous fluctuations in RS-fMRI signal form coherent networks (resting-state networks (RSNs)) that represent connections between brain areas of similar functionality.[11,12] Studying drug effects on brain connectivity using RS-fMRI provides direct evidence of drug-induced changes in brain dynamics and consequently on brain function.

In the current study we test the effect of low-dose ketamine *versus* placebo in healthy volunteers using a crossover and randomized design. The effect of two doses of ketamine (or placebo) on RS-fMRI and on pain scores during noxious cutaneous heat stimulation is assessed. By incorporation of pain scores in the statistical model, we will be informed on the influence of both ketamine (*i.e.* intended effect and side effects) and pain processing on brain connectivity. We hypothesize that RS-fMRI is able to detect ketamine-induced alterations in large-scale network patterns and will identify changes in connectivity for (1) brain areas involved in ketamine's pharmacodynamic profile with respect to intended (analgesia) and side effects (most importantly psychedelic effects) and (2) areas involved in pain processing.

Methods

Subjects

Twelve healthy male volunteers (age 19-36 years; body mass index: 21-27 kg/m^2) were recruited to participate in the study after approval by the local ethics committee (Commissie Medische Ethiek, Leiden, The Netherlands). Oral and written informed consent was obtained from all participants and before participation all subjects received a physical examination. Exclusion criteria for participation were: age younger than 18 years or older than 45 years; a medical disease such as renal, liver, cardiac, vascular (including hypertension) or infectious disease; presence or history of a neurologic or psychiatric disease (*e.g.* increased cranial pressure, epilepsy, psychosis); glaucoma; obesity (body mass index greater than 30 kg/m^2); history of chronic alcohol or drug abuse; use of central-acting medication; presence of metal implants (*e.g.* pacemaker, hip/knee prosthesis, cochlear implants, vessel clips); claustrophobia.

Study design

The effect of ketamine on resting-state brain function was assessed using a single-blind, randomized, placebo-controlled cross-over study design with two occasions (at least 1 week between sessions). Subjects received S(+)-ketamine (Ketanest-S, Pfizer BV, Capelle a/d IJssel, The Netherlands) on one occasion and placebo (NaCl 0.9%) on the other. No details regarding treatment effects were given apart from the possibility of experiencing "drug high" during treatment.

Upon arrival subjects were given two intravenous lines (in separate arms), one for drug infusion and one for blood sampling. A baseline blood sample; baseline measurements for heat pain, nausea, vomiting and psychedelic effects; and a baseline anatomical (T1) and RS-fMRI scan were obtained. Next, drug infusion with either S(+)-ketamine or placebo was started at t = 0, at a low-dose (20 mg/70 kg/h) for 1 hour, followed by a high dose (40 mg/70 kg/h) for another hour. During infusion heat pain rating, nausea, vomiting and psychedelic effects were scored at 15-minute intervals using visual analogue scales as described below (sections "Subjective effects" and "Pain assessment"). RS-fMRI scans were obtained during the last 10 minutes of the low-dose and high-dose infusion period. After 2 hours, drug administration was terminated. Heat pain, nausea, vomiting and psychedelic effects were monitored at regular intervals for another 1.5 hours, and two more RS-fMRI scans were performed during the drug elimination phase. The study was registered in the Dutch Trial Register under number NTR2717 (www.trialregister.nl).

Blood sampling and S(+)-ketamine and S(+)-norketamine analysis

Venous blood was collected from a venous line inserted into the arm of the subject. Blood samples were obtained at fixed time point (t = 0, 15, 30, 60, 75, 90, 120, 130, 160 and 200 minutes) after the start of drug administration. Blood was centrifuged (3500 rotations per minute for 10 minutes) within 15 minutes after collection to separate the plasma. Plasma samples were stored at -25 °C until

analysis. For the construction of S(+)-ketamine and S(+)-norketamine calibration lines, solid substances were obtained from Parke-Davis (Dallas, TX) and Tocris (St. Louis, MO), respectively. S(+)-ketamine and S(+)-norketamine concentrations were determined by high-performance liquid chromatography on a Gemini C18 column (Phenomenex, Utrecht, The Netherlands) at 40 °C. The detection of both analytes in the eluent was performed at 195 nm with a photodiode-array-detector (PDA 100, Dionex, Amsterdam, The Netherlands). The lower limit of quantification was set at 10 ng/ml for both drugs.

Subjective effects
Psychedelic effects were scored at fixed time points (t = 0, 13, 28, 58, 73, 88, 118, 128, 158 and 198 min) after the start of drug administration. Psychedelic effects were monitored using visual analogue scales ranging from 0 cm (no effect) to 10 cm (maximum effect) of the Bowdle questionnaire.[13] Three factors of psychedelic effects can be derived from this questionnaire: drug high, internal perception and external perception.[14] Internal perception reflects inner feelings that do not correspond with the reality and is derived from questions regarding the hearing of unrealistic voices or sounds and having unrealistic thoughts and paranoid or anxious feelings. The external perception indicates a misperception of an external stimulus or change in the awareness of the subject's surroundings and is derived from questions regarding the change of body parts, the change of surroundings, the altered passing of time, the difficulty of controlling thoughts and the change in color and sound intensity.

Pain assessment
Heat pain stimuli were applied between imaging sessions. Heat pain was induced using the Pathway Neurosensory Analyzer (Medoc Ltd, Ramat Yishai, Israel) at fixed time point after the start of drug infusion (t = 0, 17, 32, 62, 77, 92, 122, 132, 162, 202 minutes). A fixed location on the skin of the volar side of the nondominant arm was stimulated with a 3 x 3 cm thermal probe. To quantify the pain intensity of the noxious stimulus, subjects scored the perceived pain using a computer-connected slider on an electrical potentiometer that ranged from 0 (no pain) to 100 mm (worst pain imaginable). This allows electronic monitoring of the visual analogue scale during heat pain stimulation. To induce heat pain, baseline temperature was set at 32 °C. Next, the temperature of the probe gradually increased (0.5 °C/s) towards a preset peak temperature, after which the temperature rapidly (6 °C/s) returned to baseline. A peak temperature causing a pain score between 60 and 70 mm was used during the study for evaluation of the analgesic effect of ketamine. Individual peak temperatures were determined on every experiment day before the start of drug administration.

Resting-state fMRI
Neuroimaging was performed on a 3-Tesla Achieva Scanner (Philips Medical System, Best, The Netherlands) at fixed time points (t = –30, 45, 105, 140, 170 minutes), where drug infusion was started at time-point t = 0. The neuroimaging protocol included a high resolution T1-weighted scan (repetition time = 9.7

ms, echo time = 4.6 ms, flip angle = 8 degrees, 256 x 256 x 140, isotropic resolution 2 mm, 4 minutes) and 5 RS-fMRI series (each 220 T2*-weighted, whole-brain volumes obtained with a gradient echo planar with repetition time = 2180 ms, echo time = 30 ms, flip angle = 80 degrees; 64 x 64 x 38, isotropic resolution 3.44 mm, 8 minutes). Because the subjects were taken out of the scanner between scans, and because only one anatomical T1-weighted image was acquired, each RS-fMRI was accompanied with a high resolution T2*-weighted echo planar (~ 30 seconds) in order to facilitate registering the RS-fMRI data to the anatomical image. During scanning, heart rate was monitored with a magnetic resonance imaging-compatible pulse oximeter (INVIVO MRI 4500, Siemens Healthcare, Erlangen, Germany). Respiratory rate was recorded and registered using a flexible pressure belt (Philips Medical System, Best, The Netherlands).

Data and statistical analysis

To assess the effect of ketamine *versus* placebo, a repeated measures analysis of variance was performed on pain scores, drug high effect, internal perception values and external perception with post-hoc t-testing with Bonferroni correction for multiple comparison. p-values < 0.05 were considered significant. Data are presented as mean ± SEM and 95% confidence interval (CI) unless otherwise stated. Statistical analysis was performed in SigmaPlot version 12.0 for Windows (Systat Software Inc., Chicago IL).

Each of the (12x5x2) RS-fMRI data series was preprocessed with motion correction, brain extraction, Gaussian smoothing, mean-based intensity normalization and high-pass temporal filtering with default software parameters. Then RS-fMRI data were normalized to a standard space by first registering the middle RS-fMRI volume to the high-resolution T2*-weighted image, which was registered to the subject's anatomical T1-weighted image, which was registered to the MNI152 standard template. These registration parameters were combined to obtain the parameters to put the RS-fMRI data in standard space. This produced standardized RS-fMRI data sets that were further analyzed to estimate functional brain connectivity. We applied a technique that we have used previously in a placebo-controlled crossover study involving alcohol and morphine.[10] In that study, we have shown that the proposed technique reveals drug-specific and regional changes in functional brain connectivity. This method parcellates the brain into eight networks of interest (NOIs) that are consistently present in model-free analysis of the RS-fMRI data.[15] These NOIs represent 80% of the total brain volume and include the medial (NOI1) and lateral (NOI2) visual network, the auditory and somatosensory network (NOI3), the sensorimotor network (NOI4), the default mode network (NOI5), the executive salience network (NOI6), the visual-spatial network (NOI7) and the working memory network (NOI8). We then defined functional connectivity as a measure of the correspondence of the RS-fMRI fluctuations in each brain voxel in relation to characteristic fluctuations of the RS-fMRI signal in each NOI. This correspondence is expressed in terms of regional z-scores of fitting an average NOIs RS-fMRI fluctuation. We refer to these statistical maps as RSN maps.

To examine the effect of ketamine over time on functional connectivity, voxel-wise statistics were performed on RSN maps. The analysis involved a mixed-effects general linear model with subject as random and time and drug as fixed within-subject variables. Because subjects received a pain stimulus between each RS-fMRI session, the corresponding pain relief scores (obtained 10 minutes before the RS-fMRI scan) were included as a regressor in the general linear model. This model accounts for possible confounding effects of pain per se on functional connectivity changes caused by ketamine. Voxel-wise statistical test runs the risk of false positive results because of the problem of multiple comparisons. However, true neuronal effects are likely to happen in adjacent voxels. For this reason, correction for multiple comparisons is conducted with a method that minimizes the chances of type I errors by examining both the magnitude and size of a cluster with effects in the same magnitude range.[16] Within this criterion, we set the corrected statistical significance to $p < 0.05$ after threshold-free cluster-estimation. In the post-hoc analysis, the highest value in the significant cluster (determined as explained above) was chosen to illustrate the connectivity change (z-score) over time for areas of interest. In all stages Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL 4.1, Oxford, UK) was used (www.fMRIb.ox.ac.uk/fsl).[17]

Results

Ketamine and norketamine concentrations

Plasma ketamine and norketamine concentrations are shown in figure 1. Peak ketamine and norketamine concentrations at the end of the first infusion hour were 74.9 ± 4.5 ng/ml and 26.7 ± 2.3 ng/ml, respectively. At the end of the second hour, peak ketamine and norketamine concentrations were respectively 187.5 ± 9.5 ng/ml and 93.0 ± 8.2 ng/ml. The relatively low variability in plasma concentrations indicates that the RS-fMRI data were obtained under stable ketamine and norketamine concentration conditions (between subjects).

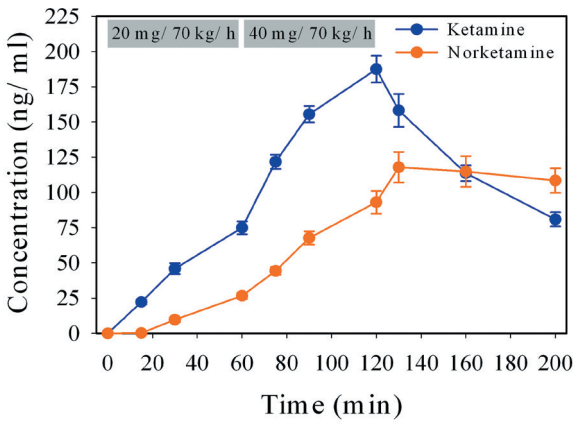

Figure 1. *The blood ketamine (blue circles) and norketamine (orange circles) concentrations in nanogram per milliliter. Ketamine was administered at 20 mg/70 kg in the first hour of infusion and at 40 mg/70 kg during the second hour. A rapid increase in blood ketamine concentration (peak 187.5 ± 9.5 ng/ml) and a slow increase in blood norketamine concentration (peak 117.9 ± 10.9 ng/ml) was observed.*

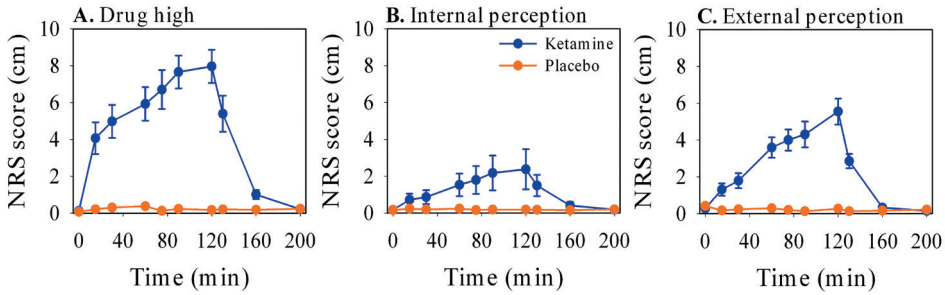

Figure 2. *Psychedelic effects observed during ketamine (blue circles) and placebo (orange circles) infusion presented as* ***A.*** *drug high,* ***B.*** *internal perception, which reflects inner feelings that do not correspond with reality and* ***C.*** *external perception, which reflects a misperception of an external stimulus or change in the awareness of the surroundings. Concentration-dependent psychedelic effects were observed. NRS = numerical rating scale.*

Subjective effects

Psychedelic effects were observed in all subjects, and scores are shown in figure 2. Mean drug high scores at 60 minutes after the start of drug infusion were 5.9 ± 0.9 cm (-0.5 to 12.4 cm) for ketamine and 0.4 ± 0.2 cm (-0.8 to 1.6 cm) for placebo ($p < 0.001$). At 120 minutes after the start of drug infusion, mean drug high scores were 8.0 ± 0.9 cm (1.8 to 14.1 cm) for ketamine compared to 0.2 ± 0.08 cm (-0.4 to 0.8 cm) for placebo ($p = 0.003$). Visual analogue scale scores for the outcome parameter internal perception for ketamine and placebo were, respectively, 1.6 ± 0.4 cm (-1.0 to 4.3 cm) *versus* 0.2 ± 0.1 cm (-0.5 to 0.9 cm; $p < 0.001$) at time point t = 60 and 2.3 ± 0.6 cm (-1.1 to 6.0 cm) *versus* 0.2 ± 0.07 cm (-0.3 to 0.7 cm; $p = 0.003$) at time point t = 120. Mean external perception visual analogue scale scores were 3.6 ± 0.8 cm (-1.3 to 8.5 cm) for ketamine and 0.3 ± 0.1 cm (-0.6 to 1.2 cm; $p < 0.001$) for placebo at time point t = 60 and 5.6 ± 1.0 cm (-1.0 to 12.1 cm) for ketamine and 0.3 ± 0.1 cm (-0.4 to 1.0 cm) for placebo at time point t = 120 ($p < 0.001$). Psychedelic effects rapidly returned to baseline (within 30 minutes) after termination of the drug infusion.

