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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/m²), 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).

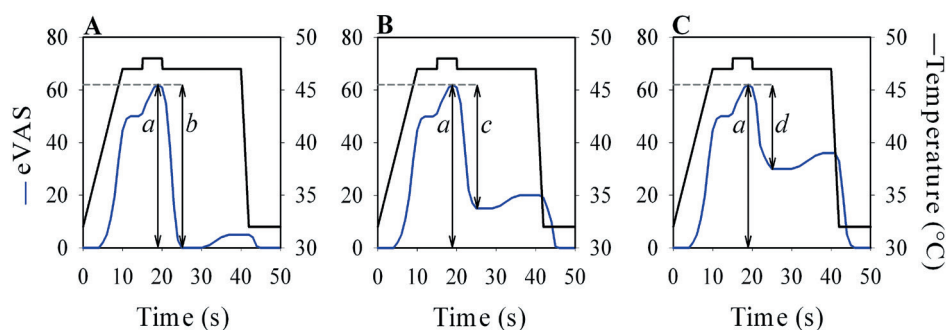


Figure 1. Calculation of the magnitude of offset analgesia (OA) relative to peak effect ($\Delta eVASc$), where $eVAS$ = electronic visual analogue score, $\Delta eVAS$ = the decrease in $eVAS$ from peak $eVAS$ value to the $eVAS$ nadir after the 1°C decrease of the test stimulus, and $\Delta eVASc = \Delta eVAS / [\text{peak } eVAS] * 100$. **A.** Peak $eVAS = a$; reduction in $eVAS$ after the 1°C decrease in noxious heat stimulation ($\Delta eVAS$) = b ; $\Delta eVASc = (b/a) * 100\% = 100\%$ because $a = b$. **B.** Peak $eVAS = a$; $\Delta eVAS = c$; $\Delta eVASc = (c/a) * 100\% = 75\%$ because $c = 0.75a$. **C.** Peak $eVAS = a$; $\Delta eVAS = d$; $\Delta eVASc = (d/a) * 100\% = 50\%$ 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 ($\Delta eVAS$), corrected for the value of the peak $eVAS$ ($\Delta eVASc = (\Delta eVAS / [\text{peak } eVAS]) * 100$) (*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 $\Delta eVASc$ ($\Delta eVAS$ corrected for peak effect) was tested by one-way analysis of variance (ANOVA) and unpaired two-tailed t-test, respectively. To compare the $\Delta eVASc$ 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 $\Delta eVASc$ 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 $\Delta eVASc$ 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 \pm 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, Δ eVAS (not corrected for peak value) per age cohort are plotted in figure 3A. It shows a trend towards a decrease in the Δ eVAS with increasing age and noticeably large variability in the response in the oldest cohort: 6-12 years: Δ eVAS = 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 Δ eVASc of the total population that displayed a pain response greater than zero ($n = 78$) was $97 \pm 1\%$ (95% CI: 95-99%). No difference was observed in Δ eVASc scores between the age cohorts (Fig. 3B): 6-12 years: $92 \pm 4\%$ (85 – 100%; $n = 17$), 13-19 years: $98 \pm 1\%$ (96 – 100%; $n = 17$), 20-39 years: $96 \pm 2\%$ (92 – 100%; $n = 14$), 40-59 years: $99 \pm 1\%$ (96 – 100%; $n = 23$) and 60-80 years: $97 \pm 3\%$ (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

