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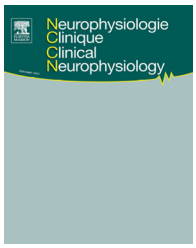

ORIGINAL ARTICLE

Cold evoked potentials: Acquisition from cervical dermatomes

Jan Rosner, Janosch Rinert, Mario Ernst, Armin Curt, Michèle Hubli*

Spinal Cord Injury Center, Balgrist University Hospital, University of Zurich, Forchstrasse 340, 8008 Zurich, Switzerland

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Summary
Introduction. — Cold evoked potentials (CEPs) represent a novel technique to assess the integrity of cold-specific pathways within the somatosensory system. So far an objective assessment of these pathways has not been implemented into the clinical routine. Specifically, CEPs may help to elucidate the pathophysiological underpinnings of altered cold processing in neurological diseases.
Objective. — To test feasibility and test-retest reliability of CEPs within two cervical dermatomes, including recording sites in glabrous and hairy skin, in order to facilitate the transition into clinical practice.
Methods. — Twenty healthy subjects received 15 cold stimuli applied by a thermode either at the hand dorsum (C6 dermatome, hairy skin), the shoulder (C4 dermatome, hairy skin) or the thenar eminence (C6 dermatome, glabrous skin). Stimuli were applied from a baseline temperature of 30°C down to a destination temperature of 25 °C at a rate of 20 °C/s. N2 latencies and N2P2 amplitudes were recorded at the vertex using a surface electroencephalogram and test-retest statistics were calculated.
Results. — Slight, innocuous cooling (Δ5 °C) from a baseline temperature of 30 °C elicited a brief percept of cooling and generated a vertex potential (N2P2) in most subjects. The latency of the vertex response is consistent with A-delta fiber activation. Based on test-retest analyses (i.e., intraclass correlation coefficients (ICCs) and Bland-Altman analyses) reliability is best within the C4 dermatome and for stimulation of hairy skin. ICCs display fair to substantial (ICCs from 0.51—0.81) reliability for amplitudes across all stimulation sites, possibly due to floor effects. CEPs latencies, however, were only poorly reliable (ICCs from —0.13 to 0.31).

* Corresponding author.
E-mail address: Michele.Hubli@balgrist.ch (M. Hubli).

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Conclusion. — The acquisition of CEPs from cervical dermatomes is feasible. Since involvement of cold-specific pathways is relevant for several pathologies in clinical neurology, the application of CEPs may complement existing techniques like contact heat and laser stimulation in the assessment of peripheral and central nervous system disorders. Future studies employing different stimulation paradigms using faster cooling are warranted in order to improve the signal-to-noise ratio.

Introduction

The somatosensory system encompasses various modalities, of which only a subset can currently be reliably assessed using evoked potentials [2,45]. In particular the nociceptive pathways mediating noxious heat can be objectively studied using contact heat or laser evoked potentials (CHEPs, LEPs) [7,26,31,33,40,46]. Both methods constitute objective assessments of peripheral and central nociceptive afferents and have been widely employed in the examination of peripheral A-delta and C fiber function [33] as well as spinothalamic integrity across various conditions [4,24,27,42,49].

Whereas LEPs/CHEPs already yield sound readouts of nociceptive pathways, objective assessments of cold-specific pathways may provide additional diagnostic value: (a) innocuous cooling is conveyed by a separate cold-specific A-delta population in the periphery [5]; (b) there is also evidence for a separate spinal pathway originating from cold-specific neurons in the dorsal horn [8,19].

Interestingly, this pathway retains a specific thalamic relay, whereas other thermo-nociceptive pathways largely converge with somatosensory pathways at the thalamo-cortical level [12]. The specific neuroanatomical organization of cold pathways may provide novel diagnostic approaches to explore sensory processing in supra-spinal lesion (i.e., after insular lesions), which are currently not readily amenable to neurophysiological testing [45].