RSN connectivity on drug effect

Resting-state network connectivity changes caused by ketamine administration were observed in relation to NOI1 (medial visual network) and NOI3 (auditory and somatosensory network). Figure 3A shows the statistical map (threshold-free cluster enhancement corrected p-value < 0.05 in yellow) of the areas where the connectivity in relation to NOI1 was increased. Affected areas include the frontal lobe, thalamus, primary and secondary somatosensory cortex, occipital cortex, optic radiation, cerebellum, and the supramarginal gyrus. The statistical map (threshold-free cluster enhancement corrected p-value < 0.05 in dark blue) of the areas involved in the cortical and subcortical connectivity decreases in relation to NOI3 is shown in figure 3B. The connectivity changes in the cortex in many areas, with the largest effects in the occipital cortex, the anterior and posterior cingulate cortex, orbital frontal cortex and insular cortex. The connectivity changes in subcortical areas were observed in the basal ganglia and limbic areas. Details

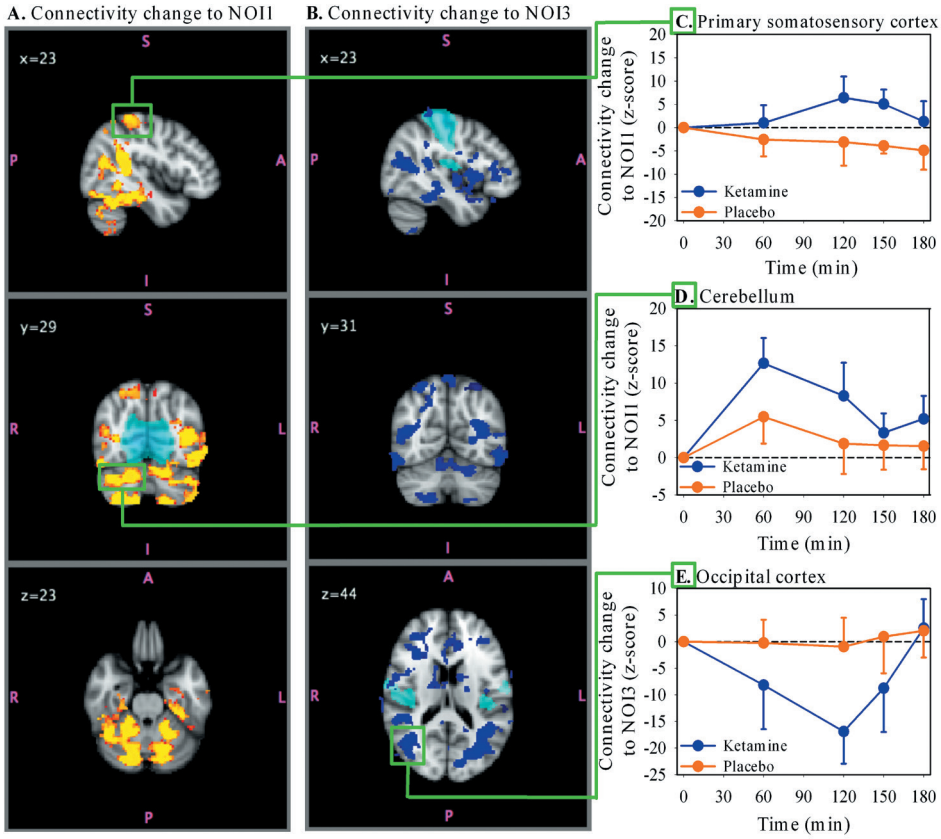

Figure 3. *Statistical maps of the ketamine induced* ***A.*** *increase in resting-state network (RSN) connectivity (yellow) in relation to network of interest 1 (NOI1; light blue) and* ***B.*** *decrease in RSN connectivity (dark blue) in relation to NOI3 (light blue; cluster P-value < 0.05). The ketamine effect (blue) on connectivity over time is shown for* ***C.*** *the primary somatosensory cortex,* ***D.*** *the cerebellum and* ***E.*** *the occipital cortex. A = anterior; I = inferior; L = left; P = posterior; R = right; S = superior.*

regarding cluster size, t-value of the cluster peak and cluster peak location of the affected areas are provided in table 1. The ketamine effects over time on the primary somatosensory cortex and the cerebellum in relation to NOI1 are shown in figure 3C en 3D respectively; the effect over time for the occipital cortex in relation to NOI3 is shown in figure 3E.

RSN connectivity on pain processing

Mean baseline pain scores are shown in figure 4A and were 63.9 ± 4.9 mm (30.6 to 97.2 mm) for the ketamine study day *versus* 62.3 ± 4.9 mm (28.8 to 95.7 mm) for the placebo study day ($p = 0.621$). Corresponding testing temperatures were 48.7 ± 0.6 °C (44.9 to 52.5 °C) and 48.4 ± 0.7 °C (43.7 to 53.0 °C) for ketamine and placebo respectively ($p = 0.296$). At the end of the first infusion hour (t = 60 min), 20.1% ketamine-induced pain relief was observed with mean pain scores of

Table 1. *Ketamine effect on resting-state network connectivity (cluster P-value < 0.05)*

	Location	Cluster size (voxels)	t-value	x	y	z
NOI1: Medial Visual Network Includes: calcarine, inferior precuneus and primary visual cortex. Relays visual input through thalamus to primary visual area.	L Thalamus	11	3.30	45	53	37
	R Frontal lobe	40	3.61	20	89	29
	R Cerebellum	21098*	4.17	36	22	14
	*cluster also includes:					
	R Primary somatosensory cortex		4.42	23	47	64
	L Secondary somatosensory cortex		4.34	75	57	45
	L Occipital cortex		3.82	64	29	41
	L Optic radiation		4.40	62	41	46
	R Supramarginal gyrus		5.28	19	42	42
NOI3: Auditory and somatosensory network Includes: superior temporal cortex, insula, operculum, dorsocaudal anterior cingulate cortex, somatosensory cortices and bilateral thalamus.	R Hippocampus	5	3.18	30	54	25
	L Precuneus cortex	12	2.85	48	40	67
	R Primary motor cortex	16	4.02	41	50	71
	L Orbitofrontal cortex	17	3.52	56	77	23
	L Premotor cortex	126	4.27	55	53	72
	R Middle temporal gyrus	28971*	5.64	16	51	33
	*cluster also includes:					
	R Thalamus		2.76	43	51	40
	L Cerrebellum		3.75	48	27	28
	L Primary auditory cortex		2.68	70	52	38
	L Caudate nucleus		3.69	51	64	45

Table 1. *Ketamine effect on resting-state network connectivity (continued)*

	Location	Cluster size (voxels)	t-value	x	y	z
NOI3: Auditory and somatosensory network Includes: superior temporal cortex, insula, operculum, dorsocaudal anterior cingulate cortex, somatosensory cortices and bilateral thalamus.	*cluster also includes (continued):					
	R Caudate nucleus		4.45	42	69	39
	L Anterior cingulate cortex		3.76	46	61	52
	L Lateral occipital cortex		4.67	72	31	27
	L Posterior cingulate cortex		4.28	50	52	53
	L Amygdala		3.19	60	63	26
	L Superior longitudinal fasciculus		4.48	62	48	53
	R Insular cortex		4.36	24	67	35
	R Occipital cortex		3.78	17	30	26

Voxel dimension is 2 mm x 2 mm x 2 mm (voxel volume 0.008 ml); L = left; NOI = network of interest; R = right.

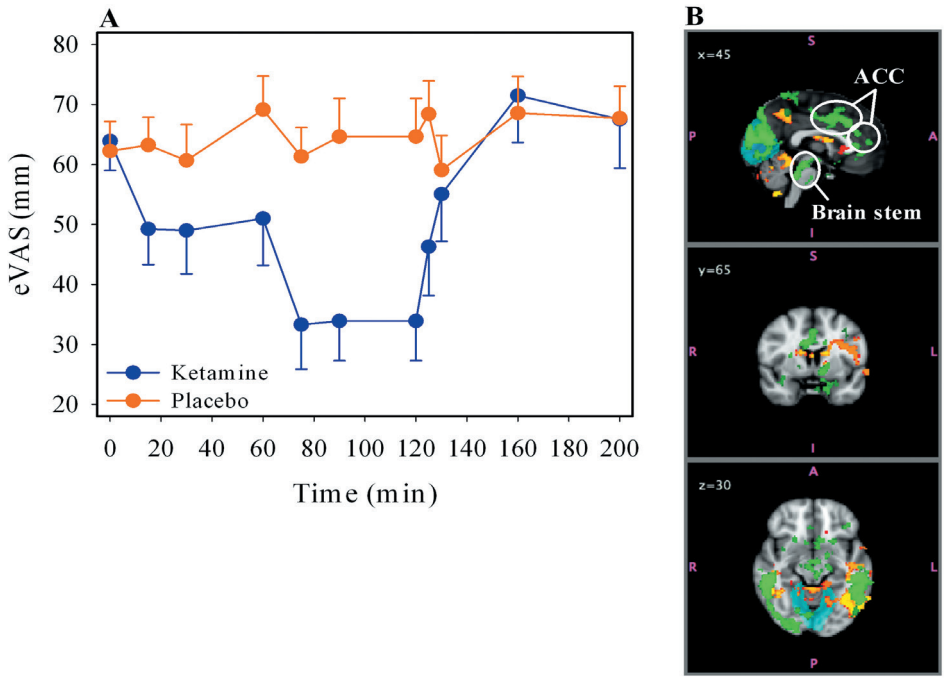

Figure 4. A. *Pain scores to the fixed heat stimulus during and after ketamine (blue circles) and placebo (orange circles) infusion. Concentration dependent analgesia (46.9% at t = 120 minutes) was observed during ketamine infusion, which rapidly disappeared after termination of the infusion. No analgesia was observed during the placebo infusion.* ***B.*** *Statistical maps of the variations in connectivity explained by the drug effect (yellow) and by pain relief (green). The network of interest is represented in light blue. A = anterior; ACC = anterior cingulate cortex; eVAS = electronical visual analogue scale; I = inferior; L = left; P = posterior; R = right; S = superior.*

51.0 ± 7.8 mm (9.6 to 92.4 mm) for ketamine compared to 69.2 ± 5.6 mm (31.2 to 107.2 mm; $p = 0.027$) for placebo. Mean pain scores for ketamine and placebo at t = 120 (end of second infusion hour) were 33.9 ± 6.6 mm (-7.2 to 75.0 mm) and 64.7 ± 6.4 mm (21.5 to 107.8 mm; $p = 0.001$) respectively, with a pain relief of 46.9% by ketamine. During the drug elimination phase, ketamine pain scores rapidly returned to baseline (in approximately 20 minutes).

Since we included pain scores as a regressor in the general linear model, connectivity maps were obtained that assist in separating brain areas affected by ketamine from regions involved in the processing of pain. Figure 4B illustrates the statistical map of regions where the amount of connectivity variations is explained by the drug effect (threshold-free cluster enhancement corrected p-value < 0.05 in yellow) and by the fluctuations in pain scores (threshold-free cluster enhancement corrected p-value < 0.05 in green). Using this model, an increase in RSN connectivity explained by pain processing was observed only in relation to NOI1 for the anterior cingulate cortex, insula, orbitofrontal cortex and the brain stem. No decrease in RSN connectivity was observed for any of the NOIs.

Discussion

In the last decade, neuroimaging studies increasingly focused on the spontaneous RS-fMRI signal to study large-scale brain interactions within RSNs.[11,12,18] RS-fMRI can be used to evaluate the effect of psychoactive drugs on RSN connectivity.[10,19] In this study, we assessed the effect of low-dose S(+)-ketamine on brain connectivity. The use of low-dose (*i.e.* subanesthetic) ketamine has increased significantly since 1990, mainly for treatment of chronic neuropathic pain syndromes (and since a few years for the treatment of therapy-resistant depression).[1] Thus, knowledge on the effect of low-dose ketamine on brain areas involved in pain processing is of importance, providing additional insight into the mechanisms of action of this increasingly popular analgesic.

Ketamine acts via antagonism of the excitatory glutamatergic NMDA receptor. The NMDA receptor has a high expression in the temporal cortex, hippocampus, basal ganglia, cerebellum and brain stem, all regions significantly affected by ketamine in our study.[20,21] Ketamine most significantly affected the cerebellum (relative to NOI 1 and 3). The cerebellum has an important role in motor learning and coordination,[22] and we showed previously in rats that ketamine produces motor dysfunction.[23] Because of its connections with nonmotor cortical and subcortical areas, including the limbic system and prefrontal cortex, the cerebellum is also thought to play a role in emotional processing (mainly anxiety) and thought coordination.[22] Therefore, alterations in connectivity of the cerebellum (increases to NOI1 and decreases to NOI3) may be related to some of the psychedelic side effects observed during ketamine infusion. Large connectivity changes were also observed in the visual cortex and the optic radiation to NOI1, which may explain the visual hallucinations observed during ketamine infusion and perhaps the symptoms of blurry and double vision.[5,24,25] Additional connectivity changes to NOI3 were observed in the (pre)frontal cortex, orbitofrontal cortex, temporal cortex and gyrus, anterior and posterior cingulate cortex, thalamus and precuneus cortex. In agreement with our observations, task-related fMRIs, evaluating ketamine-induced psychedelic effects as model for schizophrenia, showed similar activity changes.[3,5,24-30] Indeed, ketamine has long been recognized as a model for schizophrenia because many of the ketamine-induced psychedelic side effects show similarities with the positive (psychotic symptoms like hallucinations) and negative (emotional blunting, lack of initiation) symptoms of schizophrenia.[26]

Ketamine decreased resting-state connectivity in most of the known pain-processing related structures to NOI3, including the thalamus, amygdala, insula, anterior and posterior cingulate cortex, orbitofrontal cortex and primary and secondary sensory cortices. The observed effect on the amygdala (which is part of the limbic system) may explain ketamine's effect on the loss of the affective component of pain. Our findings are in close agreement with positron emission topography and task-related fMRI studies, which show the involvement of the brainstem, thalamus, amygdala, insula, anterior and posterior cingulate cortex, orbitofrontal cortex and the sensorimotor cortices in pain processing.[31-33] Overall,

these data indicate that RS-fMRI is a reliable and relatively simple (more efficient) method for identifying how drugs affect the brain. A single RS-fMRI study can recognize all pharmacologically affected regions in the brain simultaneously in contrast to task-related fMRI studies.

Including pain scores as regressor in the statistical model for testing the ketamine *versus* placebo fingerprint on the brain revealed brain areas whose connectivity was explained by pain processing aside from drug effect. Pain relief scores were associated with increased connectivity in relation to NOI1 in the anterior cingulate cortex, orbitofrontal cortex, insula and brain stem, all of which are regions involved in pain sensing and processing.[31-33] Various studies have demonstrated the involvement of these same brain areas in descending inhibition of pain.[34-39] Descending inhibition of pain is a modulatory system originating at spinal and supraspinal sites, modifying afferent pain signal propagation. Examples of this system are conditioned pain modulation (which is the central inhibition of a focal pain stimulus by administering a second noxious stimulus at a remote area), placebo analgesia and stress-induced analgesia.[34,35] These top-down pain modulatory pathways dampen pain signal propagation at the level of the spinal cord dorsal horn and are thought to be defective in various chronic pain states.[40,41] The current study suggests a modulatory role for ketamine on descending pain inhibition. We previously tested the effect of ketamine treatment on endogenous pain modulation in patients with chronic pain and observed an increase in descending inhibition as tested by conditioned pain modulation (unpublished observation by Marieke Niesters MD MSc, Leiden, The Netherlands; April 2011). Our current study corroborates the hypothesis that ketamine is able to influence endogenous pain modulation.

An important potential of the current technique lies in the development of new NMDA receptor antagonists for treatment of chronic pain. By applying the current RS-fMRI paradigm, new agents can be evaluated on their effects on the brain areas currently identified as involved in analgesia *versus* those involved in the side effects of ketamine, the most important, potent and prototypical NMDA receptor antagonist currently available. One such agent could be traxoprodil (Pfizer, NYC, NY), a selective NR2B NMDA receptor antagonist.[21] In rats, we previously showed that this drug produces analgesic effects similar to that of ketamine but without significant side effects (such as absence of motor dysfunction and agitation). It would be of interest to assess whether this drug is effective in humans and whether the lack of side effects coincides with the absence of alterations in activity (connectivity) in the cerebellum, frontal cortex and visual cortex. The current study provides support for the use of the RS-fMRI technique to evaluate even newer NMDA receptor antagonists, which may allow prediction of the toxicity-efficacy balance prior to application in patients.