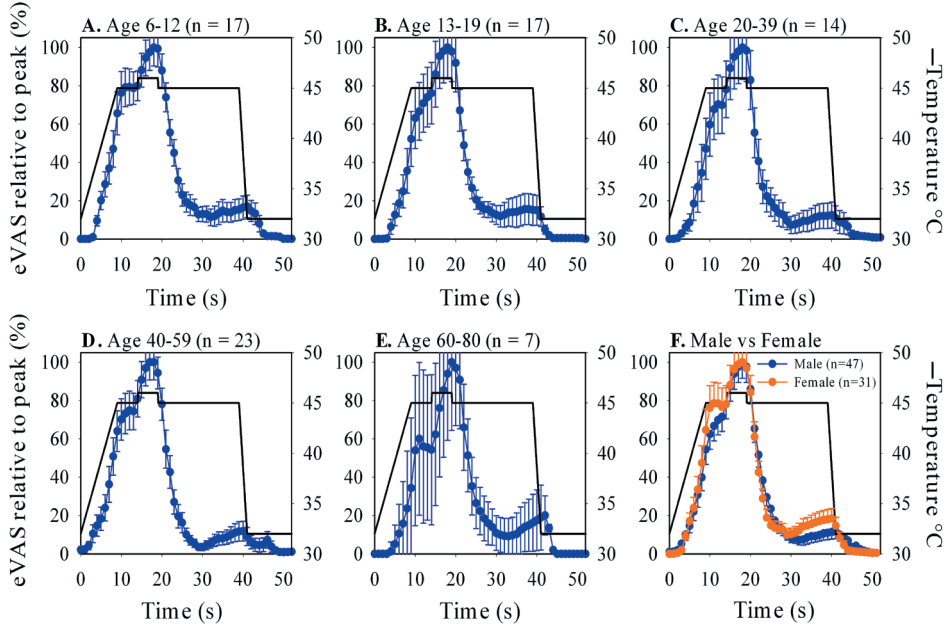


Figure 2. Offset analgesia (OA) responses to a heat stimulus paradigm (black line) in a random study population of healthy volunteers of different age categories (A-E). In panel F the effect of the offset analgesia responses in men (blue) and women (orange) are compared. Values are mean \pm SEM.

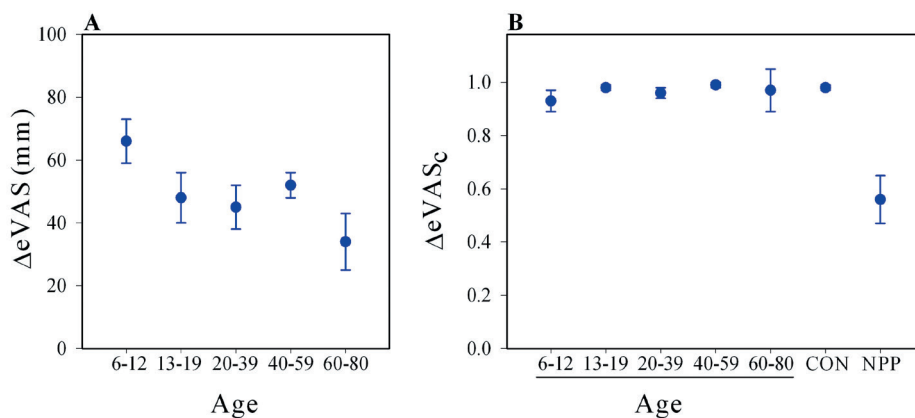


Figure 3. **A.** Absolute magnitude of offset analgesia (OA) in mm ($\Delta eVAS$) of the different age categories. The age effect was not significant ($p = 0.054$). **B.** OA response relative to peak effect ($\Delta eVASc$) of the different age categories. $\Delta eVASc$ scores range from 92 to 99%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

mm; $p = 0.57$; Fig. 2). However, a small but significant difference in difference in $\Delta eVASc$ was observed: male $98\% \pm 1\%$ (97 – 100%) and female $94 \pm 2\%$ (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 $\Delta eVASc$ $98 \pm 1\%$ versus female $93 \pm 2\%$; $p = 0.185$) but persistent differences in the 20+ cohorts (20-80 years: male $\Delta eVASc$ $99 \pm 1\%$ versus female $95 \pm 2\%$; $p = 0.002$; Fig. 4).