In the past a number of researchers tried to develop contact cold stimulators (for review see [2]). These devices were usually custom-built and lacked standardized stimulation parameters, or at least they were hard to control. In the 1970s, CEPS were first recorded with quickly operating valves regulating rapid in- and outflow of cold or hot water into a special probe placed onto the skin [13]. Small decrements of merely 3 °C already elicited reproducible cortical potentials. Similar results were later reproduced as vertex potentials after stimulation of the hand and face [16]. More recently, newer methods have been published exploring mechanical pain pathways using pinprick evoked potentials (PEPs) [23,50] and cold pathways employing innocuous cold stimulation (for CEPs) [11,21]. These novel multi-modal neurophysiological methods will likely not only improve the diagnostic work-up of patients with neuropathic pain conditions but may also aid in the topographical diagnosis of lesions in peripheral and central disorders, thus improving clinical practice in neurology.

The recording of CEPs from different body sites using a contact thermode was demonstrated by Hüllemann and colleagues [21]. Cortical potentials could be recorded after brief stimulation (cooling from 30 °C to 25 °C) of cold-sensitive afferents in glabrous as well as hairy skin. The responses were shown to be dependent on peripheral A-delta fibers, and could be reversibly abolished using an ischemic A-fiber block [21]. Cortical potentials consistent with A-delta fiber activation were also reported by other authors [11,14].

Cold deficits have been reported for a number of neurological conditions. Patients with polyneuropathies suffer from cold deficits in about 60% of cases according to a multicenter study employing quantitative sensory testing [34]. In Fabry's disease, a metabolic disorder manifesting from early on with small fiber pathology, loss of cold sensation is among the first symptoms [37,47]. Moreover, for cold allodynia in central pain conditions, disrupted cold processing is also implicated as a potential mechanism [9,35]. Cold allodynia is also a characteristic feature of oxaliplatin-induced neuropathy and has been shown to be mediated by A-delta fibers [15,37]. Furthermore, preservation of cold sensation in patients with discomplete spinal cord injuries and residual spinothalamic integrity was shown to be associated with the development of neuropathic pain [53]. Loss of CEPs in a patient with small fiber neuropathy and a patient with syringomyelia was presented by Jamal and colleagues [25]. Recently, we presented a case study where CEPs and CHEPs were lost in parallel, while dorsal column function remained intact, in a patient with a spinal cord injury indicating a conduction of CEPs within the spinothalamic system [39].

In order to implement CEPs as a diagnostic tool in the clinical routine, reliability over time needs to be demonstrated. In the present study we recorded CEPs from two cervical dermatomes (C4 and C6), including recording sites in glabrous and hairy skin. These sites are often affected in cervical myelopathy [27,48,49], and some polyneuropathies can also present with palmar symptoms (e.g., oxaliplatin-induced polyneuropathy) [51,52]. The objective of this study was to assess feasibility and reliability of CEPs for the different spinal segments and skin types. We hypothesized that the acquisition of CEPs in a segmental fashion is feasible and that stimulation of hairy skin shows better test-retest reliability.

Material and methods

Subjects

Twenty healthy subjects (10 females, 10 males) were recruited between November 2016 and October 2017. A retest on a subset of 15 out of these 20 subjects was planned approximately 30 days after the first appointment. Inclusion criteria were (1) native language either German or English and (2) age between 18 and 60 years. Exclusion criteria included (1) intake of any psychoactive medication, (2) pregnancy and (3) any neurological condition. Each subject provided written informed consent, and all procedures described below were in accordance with the Declaration of Helsinki. This study has been approved by the local ethics board ''Kantonale Ethikkommission Zürich, KEK'' (EK-04/2006, PB_2016-02051, clinicaltrial.gov number NCT02138344).