Only a limited number of drugs has been tested using RS-fMRI.[10,19,42-44] Previously we assessed the effect of morphine and alcohol in healthy volunteers using the same paradigm as applied in the current study.[10] Morphine influenced

resting-state connectivity in all NOIs, with the most extensive effects between NOI4 and NOI6 and the thalamus, brain stem, insula, putamen and cerebellum, some of which are areas involved in descending inhibition of pain. Alcohol effects were limited, with the most important changes relative to NOI1, NOI3 and NOI4 (areas: posterior parietal cortex, cerebellum, brain stem and visual cortex). Although some overlap in connectivity changes was present between morphine and alcohol *versus* ketamine, the overall connectivity change pattern observed after ketamine administration was different from that of morphine and alcohol. Further RS-fMRI studies include a study with psilocybin, a psychedelic found in mushrooms, which at a dose causing changes in consciousness (sedation) produced a decrease in connectivity between medial prefrontal cortex and posterior cingulate cortex. This may be related to its psychedelic effects.[19] Anesthetic doses of propofol caused changes in corticocortical and thalamocortical connectivity relative to frontoparietal networks. These propofol changes correlated linearly with the level of consciousness.[42-44] Although we do not know the biologic meaning of the observed connectivity changes, the finding of drug and state-of-consciousness specific changes in connectivity are plausibly related to drug-specific neuronal modulation (*i.e.* a drug-specific fingerprint of the brain).

In the current study we applied two low (subanesthetic) doses of ketamine. In previous studies we showed that in volunteers and patients these doses had no effect on the level of consciousness.[45-47] We cannot exclude that some (minor) sedative effects did occur in our subjects that may have affected our results. However, we did not find any changes in brain connectivity relative to the default- and executive-control networks. Connectivity changes relative to these frontoparietal networks play an important role in the generation of sedation and unconsciousness.[45,46] Both respiratory stimulation and depression have been reported after ketamine administration.[48-50] Changes in carbon dioxide concentration may affect the cerebral blood flow and possibly RS-fMRI connectivity globally in the brain. In our study, we observed no changes in cerebral blood flow in the areas of the brain where we report connectivity changes, as measured by arterial spin labeling (data not shown). Furthermore, we measured respiratory frequency during imaging and observed no changes during ketamine infusion relative to placebo. Finally, we incorporated breathing frequency as a regressor in our statistical model. This did not affect the ketamine-induced changes in RS-fMRI connectivity values.

Conclusions

In conclusion, RS-fMRI is a useful and efficient method for assessing drug effect on the brain. In the current study, this was exemplified by assessing the effect of the NMDA receptor antagonist ketamine on resting-state brain connectivity in healthy volunteers. Low-dose ketamine induced connectivity changes in brain areas involved in motor function, psychedelic effects and pain processing. With respect to pain processing, ketamine's analgesic effect may arise from multiple pathways. We observed a decreased connectivity in regions of the pain matrix responsible for the perception of pain (pain sensing) and the affective processing

of pain. In addition, ketamine affected connectivity in brain areas involved in endogenous pain inhibition.

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Chapter 7

Effect of Deafferentation from Spinal Anesthesia on Pain Sensitivity and Resting-state Functional Brain Connectivity in Healthy Male Volunteers

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Introduction

Deafferentation is the disruption of afferent and efferent signals between the central and peripheral nervous system.[1] Several experimental human and animal studies show that when peripheral sensory and motor input is removed (for example by application of ischemia or local anesthetic-induced nerve blocks, cutaneous anesthesia or peripheral nerve damage) detectable functional changes in the cortex occur.[1-6] Also subcortical areas, including the thalamus, show changes upon deafferentation.[1,7-9] These changes are best described as reorganization of neuronal interactions due to a rebalancing of excitatory and inhibitory factors that mediate adaptation and neuronal plasticity.[3] For example, the loss of sensory and motor input from the hand by peripheral nerve blockade is associated with supraspinal excitatory changes possibly mediated by disinhibition of unmasked (interhemispheric) cortical neuronal connections, and explains the enhanced functionality of the contralateral hand.[4,5] These cortical changes coincide often with perceptueal changes.

One form of deafferentation that is performed yearly in millions of patients world-wide is spinal anesthesia where the sensory information from the lower part of the body is temporary removed to allow surgical intervention without the perception of pain. It is well known that spinal anesthesia may coincide with sensory distortions. For example, some patients report body image distortions (such as swelling of the legs, illusionary limb position and changes of the length of the limbs) during regional (including spinal) anesthesia.[10-13] Additionally, the affected limbs are often perceived as warm upon the administration of the local anesthetic, while some patients perceive paradoxical heat sensations above the level of the anesthetic block (*i.e.* a cold stimulus is perceived as warm) during the assessment of the spread of the anesthetic.[12,14] These observations are typically made during the initial rapid rise of the anesthetic level and are suggestive of changes in central sensory modulation, possibly related to the deafferentation from the spinal block. There is further the observation that epidural anesthesia (another form of deafferentation) can lead to occurrence of painful sensations in the deafferented area in an otherwise healthy individual.[15] Existing evidence presented above suggests that deafferentation from spinal anesthesia would lead to a change in functional organization of cortical and subcortical networks involved in sensory motor perception and pain. Possibly the altered sensory perceptions during spinal anesthesia and functional changes in cortical and subcortical areas of the brain are causally related. Some evidence to that suggestion comes from data in patients where hyperexcitability of thalamic neurons coincides with neuropathic deafferentation pain.[13]

A well-known paradigm to evaluate the efficacy of the endogenous pain modulatory system is "conditioned pain modulation" or CPM.[17,18] The CPM paradigm assumes that adding afferent nociceptive input at a remote area of the body inhibits the intensity of primary focal pain stimulus ("pain inhibits pain") through activation of supraspinal centers including the anterior cingulate cor-

tex (ACC), the insula and the prefrontal cortex. Therefore, it would be plausible that blockade of afferent input would have the reverse effect on pain perception. This means that if afferent input becomes "disconnected", then pain perception would become more intense. However, there are no human studies assessing the effect of pain perception on areas remote from deafferentation sites (such as pain perception on the arm during spinal anesthesia). We aim to use this model of acute deafferentation by spinal anesthesia in healthy participants to further understand the mechanisms involved in endogenous modulation of pain.

Our placebo (sham-spinal anesthesia), crossover, randomized study investigates (1) whether pain perception above the level of the anesthetic is altered by spinal deafferentation, and (2) whether we can detect an coinciding change in resting-state functional connectivity of cortical and thalamic networks in healthy humans. The thalamus was chosen as region of interest as it is an important pain modulatory center that receives input from multiple ascending pain pathways and projects to various (sensory and affective) pain modulatory regions of the cortex and limbic system.[19,20] In the current study we obtained repeated resting-state functional magnetic resonance images (RS-fMRI) in two sessions (spinal and sham-spinal peripheral anesthesia). It has been shown that this technique can be reliably used to evaluate alterations in intrinsic brain connectivity following pharmacological interventions in humans, and deafferentation in rats.[21-24]

Methods

Subjects

Twelve right-handed, healthy, male volunteers (age: 23.7 ± 3.4 years (mean ± SD); body mass index: 21.3 ± 2.4 kg/m^2) were enrolled in the study after approval by the local ethics committee of the Leiden University Medical Center in Leiden, the Netherlands. All participants gave oral and written informed consent. The study was performed according to GCP guidelines and the ethical principles for medical research involving human subjects of the International Association of the Study of Pain (http://www.iasp-pain.org/Education/Content.aspx?ItemNumber=1213) and according to the Declaration of Helsinki, (http://www.wma.net/en/30publications/10policies/b3/; amended in 2013). Before participation, all subjects were screened to exclude the presence or history of any disease or their inability to maintain a regular diurnal rhythm, the presence or history of alcohol or drug abuse, the presence of metal implants (*e.g.* pacemaker, hip or knee prosthesis, cochlear implants, vessel clips) and claustrophobia. Additional exclusion criteria included: < 18 or > 45 years of age and a body mass index > 30 kg/m^2. The study was registered in the Netherlands' Trial Register (NTR at www.trialregister.nl) under number NTR3491.

Study design
The study was performed using a randomized crossover design. Upon arrival, an intravenous line was placed in the right hand to allow fast administration of emergency medication when necessary. Next, baseline anatomical MRI (T1-weighted) and baseline RS-fMRI scans were obtained followed by baseline heat pain measurements. After baseline measurements were complete, subjects received an intrathecal injection with a local anesthetic on one occasion and a sham procedure on the other as described below (time of injection is t = 0). Responses to heat pain and the height of the sensory block (measured by the response to a cold 4 cm^2 surface applied to the skin in the left and right mid-axillary line) were measured at 15-minute intervals. Additional RS-fMRI scans were obtained 1 and 2 hours after the spinal injection or sham procedure. At the end of the study, subjects were monitored until fully recovered from the spinal anesthetic, as defined by return of motor functions and diuresis, and then allowed to go home.

Intrathecal injection and sham procedure
The intrathecal injection was performed at the interspace between vertebrae L3 and L4 with 3 mL bupivacaine 5 mg/mL (AstraZeneca, Zoetermeer, the Netherlands) after the skin was locally infiltrated with 1-2 mL lidocaine 10 mg/mL (AstraZeneca, Zoetermeer, the Netherlands). For the spinal puncture a 27 Gauge Whitacre needle (Vygon, Valkenswaard, the Netherlands) was used to minimize the risk of post-spinal headache. The sham procedure was performed by insertion of a spinal needle at the interspace between vertebrae L3 and L4 through the skin, after the skin was locally infiltrated with 1-2 mL lidocaine 10 mg/mL. The dura mater was not punctured and no bupivacaine was injected. An independent anesthesiologist, who was not involved in conducting or analyzing other measurements made during the study, performed the injections. The instructions to the subject were similar on both occasions so that the subject and the investigators did not know which treatment was given at the moment of injection.

Pain assessment
Heat pain was induced on the lower part of the non-dominant arm with a 3 x 3 cm thermal probe of the Pathway Neurosensory Analyzer (Medoc Ltd., Ramat Yishai, Israel). Baseline temperature was set at 32 °C. During heat pain tests the temperature of the probe gradually increased (1.5 °C/s) towards a pre-set destination temperature that was held constant for 30 seconds and then rapidly returned (6 °C/s) to baseline temperature. To quantify pain intensity of the heat pain stimulus, subjects rated the perceived pain stimulus using a computer-connected slider on an electrical potentiometer that ranged from 0 mm (no pain) to 100 mm (worst pain imaginable). This allowed for continuous electrical monitoring of the visual analogue scale during the noxious stimulation. The target temperature of the heat stimulus was determined at the start of each study day and was intended to evoke an electronic visual analogue scale (eVAS) of 40 mm. To evaluate pain responses after the intrathecal injection or sham procedures, pain tests were applied between imaging sessions at fixed time points: t = 15, 30,

45, 90, 105 and 150 minutes.

Resting-state functional magnetic resonance imaging acquisition
A 3-Tesla Achieva Scanner (Philips Medical System, Best, The Netherlands) was used to acquire functional data at fixed time points (baseline, t = 60 and t = 120 min). The neuroimaging protocol included a high-resolution T1-weighted scan (repetition/echo time = 9.7/4.6 ms, flip angle = 8 degrees, 1 mm isotropic, 4 min) and 3 RS-fMRI series (each 220 T2*-weighted whole-brain volumes, obtained with a gradient echo planar with repetition/echo time = 2180/30 ms, flip angle 80 degrees, 3.44 mm isotropic, duration 8 min; subjects were instructed to keep their eyes open and relax). A high resolution T2*-weighted scan (~ 30 seconds) was acquired at the end of each repeated RS-fMRI in order to facilitate registering the functional data to the anatomical image.

RS-fMRI analysis
The following pre-statistics processing was applied using FSL software on all individual RS-fMRI scans: motion correction; registration to standard space by applying 6 rigid-body transformations between RS-fMRI and high-resolution T2*, and high resolution T1, followed by an affine registration to the MNI152 template with 2 mm resampling;[25] brain extraction; spatial smoothing using a 5-mm full width at half-maximum Gaussian kernel; grand-mean intensity normalization; and high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with a 0.01 Hz cut-off).

Functional connectivity was assessed using two different approaches. First, to evaluate the general effects of deafferentation from spinal anesthesia on functional brain connectivity, we used a model-free analysis of eight predefined networks of interest (NOIs) as previously described by Khalili-Mahani *et al*.[22] These canonical networks represent 80% of the total brain volume and are described based on their general function as the medial and lateral visual network, the auditory and somatosensory network, the sensorimotor network, the default mode network, the executive salience network, the visual-spatial network and the working memory network.[26] As we have previously shown for morphine, alcohol, δ(9)-tetrahydrocannabinol and ketamine, RS-fMRI data analysis using these networks reveals localized and drug-specific changes in functional brain connectivity.[21-24]

The second functional connectivity analysis focused on functional connectivity in relation to the thalamus. The thalamus was chosen as it receives projections from multiple ascending pain pathways, is involved in processing nociceptive information, and projects the information to various parts of the limbic and cortical structures involved in sensory discriminative and the affective dimensions of pain perception.[19] We used 7 thalamic subregions according to the Oxford thalamic connectivity atlas.[27] Our choice of this atlas is motivated by our principle to develop objective, easily reproducible and standardized procedures for replication studies. The important advantage of this atlas is that it is constructed

based on probabilistic diffusion tractography that describes the probability of corticothalamic white matter fibers connection between thalamic subregions and cortical segments (prefrontal cortex, temporal cortex, pre-motor cortex, primary motor cortex, sensory cortex, posterior-parietal cortex and the occipital cortex). We refer to the resulting functional networks as the thalamo-prefrontal, thalamo-premotor, thalamo-primary motor, thalamo-sensory, thalamo-parietal, thalamo-occipital network, and thalamo-temporal network to indicate the reference region.

In both analyses, we used a dual regression analysis to define resting-state networks (RSNs) in relation to reference regions (the 8 canonical NOIs or the 7 subthalamic segments).[28] Briefly, dual regression involves multiple-regression of RS-fMRI time-series against several NOIs or thalamic subregions to estimate a representative vector of BOLD fluctuations within each reference region, and next regressing the RS-fMRI time-series against the time vector to identify spatial representations of RSNs, *i.e.* brain areas with similar fluctuations patterns as the reference regions. Nuisance variables corresponding to fluctuations in the deep white matter (measured from the center of the corpus callosum) and cerebrospinal fluid (measured from the center of the lateral ventricles) were included in the dual regression analysis to account for non-specific and physiological variations.[29] This resulted in statistical maps of z-scores, where each voxel of the brain represents the functional connectivity between that voxel and each of the NOIs or the thalamic subregions. These statistical maps were later used for voxel-wise inference testing of the spinal anesthesia on each network.

Data, power and statistical analyses

To quantify pain intensity, the area-under-the-curve (AUC) of each eVAS response was calculated and presented relative to the baseline measurement.[30] The study was powered to detect a 50% treatment difference in the eVAS AUC at peak spinal level (estimated SD 35%, alpha = 0.05, 1-beta = 0.9). The effect of spinal anesthesia on pain perception was tested by a repeated measures analysis of variance with *post-hoc* Bonferroni correction on the AUC values relative to baseline. The statistical analysis was performed in SigmaPlot version 12.0 (Systat Software Inc., Chicago, IL) and p-values < 0.05 were considered significant. Data are presented as mean ± SEM unless otherwise stated.