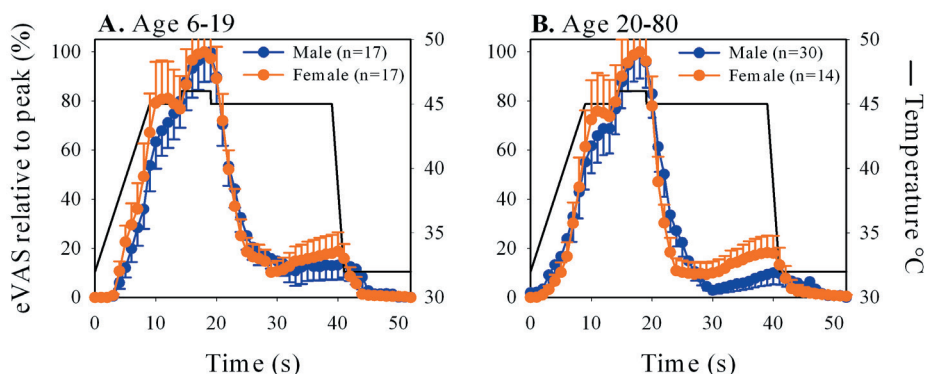


Figure 4. **A.** Offset analgesia (OA) responses in male versus female volunteers in the age category of 6-19 years. **B.** OA responses in male versus female volunteers in the age category of 20-80 years. Responses are percentage of peak response. All value are mean \pm SEM. $eVAS$ = electronic visual 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: acetaminophen, non-steroidal anti-inflammatory drugs, tramadol, gabapentin, pregabalin and amitriptyline. All participants

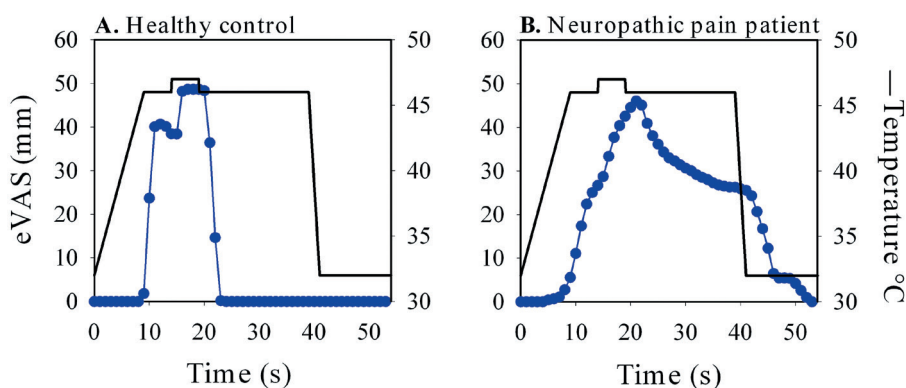


Figure 5. Example of the offset analgesia (OA) response in a **A.** healthy control and a **B.** neuropathic pain (NP) patient. The healthy control shows OA with a $\Delta eVASc$ of 100%. The NP patient clearly shows an aberrant response to the heat stimulus paradigm (black line) with a delayed onset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

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

Neuropathic pain patients	Δ eVASc (%)	Age- and sex-matched controls	Δ eVASc (%)
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; Δ eVASc = 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 Δ eVASc was significantly greater than in patients with pain; the Δ eVASc averaged to $98 \pm 1\%$ (96 – 100%) in controls versus $56 \pm 9\%$ (38 – 73%) in NP patients (Fig. 6, $p < 0.001$). Individual values of the eVASc of patients and controls are given in table 2.

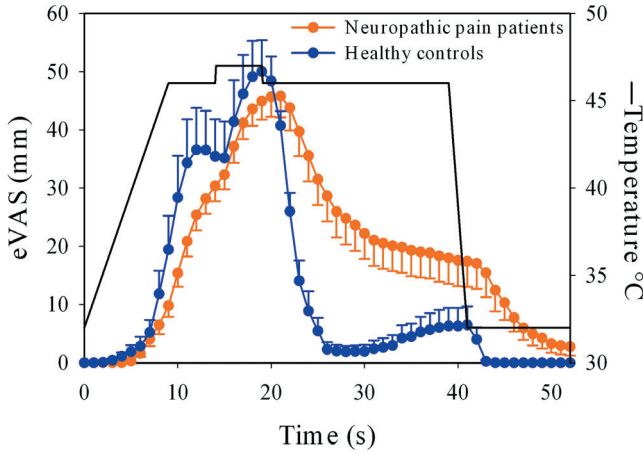


Figure 6. eVAS responses to the three-temperature paradigm (black line) in neuropathic pain patients ($n = 10$, orange line) and age-matched healthy controls ($n = 10$, blue line). Values are mean \pm SEM. eVAS = electronic visual analogue score.