Study design

Prior to the acquisition of CEPs, a thorough medical history was taken. In addition, cool perception using a tip therm (Tip Therm® GmbH, Germany) as well as mechanoreception and nociception were semi-quantitatively assessed by applying light touch and pinprick, respectively, according to the grading system of the International Standards for Neurological Classification of Spinal Cord Injury [28]. Three skin sites on the left or right upper extremity were chosen for contact cold stimulation: dorsal side of thumb (i.e., hairy skin; dermatome C6), palmar side of thumb (i.e., glabrous skin; dermatome C6), and shoulder (dermatome C4). The sequences of tested skin sites and body side were randomized for each subject. Contact cold stimulation was delivered from a baseline temperature of 30°C to a temperature nadir of 25°C [21]. Room temperature was maintained constant. For the entire sensory assessment and CEP acquisition, subjects were lying comfortably in a supine position. In order to reduce artefacts during CEP acquisition, subjects were instructed to fix a point on the ceiling and to remain relaxed. Prior to the CEP acquisition, subjects were familiarized with the entire procedure, including the presentation of a cold stimulus on the contralateral volar forearm. Subjects had to adapt to a baseline temperature of 30°C (i.e., measurements were started after the thermode's temperature felt neutral). To this end, measurements were started after the thermode had been in a fixed position for at least one minute. Then a total of 15 rapid cold stimuli (Δ5°C) were applied per site with an inter-stimulus interval that randomly varied between 8 and 12s. Based on preliminary experiments employing 15 and 20 stimuli in five individuals we concluded that the signal-to-noise ratio is sufficient (i.e., 4.1 vs. 4.5) using 15 stimuli, thus facilitating a clinical implementation in terms of time expenditure.

Artefact correction was performed in two steps. During the experiment, the experimenter closely observed the subject for any movements/blinking and cleared those traces ''online'' during the course of the experiment. In a subsequent step, i.e., during an offline analysis, individual traces were inspected by two independent examiners for any further artefacts. In order to recognize and be able to distinguish ocular movement, blinking and jaw movement associated artefacts, subjects were asked to blink, move their eyes in different positions and clench their teeth prior to the recording of the evoked potentials. On average 2.3 trials had to be excluded across all dermatomes due to artefact contamination and additional stimuli were delivered in order to consistently acquire 15 stimuli per site.

Subjects were asked to rate each stimulus in terms of perceived intensity on a numeric rating scale (NRS) from 0 (no coldness) to 10 (most imaginable coldness) upon an auditory signal that was triggered 4 seconds after cold stimulus delivery. The thermode was attached to the sites to be tested with an elastic strap. The retest was conducted analogously.

Acquisition of CEPs

Contact cold stimuli were applied using a thermode of 27mm diameter (Pathway Pain & Sensory Evaluation System, Medoc Ltd., Ramat Yishai, Israel). This set-up allows to reach a cooling ramp of 20°C/s. The system was initially conceived for elicitation of CHEPs, however, with an integrated CEP module the baseline temperature can be lowered below 30°C. The cortical potentials were registered by electroencephalogram electrodes according to the 10—20 system. The active electrode was placed to Cz with references to both earlobes (A1—A2). Skin was prepared with Nuprep® Skin Prep Gel (D.O. Weaver & Co, Aurora, USA) and Softasept® alcohol. Ag/Cl cup electrodes (Medtronic, Minneapolis, USA) filled with conductive adhesive Elefix gel (Nihon Kohden, Tokyo, Japan) were installed. The subject was grounded ipsilateral to the stimulation.

The signals were acquired at 2000Hz using a preamplifier (20000x, ALEA Solutions, Zurich, Switzerland) and bandpass filtered in the range of 0.5—30Hz. CEPs were registered with a pre- and post-stimulus time interval of 100ms and 1s, respectively. Each stimulus released by the Pathway system released customized recording software (V1.43 CHEP, ALEA Solutions, Zurich, Switzerland) in parallel, which displayed a single trace in response to each stimulus as well as an averaged trace over all stimuli for the respective body site (i.e., 15 stimuli per site; see above). The N2P2 amplitude (i.e., peak to peak amplitude) was then visually detected in the averaged trace. Prior to statistical analyses, offset-correction based on the pre-stimulus time interval was employed on all averaged traces in LabVIEW based customized software (Soleasy 4.1, ALEA Solutions, Zurich, Switzerland).

Data analysis and statistics

Statistics were conducted using IBM SPSS Statistics software (version 23) for Windows. All data was tested for normal distribution by visual inspection of histograms and using Shapiro-Wilk tests. Statistical significance was determined at $\alpha = 0.05$.

CEP parameters (i.e., N2 latency, P2 latency, N2P2 amplitude and cold rating) were extracted from the subjects' averaged traces. All tests were performed specifically for each parameter, for both stimulation sites (i.e., C6 hairy skin and C6 glabrous skin) as well as for test and retest.