To determine the effect of deafferentation from spinal anesthesia on resting-state functional connectivity a mixed-effects analysis was applied with subject as random and time and drug as fixed within-subject variables. Voxel-wise statistical analysis on the z-score connectivity maps was performed using a permutation-based statistical inference with 5000 permutations. Statistical significance was set at p-value < 0.05 after family wise error cluster-based correction (with cluster forming voxelwise thresholds set at $p < 0.01$).[31] To further control for spurious effects, we report clusters that included a minimum of 10 adjacent voxels. We also performed a stepwise regression (without and with pain score as regressor in the model) to examine brain regions whose connectivity was modulated

by the subjective perception of pain. In all stages of MRI analyses the FMRIB Software Library was used (FSL 4.1, Oxford, United Kingdom).[25]

Results

Spinal anesthesia
All subjects completed the study without the occurrence of major side effects. Peak sensory blockade was achieved after 45 minutes with 17.5 ± 1.0 blocked segments corresponding to a sensory block level from dermatomes S5 to Th5. This sensory block persisted throughout the whole study period. The mean time of spinal anesthesia to full recovery of diuresis and motor function was 369 ± 11 minutes. No sensory blockade was observed after the sham procedure in any of the subjects. The spinal anesthetic and sham procedure did not result in significant cardiorespiratory changes. Blood pressure remained within 5% of control values. Due to the absence of spinal block following the sham procedure, blinding of the study was rapidly lost to both investigators and volunteers.

Pain responses
The mean eVAS responses prior to treatment and at peak spinal anesthetic level and sham experiments are given in figure 1A and B. Spinal anesthesia significantly increased pain sensitivity on the skin of the lower forearm. Mean AUC values at baseline were 844.7 ± 63.2 mm·sec on the study day with spinal injection and 898.6 ± 122.6 mm·sec on the day of the sham procedure ($p = 0.644$). Mean AUC values at peak spinal level were 1165.0 ± 71.0 mm·sec after spinal injection and 877.1 ± 105.8 mm·sec after the sham procedure ($p = 0.005$). Mean AUC values over time are shown in figure 1C showing that the hyperalgesic responses lasted for at least 3 hours (end of the study). There was no effect of study order on the pain AUC values (Fig. 1D).

Effect of spinal anesthesia on predefined general resting-state networks
Spinal anesthesia induced significant changes in functional connectivity in relation to three of the eight canonical NOIs: the medial visual network (increase), the somatosensory network (decrease) and the default mode network (increase). Regions that demonstrate functional connectivity changes in relation to these three networks are given in table 1 and include amongst others the thalamus, primary somatosensory cortex, primary motor cortex, premotor cortex, anterior cingulate cortex, caudate nucleus and the cerebellum. Figure 2A demonstrates the statistical connectivity map (cluster corrected; $p < 0.05$) of the brain areas with a decrease in functional connectivity in relation to the somatosensory network. The effects of spinal anesthesia on functional connectivity over time for the premotor cortex, primary somatosensory cortex and thalamus are shown in figure 2B-D. Details regarding cluster size, z-score and location of the areas that show functional connectivity changes are provided in table 1. Adding treatment order (sham first *vs.* spinal first) as a covariate to the statistical model did not affect the number, extent and location of these regions.

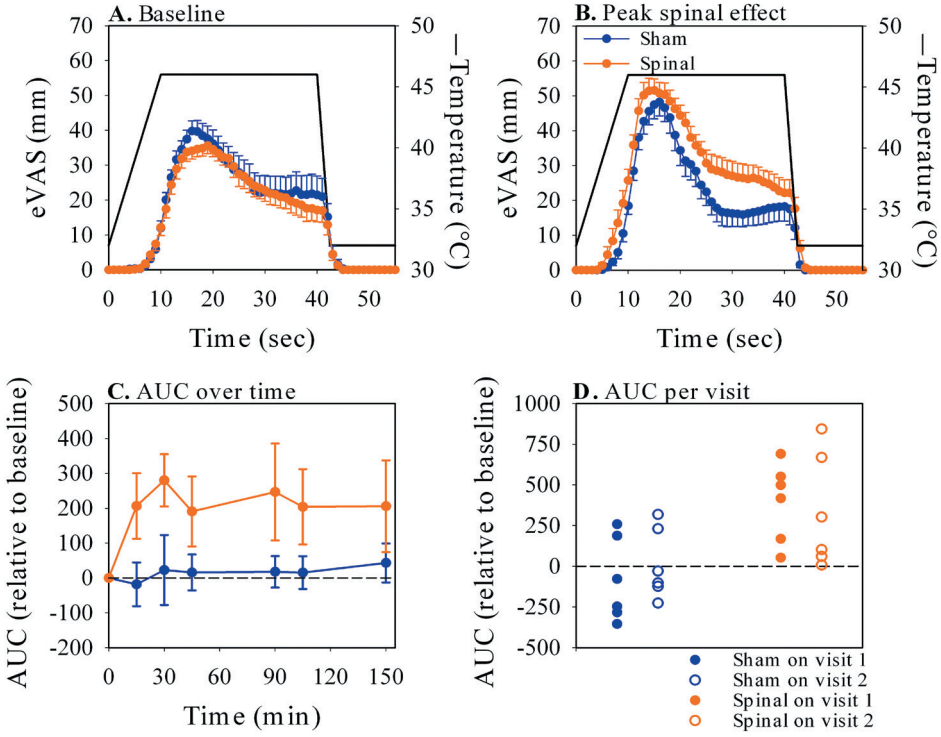

Figure 1. A. *Pain responses upon thermal stimulation on the skin of the lower arm at baseline and* ***B.*** *at peak deafferentation effect (45 minutes).* ***C.*** *Pain presented as area-under-the-curve (AUC) relative to baseline over the whole study period. The orange circles represent the pain sensitivity during spinal anesthesia; the blue circles represent the pain perception after the sham procedure. Spinal anesthesia induced a significant increase in pain sensitivity ($p < 0.001$).* ***D.*** *Pain responses in subjects receiving sham (placebo) treatment on visit 1 or spinal treatment on visit 1 (closed symbols), and the pain responses of the second visit for sham and placebo (open symbols). No order effect was observed. eVAS: electronic visual analogue scale.*

7

Thalamic resting-state networks

Cortico-thalamic connectivity maps are shown in figure 3A. To evaluate whether anatomically distinguishable networks were produced by the dual regression analyses of the thalamic subregions, average functional connectivity maps were obtained for each thalamic resting-state network. This was done by first thresholding and binarizing each functional connectivity map at a z-score > 4.0 and next computing a probability map (with probabilities of connectivity > 50%). Figure 3B represents the average functional connectivity probability maps of the RS-fMRI data acquired at baseline for all 7 thalamic subregions. With one exception, all thalamic subregions were functionally connected to cortical areas as expected according to the anatomical atlas. The exception was one thalamic subregion that instead of predominantly connecting to the premotor cortex (as expected from the atlas) demonstrated functional connectivity to the occipital cortex and cerebellum (Fig. 3B (yellow areas)).

Table 1. *Effect of spinal anesthesia on functional connectivity in relation to the general resting-state networks (cluster P-value < 0.05)*

	Location	Cluster size (voxels)	Cluster *p*-value	*z*-score	x	y	z
Medial Visual Network Includes: calcarine, inferior precuneus and primary visual cortex. Relays visual input through thalamus to primary visual area.	L Caudate nucleus	1737*	0.04	5.0	50	70	34
	*cluster also includes:						
	B Anterior cingulate cortex			3.3	43	81	36
	B Paracingulate gyrus			3.8	48	86	36
	L Nucleus accumbens			4.2	49	67	32
	L Frontal pole			4.2	61	92	37
Auditory and somatosensory network Includes: superior temporal cortex, insula, operculum, dorsocaudal anterior cingulate cortex, somatosensory cortices and bilateral thalamus.	L Putamen	4964§	0.006	-4.1	57	72	31
	§cluster also includes:						
	B Thalamus			-3.5	53	51	42
	R Primary somatosensory cortex			-2.5	32	42	64
	R Primary motor cortex			-2.2	29	50	64
	B Premotor cortex			-4.9	45	57	61
	R Caudate nucleus			-3.6	38	65	44
	L Occipital lobe	3354	0.02	-2.6	54	29	33
	B Frontal pole	2537#	0.03	-2.5	47	90	30
	#cluster also includes:						
	B Anterior cingulate cortex			-5.2	43	67	54
	B Paracingulate gyrus			-2.6	43	87	34
Default mode network Includes: rostral medial prefrontal cortex and precuneal and posterior cigulate cortex areas	B Cerebellum	2269**	0.03	2.9	52	29	10
	**cluster also includes:						
	B Brain stem			2.4	45	44	17

Voxel dimension is 2 mm x 2 mm x 2 mm (voxel volume 0.008 ml); regions can be located within or outside the resting-state network; B = bilateral; L = left; R = right

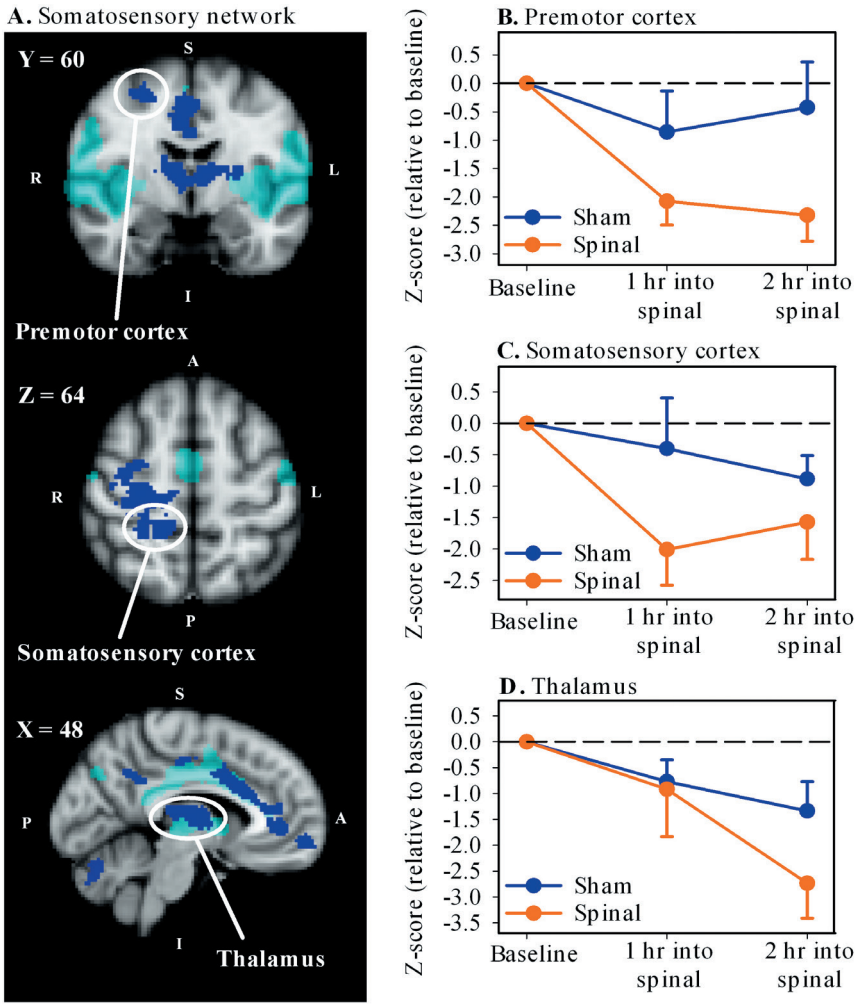

Figure 2. A. *Statistical connectivity map ($p < 0.05$; cluster corrected) of the decrease in resting state network connectivity (dark blue) induced by spinal anesthesia in relation to the auditory and somatosensory network (light blue). The effect of spinal anesthesia on connectivity over time is shown for the* ***B.*** *premotor cortex,* ***C.*** *somatosensory cortex and* ***D.*** *the thalamus. A: anterior; I: inferior; L: left; P: posterior; R: right; S: superior.*

Effect of spinal anesthesia on thalamic resting-state networks

Spinal anesthesia induced a significant increase in functional connectivity in relation to three of the seven thalamic networks: the thalamo-prefrontal, the thalamo-parietal and the thalamo-temporal network. These networks are involved in the sensory discriminative (*i.e.* pain intensity) and affective components of pain.[19] Regions that show connectivity changes in relation to the three networks are given in table 2 and include (partly) similar regions observed in the general RSN analysis: thalamus, primary somatosensory cortex, primary motor cortex

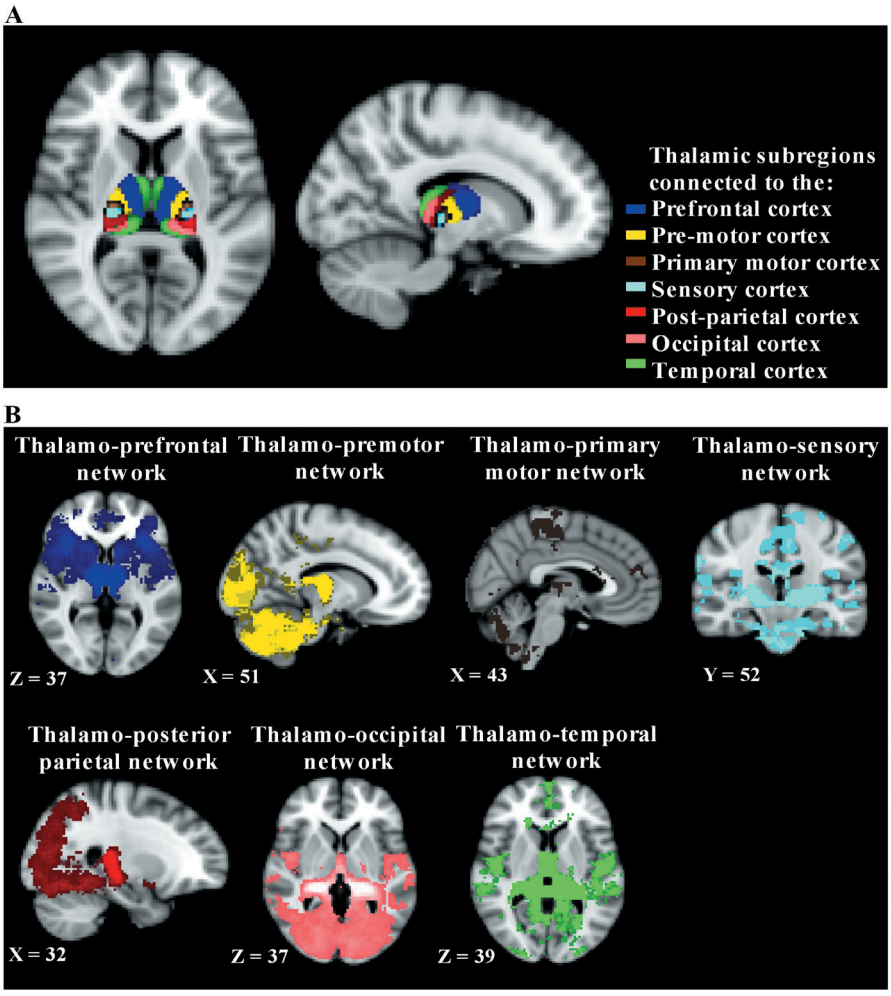

Figure 3. A. *Illustrative map of the 7 thalamic subregions used for connectivity analysis based on the oxford thalamic anatomic connectivity probability atlas.* ***B.*** *Probabilistic connectivity map of the seven thalamic resting-state networks at baseline (> 50% probability that a functional connection between the thalamic subregion and the cortex was present at Z > 4.0). All thalamic subregions (except for the region that anatomically connects to the premotor cortex) demonstrated functional brain connectivity to parts of the cortex to which they should connect according to the anatomical atlas.*

and ACC. Additional affected regions are the insula, precuneus cortex, the frontal lobe and the posterior cingulate gyrus. The effect of deafferentation from spinal anesthesia in relation to the thalamo-prefrontal network is presented in the statistical connectivity map of figure 4A, which shows a significant increase in thalamic connectivity (in red) overlapping the subregion of the thalamus that functionally and anatomically connects to the prefrontal cortex (in green). The main effect of treatment over time in this thalamic region is shown in figure 4B.