A receiver-operating characteristic curve was constructed to determine a Δ eVASc 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 (\pm 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,

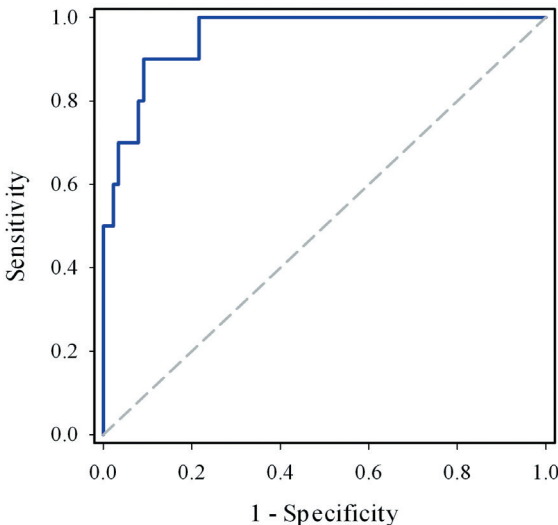


Figure 7. Receiver operating characteristic (ROC) curve constructed to determine a Δ eVASc cutoff value between healthy subjects (volunteers and controls, $n = 88$) and patients with neuropathic pain. eVAS = electronic visual analogue scale.

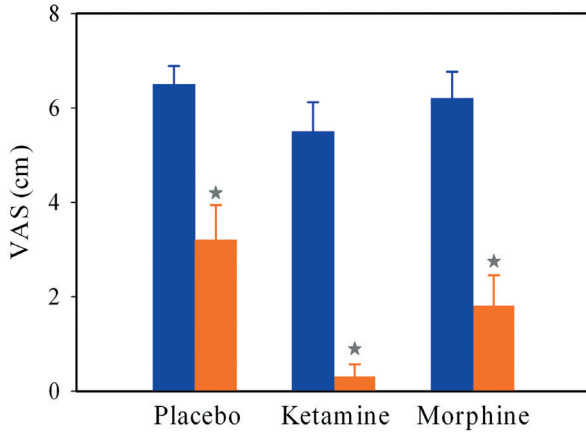


Figure 8. Effect of placebo, ketamine and morphine treatment on spontaneous pain scores in neuropathic pain patients. The blue bars represent the pain scores before treatment, the orange bars represent the scores directly after treatment. All three treatments produced significant pain relief ($p < 0.01$). Values are mean \pm 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 $\Delta eVASc$ scores were $51 \pm 1\%$ (49 – 53%), $55 \pm 1\%$ (53 – 57%) and $34 \pm 1\%$ (32 – 36%) for placebo, ketamine and morphine treatment, respectively ($p = 0.51$).

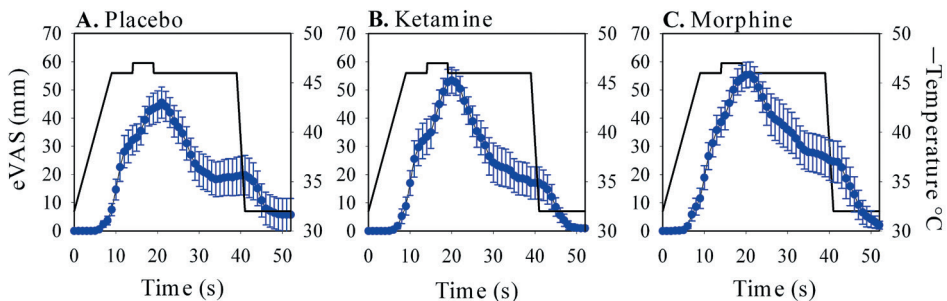


Figure 9. Effect of A. placebo, B. ketamine and morphine C. treatment on the eVAS responses in neuropathic pain patients. $\Delta eVASc$ scores were not different among treatments ($p = 0.51$). Values are mean \pm SEM. eVAS = electronic visual analogue scale; $\Delta eVAS$ = the decrease in eVAS from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

Discussion

Offset analgesia

Offset analgesia, first described in 2002, is the phenomenon where a disproportionately 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 $\Delta eVASc$ 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 $\Delta eVAS$ 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 $\Delta eVASc$ (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 (Δ eVASc: 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 A δ 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 disproportionately large decrease in pain scores, as was observed in healthy controls. On average, the Δ eVASc was 56% in patients *versus* 98% in controls. The receiver-operating characteristic analysis yielded a Δ eVASc 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, temporomandibular 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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