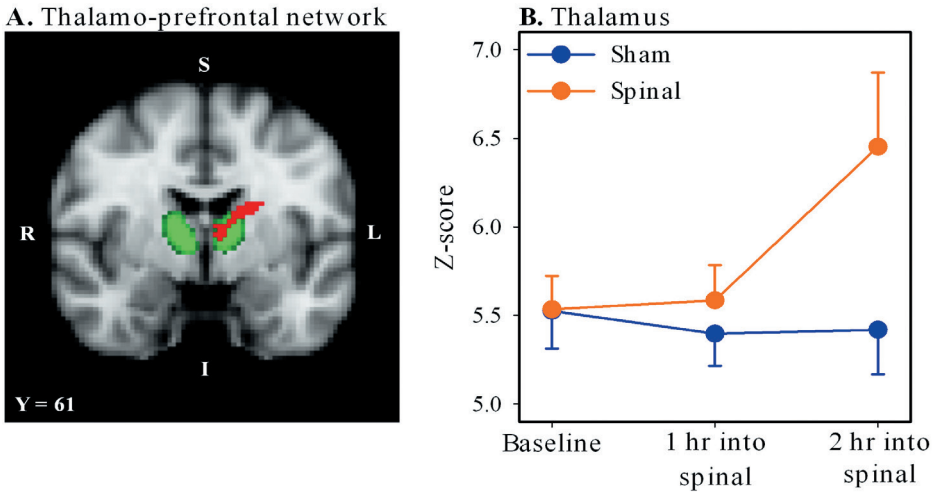

Figure 4. A. *Statistical connectivity map ($P < 0.05$; cluster corrected) of the spinal anesthesia induced increase in resting-state network connectivity in relation to the thalamo-prefrontal network. It shows the subregion of the thalamus that functionally and anatomically connects to the prefrontal cortex (green) and the thalamic area with increased connectivity due to deafferentation (red).* ***B.*** *The effect of spinal anesthesia on connectivity over time is shown for the thalamic subregion anatomically and functionally connected to the prefrontal cortex. I: inferior; L: left; R: right; S: superior.*

The significant effect from deafferentation from spinal anesthesia in relation to the thalamo-posterior parietal network is presented in the statistical map of figure 5A. The main effect of treatment over time for the ACC, the posterior cingulate gyrus and the insula is given in figure 5B-D, with significant treatment changes during the complete course of measurement. Details regarding cluster size, z-score and location of the affected areas are provided in table 2. Study order did not affect the location and extent of these clusters.

Correlations between pain and functional resting-state connectivity

Table 2 lists brain areas whose connectivity was altered by including the absolute AUC pain scores as a covariate in the permutation testing (*i.e.* connectivity changes increased with greater pain scores). Illustrative examples of significant correlations observed between functional connectivity changes and pain responses are given in figure 6 for the thalamus (in relation to the thalamo-prefrontal network, *i.e.* intra-thalamic), ACC and insula (both in relation to the thalamo-parietal network).

Discussion

Our hypothesis that spinal deafferentation would enhance pain sensitivity was confirmed by our finding that nociceptive stimuli applied to dermatomes above the level of spinal deafferentation were perceived as hyperalgesic. This observa-

Table 2. *Effect of spinal anesthesia on functional connectivity in relation to the thalamic resting-state networks (cluster p-value < 0.05)*

	Regions that show connectivity changes in relation to the thalamic networks	**Cluster size (voxels)**	**Cluster *p*-value**	***z*-score**	**x**	**y**	**z**
Thalamo-prefrontal network Includes: thalamus, prefrontal cortex, anterior cingulate cortex, insula, brain stem, caudate nucleus, putamen and pallidum	B Frontal pole	7478*	0.01	3.6	65	85	29
	*cluster also includes:						
	L Frontal lobe			2.1	32	65	62
	B Premotor cortex			2.5	67	62	60
	B Paracingulate cortex			4.4	47	76	57
	L Thalamus†			6.5	48	58	41
	L Caudate nucleus			4.7	51	71	38
Thalamo-parietal network Includes: thalamus, posterior parietal cortex with part somatosensory cortex and occipital cortex	B Posterior cingulate gyrus†	3678#	0.04	2.5	45	48	55
	#cluster also includes:						
	B Precuneus cortex†			2.1	42	32	57
	R Insula†	7312‡		3.0	25	61	29
	‡cluster also includes:						
	R Anterior cingulate cortex†			2.8	43	75	46
	L Caudate nucleus			2.1	53	69	43
	L Orbitofrontal cortex			2.6	60	81	30
	B Frontal pole†			3.8	60	90	45
Thalamo-temporal network Includes: thalamus, temporal lobe, pre-parietal lobe with premotor and primary motor cortex, precuneus cortex, hippocampus and paracingulate cortex	B Frontal pole	5359§	0.01	2.7	28	82	43
	§cluster also includes:						
	L Anterior cingulate cortex			3.7	40	65	56
	R Primary motor cortex			2.4	26	50	63
	B Premotor cortex			2.6	31	56	63
	B Parietal lobe			2.1	26	34	62

Voxel dimension is 2 mm x 2 mm x 2 mm (voxel volume 0.008 ml); regions can be located within or outside the resting-state network; B = bilateral; L = left; R = right; † Regions that disappear when pain is added as covariate to the statistical model.

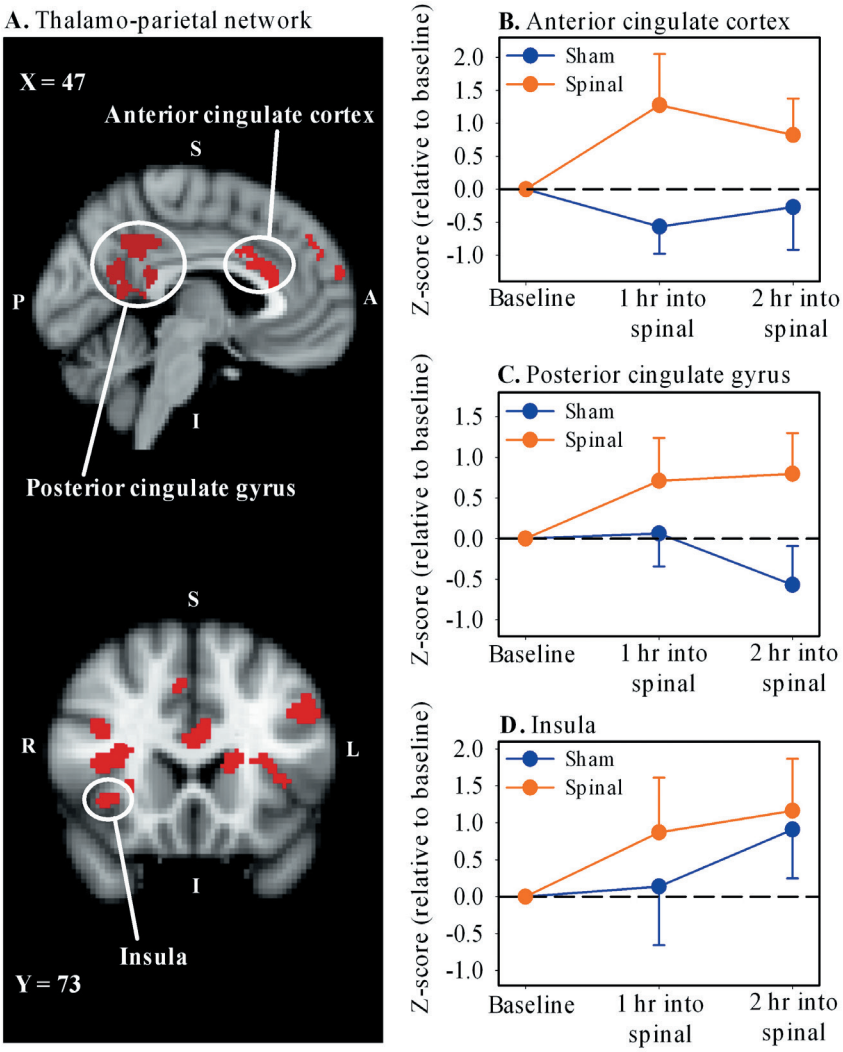

Figure 5. A. *Statistical connectivity map (P < 0.05; cluster corrected) of the spinal anesthesia induced increase in resting-state network connectivity in relation to the thalamo-parietal network.* ***B.*** *The effect of spinal anesthesia on connectivity over time is shown for the anterior cingulate cortex,* ***C.*** *the posterior cingulate gyrus and* ***D.*** *the insula. A: anterior; I: inferior; L: left; P: posterior; R: right; S: superior.*

tion is suggestive of transient central cortical and subcortical changes in neuronal organization. Indeed, we observed spinal deafferentation-induced connectivity changes in brain networks involved in the sensory discriminative dimension (*e.g.* thalamus, insula and somatosensory cortex) and in the affective dimension (*e.g.* brainstem, thalamus, insula and ACC) of pain perception from two independent analyses of canonical NOIs and thalamic networks.[32,33] Furthermore, the increased pain sensitivity at non-deafferentated skin areas was correlated to

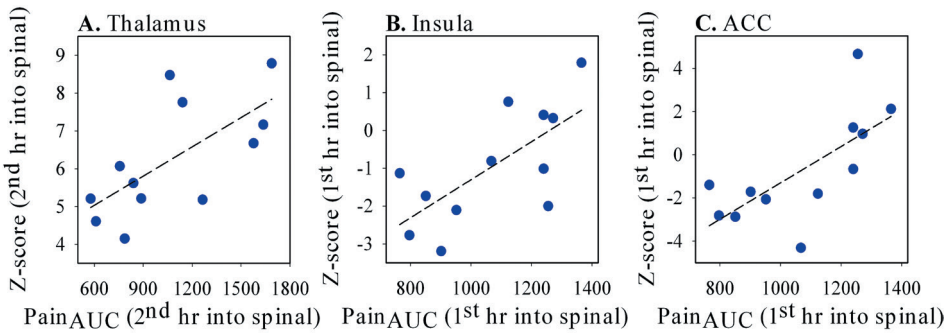

Figure 6. *Scatterplots of the pain response area-under-the-curve scores in relation to the absolute connectivity Z-scores for* ***A.*** *the thalamic subregion functionally connected to the prefrontal cortex,* ***B.*** *the insula and* ***C.*** *the anterior cingulate cortex (ACC).*

thalamo-cortical connectivity changes within the thalamus, ACC and insula. Our findings are in agreement with earlier animal and human studies showing that deafferentation is associated with changes in neuronal organization in the cortex and subcortical areas. These changes are associated with warm and referred sensations, perceptual illusions, neuropathic pain and enhanced sensorimotor function of non-deafferented areas.[2,4-6,12-16,34-36]

Effect of deafferentation on canonical resting state networks

In the current study, the RS-fMRI technique was successfully used to evaluate deafferentation-induced changes in brain connectivity in awake humans. The changes in RSN connectivity induced by the symmetric spinal deafferentation included areas involved in the sensory discriminative components of pain perception (sensory cortex, (pre)motor cortex, brainstem, thalamus) and the affective dimension of pain (insula, caudate nucleus, frontal pole, ACC, thalamus, brainstem and cerebellum), in relation to the medial visual network (increase in RSN connectivity), the somatosensory network (decrease) and the default mode network (increase) (see Table 1).

In two previous studies in an anesthetized rat model the effects of traumatic peripheral nerve- or spinal cord injury-related deafferentation were studied using RS-fMRI.[1,37] Both studies show changes in connectivity between the thalamus and cortical and subcortical areas of the brain (*e.g.* the primary somatosensory cortex). The authors argue that these changes are related to the loss of inhibitory influences within these brain neuronal networks. There is general agreement in the literature that deafferentation causes a rebalancing of excitatory and inhibitory neuronal activity towards disinhibition exposing formerly masked excitatory connections.[1-7,9,37] Krupa et al.[8] further show that also feedback from cortex to thalamus plays an important role in plastic changes due to deafferentation (see also ref. 3). These deafferentation-related changes may be adaptive and due to alterations in neuronal activity, such as due to reduced GABAergic inhibitory activity and/or enhanced glutamatergic excitatory activity, or due to changes in

microcirculation, where reduced afferent input changes the neurovascular coupling.[1,38,39] Synaptic sprouting and development of structural changes between brain areas takes more time to develop and seems to play a role in chronic deafferentation (in SCI, peripheral nerve injury or amputation).[1,35,36] Given the fact that we are unable to determine from the RS-fMRI analyses whether changes in connectivity coincide with increases or decreases in neuronal activity, attribution of the observed changes in RS-fMRI connectivity during spinal anesthesia to a shift from inhibitory towards excitatory nociceptive pathways is currently at best speculative.

We observed changes in connectivity relative to medial visual, somatosensory, and default mode networks. The reason for the selective association of spinal deafferentation with connectivity changes relative to these specific canonical networks cannot be deduced from our study. Possibly compared to the other networks, these networks are most sensitive to loss of peripheral afferent input. Irrespective of the mechanism, we argue that the observed changes may cause specific behaviors associated with neuraxial blockade. For example, epidural anesthesia is associated with block-height dependent sedation and reduced brainstem auditory evoked potentials.[40] Further, several studies show that neuraxial blockade coincides with sedation and consequently reduced (volatile and intravenous) anesthetic requirements.[41,42] These effects may be related to connectivity changes relative to the default mode and medial visual networks.[43,44] Particularly the default mode network seems important in altered states of consciousness (anesthesia, coma, vegetative state, epileptic loss of consciousness and somnambulism).[43,45] We did not measure the arousal state in our study. Due to this limitation we cannot conclude whether in our population a change in arousal state occurred. Changes observed relative to the somatosensory network may be associated with nociceptive sensations (warm sensation/paradoxal heat sensation, and as observed here: hyperalgesia) and illusions of abnormal bodily position and recognition.[12,13]

Effect of deafferentation on pain responses and thalamic resting state networks

An important observation of this study is that pain sensitivity increased during spinal deafferentation. Similar observations were made in rats following experimental spinal cord injury (SCI) where allodynia is perceived at dermatomes above the transection level in a majority of animals.[46,47] Gerke et al.[47] further showed increased spontaneous firing of thalamic neurons in rats following SCI. Several other studies show spatio-temporal changes and neuronal hyperactivity in the thalamus upon deafferentation (either in experimental animal models or in patients with deafferentation pain), with augmented connections between the primary somatosensory cortex and the thalamus.[37,48] Consistent with these findings, we observed changes in functional connectivity within the thalamus in our general RSN analysis (Fig. 2D). The more specific analysis of the thalamic subnetworks revealed significant increases in connectivity between the thalamus and regions of the brain involved in sensory and affective pain processing and perception (Figs. 4 and 5; Table 2). This indicates the importance of neuronal ac-

tivity changes in the thalamus upon deafferentation. Importantly, the enhanced pain sensitivity was also correlated with the thalamic RSN connectivity (Table 2). Positive correlations were observed between pain scores and intrathalamic and thalamo-cortical (involving the ACC and insula) functional connectivity (Figs. 6A-C), suggestive of a causal role for these networks in enhancement of pain sensitivity during acute deafferentation.

Interestingly, several brain areas that we identified in the hyperalgesic responses to deafferentation (*e.g.* thalamus, insula and ACC) are involved in endogenous modulation of pain, where activation of these supraspinal brain areas causes either facilitation or inhibition of afferent nociceptive input at the level of the spinal cord dorsal horn.[18,33] This suggest that spinal anesthesia-induced deafferentation causes the shift of the endogenous pain system towards pain facilitation. Our findings therefore support the CPM paradigm as we now observed that blockade of afferent inputs (*i.e.* the reverse of the CPM paradigm) enhances pain sensitivity.[17,18] Of interest is that You et al.[49] identified the medio-dorsal subregion of the thalamus of the rat in being involved in pain facilitation as part of the endogenous pain modulatory system. This region corresponds to the human thalamic subregion anatomically connected to the prefrontal cortex (Fig. 4). Several studies on chronic (deafferentation) pain syndromes have also observed altered functionality in these same brain regions. For example, Apkarian et al.[50] showed that chronic low back pain was associated with abnormalities (*i.e.* loss) of the thalamus (and prefrontal) gray matter density. Spinal cord injury in primates leads to a functional reduction of the GABAergic inhibitory circuitry of the thalamus, and in humans, abnormal thalamic bursting patterns and abnormal activity patterns in the ACC were observed following spinal cord injury.[51-54] Knowledge on the mechanism of both afferent and efferent signaling pathways is important for our understanding of the (ab)normal perception of pain and may lead to new insights for the treatment of pathological pain syndromes. Speculating that the enhanced pain sensitivity we observed in dermatomes above the deafferentation level is associated with excitatory changes in thalamo-cortical connectivity, a therapy focused on inhibition of these excitatory networks may be indicated. For example, pain relief may occur by reconstituting GABAergic inhibitory activity, or inhibition of glutamatergic excitatory activity. Indeed, recent studies indicate that the *N*-methyl-D-aspartate receptor antagonist ketamine induces long-term relief of neuropathic pain by improving descending pain inhibition, possibly via a central inhibitory effect on excitatory pathways.[24,55,56]

Blinding

The inability of blinding the anesthetic treatment in both subjects and investigators in our study is inevitable with the procedure and paradigm in question. Anticipation is a critical aspect of subjective pain perception and it is plausible that awareness of subjects of the nature of the effect of the spinal injection could have affected the study outcome. We controlled for possible experimental order effects and deblinding by including the order effect in our statistical model. In our small sample, we did not observe any order effect on the subjective scoring

of pain intensity (Fig. 1D), nor did we find an effect on the RS-fMRI results. This, however, is not generalizable and some effect due to differences in the attention to the thermal pain in spinal *vs.* sham sessions cannot be excluded.[57,58] Possibly such an interoceptive effect became visible in the insula signal at 2-h into the sham spinal (Fig. 5D).[59]

The insula

In this study we focused on the thalamus in relation to other brain areas to explain the observations of hyperalgesia following spinal analgesia. We are aware that other important pain areas of the brain were involved in the effect of spinal deafferentation on pain sensitivity, such as the insula and ACC. The insula is involved in the sensory and affective dimensions of pain perception as well as in the processing and modulation of interoceptive sensations.[33,59,60] Although not part of our initial protocol, we performed a secondary analysis on the effect of spinal deafferentation on the functional connectivity in relation to the insula using a similar approach as presented for the thalamus network on the complete left and right insula (as a seed region). We observed that deafferentation changed connectivities between the insula and several brain areas including the ACC, frontal cortex and hippocampus (increased connectivity) and cerebellum, occipital cortex and brainstem (decreased connectivity) (Fig. 7 and Table 3). Interestingly, connectivity changes did increase when subjects had greater pain scores although the effect size was not as large as observed for the thalamus networks (data not shown). These data indicate that apart from an effect on pain intensity, deafferentation changes the pain affect and possibly also interoceptive sensations via changes in functional connectivities in the mentioned insula networks. Since the insula is topographically organized, further studies are needed to assess the deafferentation effect on networks relative to specific insula subregions.

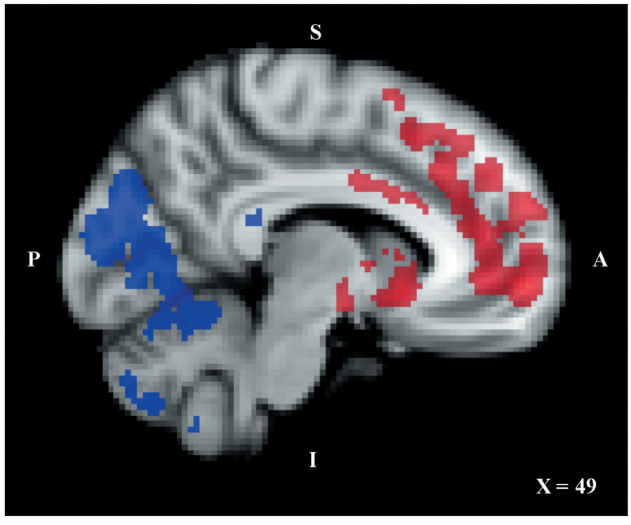

Figure 7. *Statistical connectivity map ($p < 0.05$; cluster corrected) of the increase (red) and decrease (blue) in resting state network connectivity induced by spinal anesthesia in relation to the insula.*

Table 3. *Effect of spinal anesthesia on functional connectivity in relation to the insula (cluster p-value < 0.05)*

	Regions that show connectivity changes in relation to the insula	**Cluster size (voxels)**	**Cluster *p*-value**	***z*-score**	**x**	**y**	**z**
Regions that show an increased connectivity in relation to the insula	B Frontal pole	5648	0.006	4.6	47	92	34
	B Temporal pole	2814	0.006	5.0	64	71	21
	B Insula	1310	0.006	5.7	30	73	32
	B Caudate nucleus	140	0.006	4.0	38	71	42
	B Paracingulate gyrus	98	0.006	3.1	38	86	36
	B Superior frontal gyrus	61	0.006	3.5	50	68	68
	B Anterior cingulate gyrus	57	0.006	3.3	47	62	51
	B Frontal Pole	42	0.006	3.2	59	92	42
	L Hippocampus	27	0.006	3.9	58	52	28
Regions that show an decreased connectivity in relation to the insula	B Cuneus	11132	0.003	4.7	39	21	46
	B Brain stem	1227	0.003	4.1	43	38	15
	B Cerebellum	840	0.003	3.7	52	22	13
	B Cerebellum	60	0.003	3.1	38	39	21
	B Cerebellum	45	0.003	3.2	56	20	22
	R Lateral occipital cortex	22	0.003	3.2	26	30	50

Voxel dimension is 2 mm x 2 mm x 2 mm (voxel volume 0.008 ml); B = bilateral; L = left; R = right

Conclusions

Deafferentation from spinal anesthesia is associated with connectivity changes in the brain involving both cortical and subcortical areas. Furthermore, spinal anesthesia enhanced pain sensitivity that was correlated to enhanced connectivity patterns of the thalamus, anterior cingulate cortex and insula, areas associated with endogenous modulation of pain and the sensory and affective dimensions of pain perception.

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Chapter 8

Summary & Conclusions

Summary

Endogenous pain modulation is a complex phenomenon involved in the perception of pain. It consists of top-down inhibitory and facilitatory pathways that originate at higher sites within the central nervous system and converge at dorsal horn neurons in the spinal cord, to modulate incoming afferent nociceptive information. Dysfunction of inhibitory pain pathways or a shift in the balance between pain facilitation and pain inhibition has been associated with the development of chronic pain. This thesis describes the effect of several central-acting drugs on descending control of pain in both healthy volunteers and chronic pain patients to further understand the underlying mechanism of endogenous pain control in health and disease.

In **chapter 2** the effect of the *N*-methyl-D-aspartate (NMDA) receptor antagonist ketamine on endogenous pain modulation was investigated in healthy volunteers. Ten healthy subjects (4 men/6 women) received an 1-hour placebo or S(+)-ketamine (40 mg/70 kg) infusion on two separate occasions in random order. Upon termination of the infusion the capacity to recruit descending pain inhibitory pathways was evaluated using two experimental or surrogate biomarkers for endogenous modulation of pain: conditioned pain modulation (CPM) and offset analgesia (OA). After placebo treatment significant inhibition of pain responses was present for CPM and OA. In contrast, after ketamine infusion no CPM response was observed, but rather a significant facilitatory pain response ($p < 0.01$); the OA response remained unchanged. These findings indicate that the balance between pain inhibition and pain facilitation was shifted by ketamine towards pain facilitation and suggest a modulatory involvement of the NMDA and/or other glutamatergic receptors at some level within the endogenous pain system. The absence of an effect of ketamine on OA indicates the presence of different mechanisms and neurotransmitter influences underlying OA and CPM and suggests that OA and CPM differ in their susceptibility for glutamatergic influences.

In contrast to CPM, the relatively new phenomenon offset analgesia had only been described in young healthy volunteers. In **chapter 3**, we explored OA in a large population consisting of several age categories and in ten chronic neuropathic pain patients. We defined OA by the reduction in electronic pain score upon the 1 °C decrease in noxious heat stimulus relative to the peak pain score. OA was present in healthy volunteers irrespective of age and sex (pain score decrease = 97 ± 1% (mean ± SEM), which suggests that OA is fully developed at the age of 6 years and does not undergo further maturation. In contrast, a reduced or absent offset analgesia response was observed in neuropathic pain patients (pain score decrease = 56 ± 9% *vs.* controls 98 ± 1%, $p < 0.001$). This indicates that chronic neuropathic pain patients are unable to modulate changes in pain stimulation with perseverance of pain perception where healthy subjects display signs of strong analgesia. Whether the altered OA responses contribute to the chronification of pain or are a consequence of the chronic pain process remains unknown

and requires further study. Intravenous treatment with ketamine, morphine and placebo had no effect on OA responses in patients despite sharp reductions in spontaneous pain scores, which suggests that the NMDA and μ-opioid receptors are less likely to be involved in OA mechanisms. Possibly, not central but peripheral sites may be involved in the altered offset analgesia responses in these patients.

Chapter 4 describes the effect of ketamine and morphine on CPM responses in chronic pain patients. CPM responses were obtained in 10 neuropathic pain patients (2 men/8 women), with peripheral neuropathy as defined by abnormal quantitative sensory testing. Patients were treated with S(+)-ketamine (0.57 mg/kg/h for 1 hour) and morphine (0.065 mg/kg/h for 1 hour) in a randomized, placebo-controlled double-blinded study. CPM was measured at baseline and 100 minutes after the start of treatment. Without treatment no CPM was detectable, which indicated that the descending pain inhibitory properties within this group of chronic pain patients were diminished. Treatment with ketamine, morphine and placebo produced significant CPM responses of respectively 40.2 ± 10.9%, 28.5 ± 7.0% and 22.1 ± 12.0% with no statistical difference in magnitude of CPM among treatments. However, the magnitude of the CPM responses correlated positively with the magnitude and duration of spontaneous pain relief observed after treatment. This suggests a role for CPM engagement of descending pain inhibition in analgesic efficacy of ketamine, morphine and placebo treatment in chronic neuropathic pain patients.

In **chapter 5** the effect of long-term treatment with the new analgesic tapentadol is described. Tapentadol is an analgesic agent for treatment of acute and chronic pain that activates the μ-opioid receptor combined with inhibition of neuronal noradrenaline reuptake. Both mechanisms are implicated in activation of descending inhibitory pain pathways. Twenty-four patients with diabetic polyneuropathy were randomized to receive daily treatment with tapentadol sustained-release (average daily dose 433 ± 31 mg) or placebo for 4 weeks. CPM and OA responses were measured before and on the last day of treatment. Prior to treatment none of the patients had significant CPM or OA responses. After 4 weeks of treatment, CPM was significantly activated by tapentadol slow-release (SR) and coincided with significant analgesic responses. CPM increased from 9.1 ± 5.4% (baseline) to 14.3 ± 7.2% after placebo treatment and 24.2 ± 7.7% after tapentadol SR treatment ($p < 0.001$ *vs.* placebo). Relief of spontaneous pain was also greater in patients treated with tapentadol than placebo ($p = 0.028$). Neither placebo nor tapentadol SR treatment had an effect on the magnitude of the OA responses ($p = 0.78$). These results show that patients with painful diabetic polyneuropathy who display absent CPM responses benefit from tapentadol, which induces pain relief coupled to (re)-activation of descending inhibitory pain pathways.

A relatively new approach in central nervous system drug research is resting-state fMRI (RS-fMRI), which measures intrinsic network interactions of the brain in

rest (*i.e.* not task-related). In **chapter 6** the effect of low-dose S(+)-ketamine on intrinsic brain connectivity was investigated. We aimed to identify brain regions involved in ketamine's pharmacodynamic profile with respect to intended (analgesia) and side effects (most importantly psychedelic effects) and areas involved in pain processing. Twelve healthy, male volunteers received a 2-hour intravenous S(+)-ketamine infusion (first hour 20 mg/70 kg, second hour 40 mg/70 kg). Before, during and after S(+)-ketamine administration resting-state brain connectivity was measured. Additionally, heat pain tests were performed in-between imaging sessions to determine ketamine-induced analgesia. Ketamine increased the connectivity in the cerebellum and visual cortex in relation to the medial visual network. A decrease in connectivity was observed in the auditory and somatosensory network in relation to regions responsible for pain sensing and the affective processing of pain, which included the amygdala, insula, and anterior cingulate cortex. Connectivity variations related to fluctuations in pain scores were observed in the anterior cingulate cortex, insula, orbitofrontal cortex and the brain stem, which are all regions involved in descending inhibition of pain. This study demonstrated that RS-fMRI is a useful and efficient method to assess drug effects on the brain. Low-dose ketamine induced connectivity changes in brain areas involved in motor function, psychedelic effects and pain processing. With respect to pain processing, ketamine's analgesic effect may arise from multiple pathways. We observed a decreased connectivity in regions of the pain matrix responsible for the perception of pain (pain sensing) and the affective processing of pain. Additionally, ketamine affected connectivity in brain areas involved in endogenous pain inhibition.

Descending (efferent) pain pathways are important for the normal perception of pain. However, little is known on the effect of afferent pain pathways on pain modulation. In **chapter 7**, the effect of spinal deafferentation on pain sensitivity was studied and linked to whole-brain functional connectivity as assessed by RS-fMRI. Deafferentation was induced by spinal or sham anesthesia (spinal: 15 mg bupivacaine injected at L3-4; sham: no puncture of the dura mater) in 12 male volunteers. Resting-state brain connectivity was determined in relation to 8 predefined and 7 thalamic resting-state networks and measured before, and 1 and 2 hours after spinal or sham injection in a cross-over study design. To measure the effect of deafferentation on pain sensitivity, responses to heat pain were measured at 15-minute intervals on non-deafferented skin and correlated to the RS-fMRI connectivity data. Spinal anesthesia altered functional brain connectivity within brain regions of the sensorimotor system and pain matrix in relation to somatosensory and thalamic resting-state networks. A significant enhancement of pain sensitivity on non-deafferented skin was observed after spinal anesthesia compared to sham (area-under-the-curve (mean ± SEM)): 190.4 ± 33.8 *versus* 13.7 ± 7.2; $p < 0.001$), which significantly correlated to functional connectivity changes observed within the thalamus in relation to the thalamo-prefrontal network, and in the anterior cingulate cortex and insula in relation to the thalamo-parietal network. This study demonstrated that deafferentation from spinal anesthesia was associated with rapid connectivity changes in the brain involving both cortical

and subcortical areas. These changes are best described as reorganization of neuronal interactions due to a rebalancing of excitatory and inhibitory factors that mediate adaptation and neuronal plasticity. Furthermore, spinal anesthesia enhanced pain sensitivity that was correlated to enhanced connectivity patterns of the thalamus, anterior cingulate cortex and insula, which are all areas associated with endogenous modulation of pain.

Comparison with the literature

In order to compare the results of this thesis to published data, a PubMed search was performed to identify studies evaluating the effect of central-acting drugs on CPM in healthy volunteers and chronic pain patients. From all relevant studies, on the condition that adequate quantitative data were presented, standardized effect sizes were calculated using the statistical program Comprehensive Meta

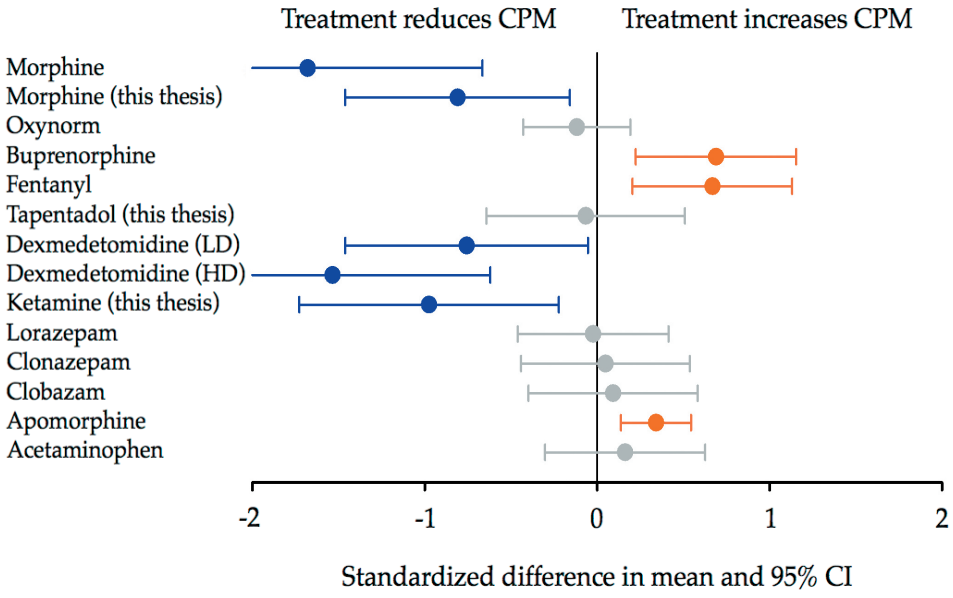

Figure 1. *Comparison of the literature on the effect of central-acting drugs on conditioned pain modulation (CPM) responses in healthy volunteers. Values are the standardized differences in mean ± 95% confidence interval (CI) calculated from CPM values relative to either placebo treatment or control (baseline or pretreatment) values. The orange symbols depict treatment that increased CPM, the blue symbols treatment that decreased CPM. The grey symbols depict treatment that caused CPM responses not different from control or placebo. The data collected from studies outside this thesis are from Le Bars et al.[1] (morphine); Suzan et al.[2] (oxycodon); Arendt-Nielsen et al.[3] (buprenorphine and fentanyl); Baba et al.[4] (dexmedetomidine); Kunz et al.[6] (lorazepam); Vuilleumier et al.[7] (clonazepam and clobazam); Treister et al.[8] (apomorphine) and Meeus et al.[5] (acetominophen). LD: low dose; HD: high dose.*

Analysis v2.2.064 (Biostat, Englewood, NJ, USA). The results for the healthy volunteers are given in figure 1. Apart from morphine, all studied drugs were tested only once. Intravenous morphine administration decreased CPM responses in both studies (this thesis and ref. 1). Single dose oxycodone and tapentadol, given orally on a single occasion, had no effect on CPM (this thesis and ref. 2). In contrast, buprenorphine and fentanyl, both administered by a continuous drug delivery transdermal patch formulation, did produce a significant increase in CPM.[3] CPM responses following treatment with non-opioid analgesics (single administration) such as ketamine and dexmedetomidine, are predominantly reduced with the exception of acetominophen.[4,5] With regard to non-analgesic central-acting drugs, no effect on CPM was observed for the single administration of GABA-ergic agonists.[6,7] The dopamine-agonist apomorphine did increase CPM responses in healthy volunteers.[8] These data indicate that drugs acting on the μ-, α_2- and NMDA-receptor influence CPM responses in healthy volunteers. However, large dissimilarities in the methods used to study CPM are present between these studies. Hence a significant part of the variability observed in study outcomes may be related to methodological issues.

The results for the chronic pain patients are given in figure 2. All studied drugs were tested only once. A significant decrease in CPM responses was observed in

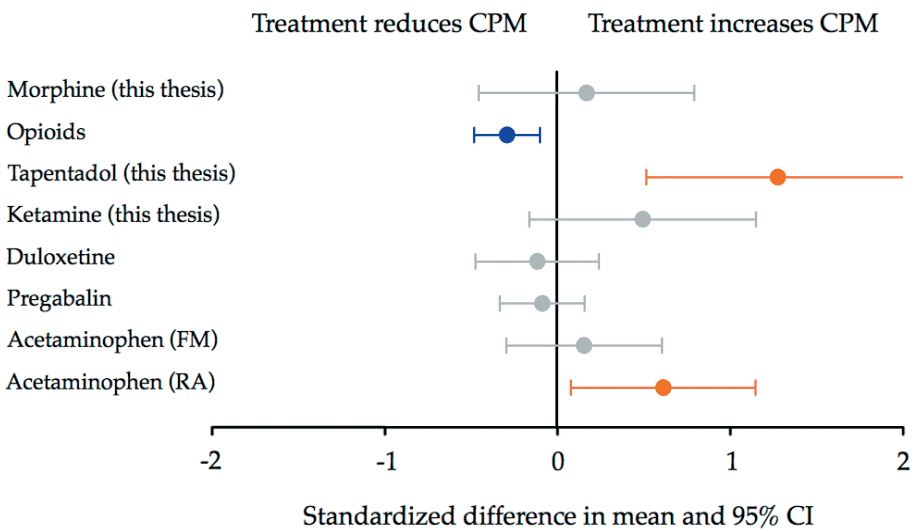

Figure 2. *Comparison of the literature on the effect of central acting drugs on conditioned pain modulation (CPM) responses in chronic pain patients. Values are the standardized differences in mean ± 95% confidence interval (CI) calculated from CPM values relative to either placebo treatment or control (baseline or pretreatment) values. The orange symbols depict treatment that increased CPM, the blue symbols treatment that decreased CPM. The grey symbols depict treatment that caused CPM responses not different from control or placebo. The data collected from studies outside this thesis are from Ram et al.[9] (opioids); Yarnitsky et al.[10] (duloxetine); Bouwense et al.[11] (pregabalin) and Meeus et al.[5] (acetominophen). FM: fibromyalgia; RA: rheumatoid arthritis.*

a group of chronic pain patients (either cancer or non-cancer related) who were treated with opioids compared to patients who were not on opioid treatment.[9] An increase in CPM response was observed in patients with chronic painful diabetic neuropathy after tapentadol treatment (this thesis) and in patients with rheumatoid arthritis after treatment with acetominophen (this effect was not observed in fibromyalgia patients).[5] And although no significant effect on CPM responses was observed after treatment with morphine, ketamine (this thesis), duloxetine and pregabalin,[10,11] a (linear) relationship was observed between the magnitude of increase in CPM and magnitude of pain relief induced by ketamine, morphine and tapentadol (this thesis). These data indicate that also in patients opioidergic and noradrenergic pathways influence CPM. The different responses between healthy volunteers and pain patients observed after treatment with morphine, tapentadol and ketamine may be related to central pathological alterations observed in pain patients (*i.e.* central sensitization and inflammation), and hence comparison of treatment effects between patients and volunteers should be done with caution. Again a large variability in study methods was present, which may have influenced the outcome of the meta-analysis.

Conclusions

From the data presented in this thesis several conclusions may be drawn:

1. In healthy volunteers, short-term ketamine treatment induces a shift in the balance between pain inhibition and pain facilitation towards pain facilitation (as measured by CPM responses). In contrast, in chronic neuropathic pain patients, in whom descending control of pain is dysfunctional, ketamine restores pain inhibitory pathways.

2. Short-term morphine treatment significantly restores CPM responses in chronic neuropathic pain patients who display dysfunctional descending inhibitory pain control prior to treatment.

3. Long-term (4-week) tapentadol treatment significantly enhances CPM responses compared to placebo in patients with chronic painful diabetic neuropathy.

4. Chronic neuropathic pain patients show an absent or diminished OA response compared to healthy volunteers. None of the central-acting drugs described in this thesis (ketamine, morphine and tapentadol) alters or restores OA responses in healthy volunteers or chronic pain patients. Whether this is because there is no central origin for OA or that other central receptors or neurotransmitter systems (which are not influenced by these drugs) are involved in this phenomenon remains unknow.

5. Resting-state fMRI is a valuable, reliable and efficient method to assess pharmacological effects on the brain.

6. Ketamine treatment and deafferentation by spinal anesthesia induce alterations in functional brain connectivity in cortical and subcortical areas. Furthermore, they both alter pain sensitivity, where ketamine induces analgesia and deafferentation induces hyperalgesia, which is correlated to alterations in functional brain connectivity in brain areas involved in descending control of pain.

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Chapter 9

Samenvatting & Conclusies

Samenvatting

Endogene pijnmodulatie is een complex fenomeen dat betrokken is bij de perceptie van pijn. Efferente inhiberende en facilitatoire zenuwbanen die hun oorsprong vinden in het brein en afdalen naar de dorsale hoorn van het ruggenmerg, moduleren afferente pijnprikkels en hiermee de pijnperceptie. Dysfunctie van deze inhiberende zenuwbanen of een verschuiving in de balans tussen pijninhibitie en pijn-facilitatie wordt geassocieerd met de ontwikkeling van chronische pijn. Dit proefschrift beschrijft het effect van een aantal centraal werkende geneesmiddelen op endogene pijnmodulatie in gezonde vrijwilligers en chronische pijnpatiënten om zo het onderliggende werkingsmechanisme van endogene pijnstilling beter te leren begrijpen.

In **hoofdstuk 2** is het effect van de *N*-methyl-D-aspartaat (NMDA) receptor antagonist ketamine op de endogene pijnmodulatie bestudeerd in gezonde vrijwilligers. Tien gezonde personen (4 mannen/6 vrouwen) kregen een 1-uur durende infusie met placebo of S(+)-ketamine (40 mg/70 kg) op twee verschillende dagen in willekeurige volgorde. Na het beëindigen van de infusie werd de effectiviteit van het endogene pijnstillingssysteem onderzocht met behulp van twee experimentele testen: "conditioned pain modulation" (CPM) en "offset analgesia" (OA). Integenstelling tot placebo, kon na infusie met ketamine geen CPM respons worden waargenomen, maar vond significante facilitatie van pijn plaats ($p < 0.01$); de OA respons was onveranderd. Deze bevindingen geven aan dat ketaminebehandeling de balans tussen pijn-inhibitie en pijn-facilitatie heeft verschoven richting pijn-facilitatie en zijn suggestief voor een modulerende rol van NMDA en/of glutamaterge receptoren in de endogene pijnmodulatie. Het afwezig zijn van een effect van ketamine op OA impliceert dat er verschillen zijn tussen CPM en OA wat betreft het onderliggende mechanisme en de neurotransmitters die hierbij betrokken zijn.

In tegenstelling tot CPM was het relatieve nieuwe fenomeen OA alleen beschreven in jonge, gezonde vrijwilligers. In **hoofdstuk 3** hebben we de aanwezigheid van OA in een grote groep vrijwilligers van verschillende leeftijden onderzocht, alsmede in chronische pijnpatiënten. De OA respons werd in vrijwilligers (n = 110) in de leeftijdscategorie 6-80 jaar onderzocht, evenals in tien neuropathische pijnpatiënten. OA werd gedefinieerd als een afname in pijnscore in reactie op een verlaging van een pijnlijke warmtestimulus met 1 °C. OA was aanwezig in gezonde vrijwilligers onafhankelijk van leeftijd en geslacht met een afname in pijnscore van 97 ± 1% (gemiddelde ± standaard error), wat aangeeft dat OA volledig ontwikkeld is op de leeftijd van 6 jaar en geen verdere veranderingen doormaakt. Echter, in neuropathische pijnpatiënten werd een verminderde of afwezige OA waargenomen (afname in pijnscore = 56 ± 9% *vs.* 98 ± 1%, $p < 0.001$). Deze data duiden erop dat chronische neuropathische pijnpatiënten niet in staat zijn om hun pijnperceptie te moduleren tijdens veranderingen in pijnstimulatie op een moment waarop bij gezonde vrijwilligers sterke analgesie waarneembaar is. Of deze veranderde OA respons bijdraagt aan de chronificatie van pijn

of een consequentie is van het chronische pijnproces is onduidelijk en vergt verder onderzoek. Intraveneuze behandeling met ketamine, morfine en placebo had geen effect op de OA respons in patiënten ondanks sterke afname in spontane pijnscores, wat aangeeft dat de NMDA en μ-opioïd receptor waarschijnlijk niet betrokken zijn bij het mechanisme van OA. Mogelijk is niet het centraal zenuwstelsel maar een perifere lokatie betrokken bij de veranderde OA respons in patienten.

Hoofdstuk 4 beschrijft het effect van ketamine en morfine op de CPM respons in chronische pijnpatiënten. De CPM respons werd bepaald in 10 neuropathische pijnpatiënten (2 mannen/8 vrouwen) met perifere neuropathie. Patiënten werden behandeld met S(+)-ketamine (0.57 mg/kg per uur gedurende 1 uur) en morfine (0.065 mg/kg per uur gedurende 1 uur) in een gerandomiseerde, placebo-gecontroleerde dubbelblinde studie. CPM werd bepaald voor infusie en 100 minuten na de start van de behandeling. Voor behandeling was geen CPM aantoonbaar, wat aangeeft dat de pijn-inhiberende mogelijkheden in deze groep patiënten sterk verminderd is. Na behandeling met ketamine, morfine en placebo werd een significante CPM respons waargenomen van respectievelijk 40.2 ± 10.9%, 28.5 ± 7.0% en 22.1 ± 12.0%. De grootte van de CPM respons na behandeling was gecorreleerd aan de mate en duur van de afname van de spontane pijnscores. Dit suggereert dat endogene pijnmodulatie een rol speelt bij het analgetische effect van ketamine, morfine en placebo in de behandeling van chronische neuropathische pijn.

In **hoofdstuk 5** wordt het effect van langdurige behandeling met de nieuwe pijnstiller tapentadol beschreven. Tapentadol is een pijnstiller geschikt voor de behandeling van acute en chronische pijn. Het werkingsmechanisme berust op activatie van de μ-opioïd receptor gecombineerd met inhibitie van neuronale noradrenaline heropname. Beide mechanismen zijn betrokken bij het activeren van efferente inhiberende pijnbanen. 24 patiënten met diabetische polyneuropathie werden gerandomiseerd voor behandeling met tapentadol (gemiddelde dagelijkse dosis 433 ± 31 mg) of placebo gedurende 4 weken. CPM en OA werden voor en aan het einde van de behandeling bepaald. Voor behandeling was geen significante CPM of OA aantoonbaar. Tapentadol behandeling activeerde de CPM respons significant en induceerde pijnstilling. CPM nam toe van 9.1 ± 5.4% (voor behandeling) naar 14.3 ± 7.2% na placebo behandeling en 24.2 ± 7.7% na tapentadol behandeling ($p < 0.001$ *vs.* placebo). Verlichting van spontane pijn was groter in patiënten behandeld met tapentadol dan met placebo ($p = 0.028$). Zowel behandeling met placebo als met tapentadol had geen invloed op de grootte van de OA respons ($p = 0.78$). Deze resultaten tonen aan dat patiënten met pijnlijke diabetische polyneuropathie en een afwezige CPM respons baat hebben bij behandeling met tapentadol. Tapentadol induceerde pijnstilling die gekoppeld was aan (re)-activatie van efferente inhiberende pijnbanen.

Een relatief nieuwe benadering van geneesmiddelenonderzoek in het centraal zenuwstelsel is *resting-state* fMRI (RS-fMRI). RS-fMRI meet intrinsieke netwerk

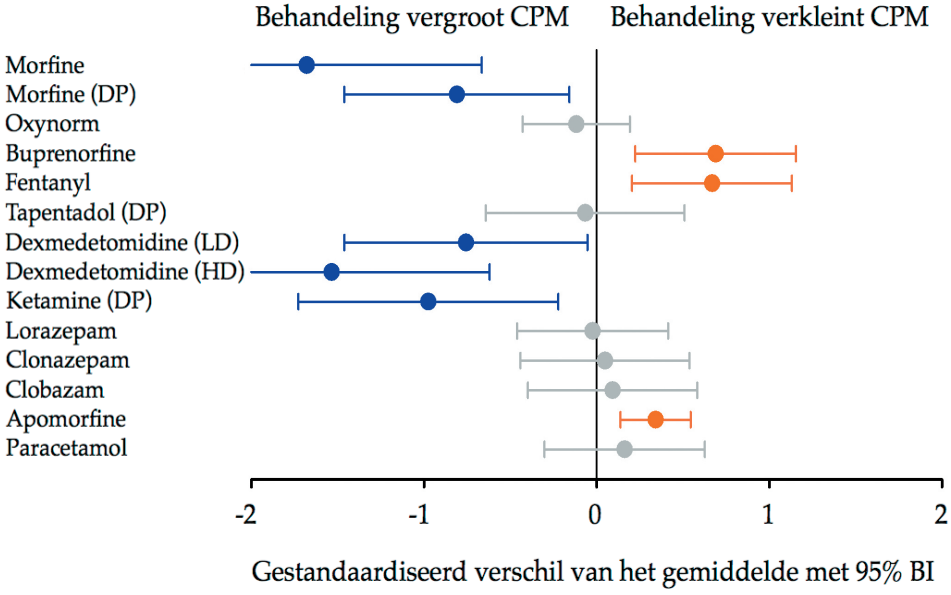
interacties in het brein in rust (niet taak-gerelateerd). In **hoofdstuk 6** is het effect van een lage dosis S(+)-ketamine op de intrinsieke breinconnectiviteit onderzocht. Het doel was om breinregio's te identificeren die betrokken zijn bij het farmacodynamische profiel van ketamine wat betreft analgesie en bijwerkingen (met name psychedelische effecten) en regio's betrokken bij pijnperceptie. Twaalf gezonde, mannelijke vrijwilligers werden intraveneus behandeld met een 2 uur durende S(+)-ketamine infusie (1e uur 20 mg/70 kg, 2e uur 40 mg/70 kg). Voor, tijdens en na toediening van ketamine werd de *resting-state* breinconnectiviteit gemeten. Tussen de scansessies door werden hitte pijntesten uitgevoerd om het analgetische effect van ketamine te bepalen. Ketamine verhoogde de connectiviteit in het cerebellum en de visuele cortex in relatie tot het mediale visuele netwerk. Een verminderde connectiviteit werd waargenomen in het auditore en somatosensore netwerk in relatie tot regio's betrokken bij pijnperceptie en de affectieve component van pijn. Hiertoe behoren de amygdala, insula en de anterieure cingulate cortex (ACC). Variaties in connectiviteit gerelateerd aan fluctuaties in pijnscores werden gezien in de ACC, insula, orbitofrontale cortex en de hersenstam, allen regio's betrokken bij endogene modulatie van pijn. De resultaten van deze studie geven weer dat RS-fMRI een bruikbare en efficiënte methode is om effecten van geneesmiddelen in het brein te onderzoeken. Een lage dosis ketamine induceerde connectiviteitsveranderingen in breinregio's betrokken bij motorfunctie, psychedelische effecten en de verwerking van pijn. Wat betreft de pijnverwerking kan het analgetische effect van ketamine worden veroorzaakt door verschillende mechanismen. Enerzijds werd een verminderde connectiviteit waargenomen in regio's van de pijnmatrix welke verantwoordelijk zijn voor de perceptie en de affectieve component van pijn. Anderzijds veranderde ketamine de connectiviteit in regio's betrokken bij endogene pijn-inhibitie.

Efferente zenuwbanen zijn belangrijk voor de normale perceptie van pijn. Er is echter weinig bekend over de rol van afferente zenuwbanen in de modulatie van pijn. In **hoofdstuk 7** werd het effect van spinale deafferentiatie op pijnsensitiviteit onderzocht en gecorreleerd aan functionele breinconnectiviteit gemeten met RS-fMRI. Deafferentiatie werd geïnduceerd door spinale of sham anesthesie (spinaal: 15 mg bupivacaïne geïnjecteerd op niveau L3-4; sham: geen punctie van de dura mater) in 12 mannelijke vrijwilligers. *Resting-state* breinconnectiviteit werd bepaald in relatie tot 8 algemene netwerken en 7 thalamus netwerken en gemeten voor, 1 en 2 uur na de spinale- of sham-injectie in een *cross-over* studiedesign. Om het effect van deafferentiatie op pijnsensitiviteit te beoordelen werd iedere 15 minuten de respons op een hitte pijnstimulus gemeten op niet-gedeafferentieerde huid en gecorreleerd aan de RS-fMRI connectiviteitswaarden. Spinale anesthesie veranderde de functionele breinconnectiviteit in regio's van het sensorimotor systeem en de pijnmatrix in relatie tot de somatosensore- en thalamusnetwerken. Na spinale anesthesie werd een significante toename in pijnsensitiviteit gezien vergeleken met de shamprocedure (*area-under-the-curve:* 190.4 ± 33.8 *versus* 13.7 ± 7.2; $p < 0.001$). Deze toename in pijnsensitiviteit was gecorreleerd aan de functionele connectiviteitsveranderingen in the thalamus in relatie tot het thalamo-prefrontale netwerk en in de ACC en insula in relatie

tot het thalamo-parietale netwerk. Deze studie laat zien dat deafferentiatie door spinale anesthesie geassocieerd is met snelle connectiviteitsveranderingen in het brein van zowel corticale als subcorticale regio's. Deze veranderingen zijn mogelijk een gevolg van reorganisatie van neuronale interacties tussen excitatoire en inhiberende factoren betrokken bij adaptatie en neuronale plasticiteit. Verder verhoogde spinale anesthesie de pijnsensitiviteit welke kon worden gecorreleerd aan versterkte connectiviteitspatronen in de thalamus, ACC en insula, allen regio's betrokken bij endogene pijnmodulatie.

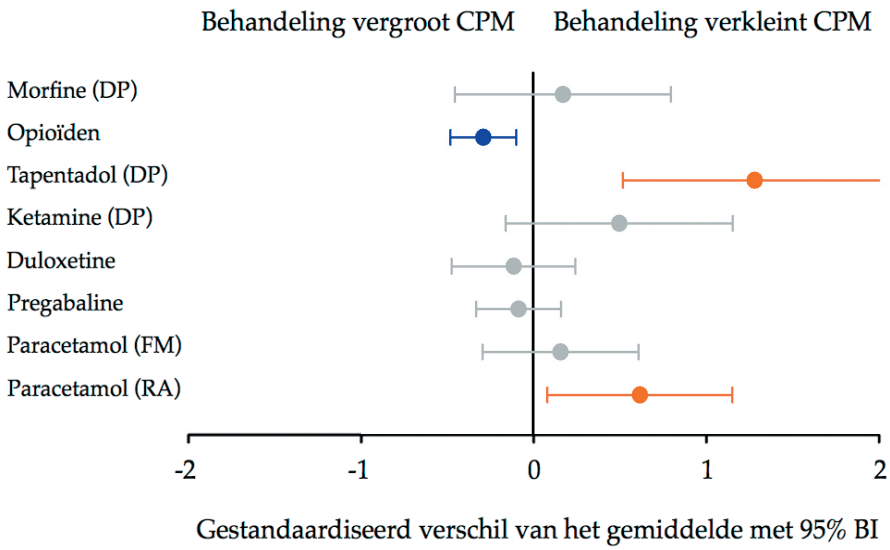
Vergelijking met de literatuur

Om de resultaten van dit proefschrift te kunnen vergelijken met eerder gepubliceerde literatuur werd een zoekopdracht in PubMed uitgevoerd met als doel studies te identificeren die het effect van centraal werkende geneesmiddelen op



Figuur 1. *Vergelijking van de literatuur naar het effect van centraal werkende geneesmiddelen op CPM in gezonde vrijwilligers. De waarden zijn gestandaardiseerde verschillen van het gemiddelde ± het 95% betrouwbaarheidsinterval (BI) berekend met relatieve CPM waarden ten opzichte van placebo behandeling of uitgangswaarden voorafgaand aan behandeling. De oranje symbolen geven behandelingen aan die CPM vergrootten, de blauwe symbolen behandelingen die CPM verkleinden. De grijze symbolen geven behandelingen aan die geen verandering in CPM induceerden. De gepresenteerde data die niet afkomstig zijn uit dit proefschrift komen van: Le Bars et al.[1] (morfine); Suzan et al.[2] (oxycodon); Arendt-Nielsen et al.[3] (buprenorfine and fentanyl); Baba et al.[4] (dexmedetomidine); Kunz et al.[6] (lorazepam); Vuilleumier et al.[7] (clonazepam and clobazam); Treister et al.[8] (apomorphine) and Meeus et al.[5] (acetominophen). DP: dit proefschrift; LD: lage dosis; HD: hoge dosis.*

CPM hebben onderzocht in gezonde vrijwilligers en chronische pijnpatiënten. Van alle relevante studies werden gestandaardiseerde effect groottes berekend met het statistische programma “Comprehensive Meta Analysis" v2.2.064 (Biostat, Englewood, VS). De resultaten van deze vergelijking voor de gezonde vrijwilligers staan in figuur 1. Voor alle geneesmiddelen geldt dat het effect op CPM in slechts één studie was onderzocht, met uitzondering van morfine waarvoor twee studies beschikbaar waren. In beide studies was een afname van CPM aantoonbaar na intraveneuze toediening van morfine (dit proefschrift en ref. 1). Een eenmalige toediening van oxycodon en tapentadol had geen effect op CPM (dit proefschrift en ref. 2). Echter, na een continue toediening van buprenorfine en fentanyl via een transdermale pleister werd een significante toename in CPM waargenomen.[3] Niet-opioïd gerelateerde analgetica zoals ketamine en dexmedetomidine verkleinde CPM, met uitzondering van paracetamol (allen na eenmalige toediening).[4,5] Centraal werkende geneesmiddelen zonder analgetische werking, zoals de GABA-erge stoffen, lieten geen effect op CPM zien.[6,7] De dopamine agonist apomorfine, daarentegen, gaf wel een toename van CPM.[8] Deze data geven aan dat geneesmiddelen met werking op de μ-, α_2- en NMDA-receptor een invloed hebben op CPM in gezonde vrijwilligers. Echter, de studies



Figuur 2. *Vergelijking van de literatuur naar het effect van centraal werkende geneesmiddelen op CPM in chronische pijnpatiënten. De waarden zijn gestandaardiseerde verschillen van het gemiddelde ± het 95% betrouwbaarheidsinterval (BI) berekend met relatieve CPM waarden ten opzichte van placebo behandeling of uitgangswaarden voorafgaand aan behandeling. De oranje symbolen geven behandeling aan die de CPM vergrootten, de blauwe symbolen behandeling die CPM verkleinden. De grijze symbolen geven behandelingen aan die geen verandering in CPM induceerden. De gepresenteerde data die niet afkomstig zijn uit dit proefschrift komen van: Ram et al.[9] (opioïden); Yarnitsky et al.[10] (duloxetine); Bouwense et al.[11] (pregabaline) en Meeus et al.[5] (paracetamol). DP: dit proefschrift; FM: fibromyalgie; RA: reumatoïde artritis.*

vertoonden grote verschillen in de gebruikte methode om CPM te onderzoeken. Dit bemoeilijkt de interpretatie van de resultaten en verklaart mogelijk voor een groot deel de variabiliteit tussen de verschillende studies.

De resultaten voor de chronische pijnpatiënten zijn weergegeven in figuur 2. Alle geneesmiddelen werden voor een langere periode toegediend en zijn onderzocht in één studie. Terwijl in kankerpatienten een afname van CPM werd gezien tijdens behandeling met morfine, werd in neuropathische pijnpatiënten en patiënten met reumatoïde artritis een toename gezien na behandeling met respectievelijk tapentadol en paracetamol (dit proefschrift en refs. 5 en 9). Ondanks dat er geen significant effect op CPM werd waargenomen na behandeling met morfine, ketamine (dit proefschrift), duloxetine en pregabaline,[10,11] kon een (lineaire) relatie worden aangetoond tussen de grootte van CPM toename en de mate van pijnstilling geïnduceerd door ketamine, morfine en tapentadol (dit proefschrift). Deze data geven aan dat ook in patiënten opioïderge en noradrenerge effecten CPM beïnvloeden. Het verschil in farmacologisch effect op CPM tussen gezonde vrijwilligers en patiënten wordt mogelijk verklaard door plastische veranderingen in het centraal zenuwstelsel die ontstaan in chronische pijnpatiënten (zoals centrale sensitisatie en inflammatie). Hierdoor is terughoudendheid geboden bij het vergelijken van behandelingseffecten tussen patiënten en vrijwilligers. Wederom was er een grote variabiliteit aanwezig in de studiemethoden tussen de verschillende onderzoeken wat mogelijk de uitkomst van deze meta-analyse heeft beïnvloed.

Conclusies

Uit de data gepresenteerd in dit proefschrift kunnen de volgende conclusies worden getrokken:

1. In gezonde vrijwilligers leidt een kortdurende behandeling met ketamine tot een verschuiving in de balans tussen pijn-inhibitie en pijn-facilitatie met als netto effect een toename van pijn-facilitatie (gemeten met CPM). Daarentegen wordt in chronische neuropathische pijnpatiënten, waarbij endogene pijnstilling aanvankelijk afwezig of gereduceerd is, een herstel of toename van pijn-inhibitie waargenomen na behandeling met ketamine.

2. Kortdurende behandeling met morfine herstelt de CPM respons in chronische neuropathische pijnpatiënten die voor behandeling een afwijkende endogene inhibitie van pijn lieten zien.

3. Langdurige (4-weken) behandeling met tapentadol vergroot de CPM respons in patiënten met chronische diabetische neuropathie.

4. Chronische neuropathische pijnpatiënten vertonen een afwezige of verminderde OA respons. Geen van de centraal werkende geneesmiddelen beschre-

ven in dit proefschrift (ketamine, morfine of tapentadol) zijn in staat om de OA respons te herstellen. Het is onbekend of dit wordt veroorzaakt door het afwezig zijn van een centraal substraat voor OA of dat andere receptoren of neurotransmitters (die niet worden beïnvloed door deze geneesmiddelen) in het centraal zenuwstelsel hierbij betrokken zijn.

5. *Resting-state* fMRI is een betrouwbare en efficiënte methode om farmacologische effecten in het brein te onderzoeken.

6. Ketamine behandeling en deafferentiatie door spinale anesthesie veroorzaken beide veranderingen in functionele connectiviteit in corticale en subcorticale breinregio's. Beide beïnvloeden de pijnsensitiviteit, waarbij ketamine analgesie induceert en deafferentiatie hyperalgesie. Beide effecten zijn gecorreleerd aan de veranderingen in functionele connectiviteit in breinregio's betrokken bij endogene pijnmodulatie.

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Addenda

Curriculum Vitae

Marieke Niesters was born on the 29th of September 1984 in Wijk bij Duurstede, the Netherlands. She obtained her VWO degree at the Caland Lyceum in Rotterdam in June 2002 and immediately started her study Biomedical Sciences at the Leiden University. Her Bachelor internship focused on differences in telomerase expression in Langerhans cell hystiocytosis at the department of Pediatrics at the Leiden University Medical Center (LUMC). She obtained her Bachelor's degree in June 2005. In September 2005 she was accepted for Medical School at Leiden University and combined this study with a Master's in Biomedical Sciences. During her first Master internship she studied the immunologic effects of blood transfusion in premature born children at the Sanquin blood bank in Leiden. Her second Master internship was performed at the department of Anesthesiology at the LUMC where she started the research described in this thesis. During this internship she was appointed as PhD student at the same department under supervision of prof. dr. A. Dahan. This appointment was financially supported by the excellent MSc-PhD grant from the board of directors of the LUMC. In June 2010 she graduated from Medical School and her Master's in Biomedical Sciences (with honor). Currently she is a resident at the department of Anesthesiology at the LUMC under supervision of prof. dr. L.P.H.J. Aarts (start date: 1 July 2013).

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