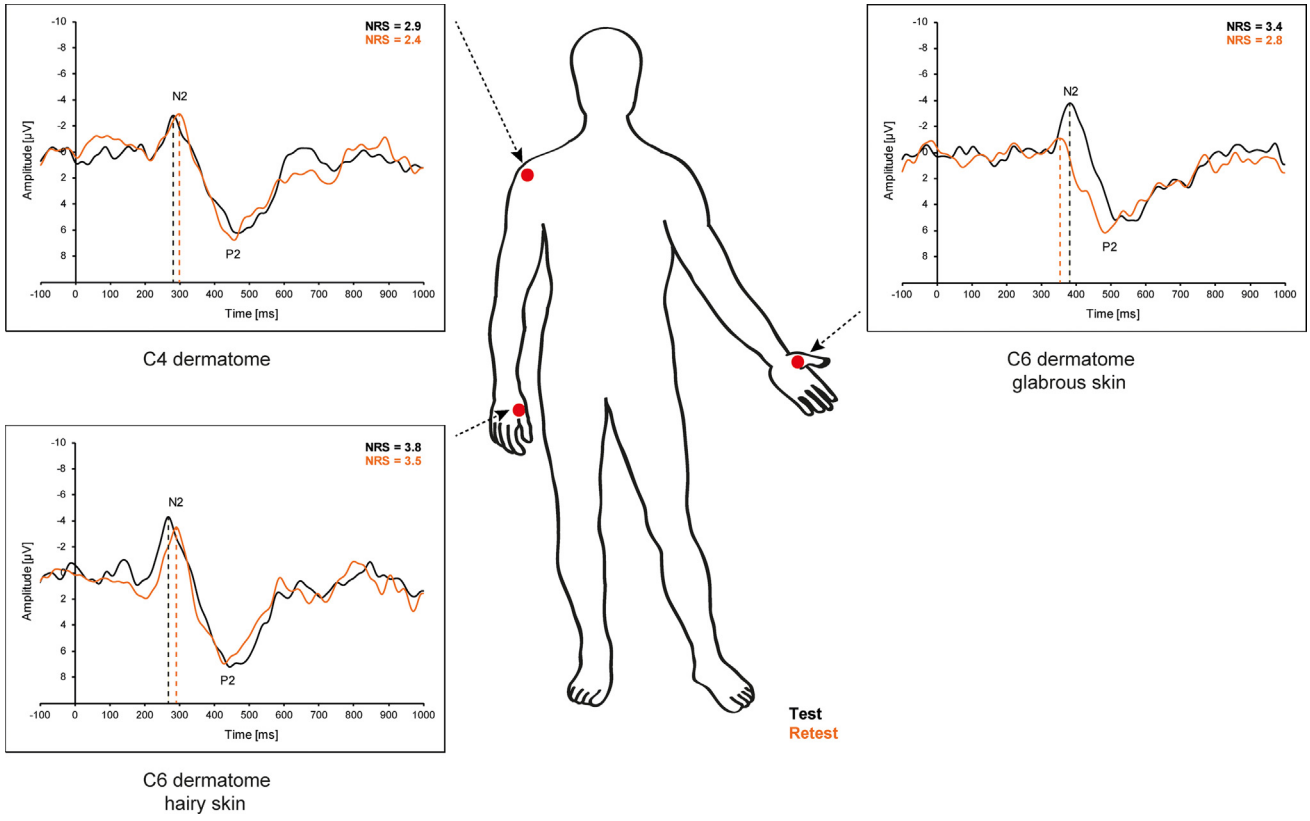

Figure 1 Grand average traces with subjective cold rating over all subjects, specifically for all tested (black) and retested (orange) sites (i.e., C4, C6 hairy skin and C6 glabrous skin).

Table 1 Proportions of detected CEPs in absolute (*n*) and percentage terms: evoked potentials (EP) and non-evoked potentials (no EP) specifically for each stimulation site and both time points.

Proportions of CEPs	C4 dermatome		C6 dermatome: hairy skin		C6 dermatome: glabrous skin	
	n	%	*n*	%	*n*	%
Test (n = 17)						
EP	17/17	100	15/17	88.2	15/17	88.2
N° EP	—	—	2/17	11.8	2/17	11.8
Retest (*n* = 15)						
EP	14/15	93.3	14/15	93.3	13/15	86.7
N° EP	1/15	6.7	1/15	6.7	2/15	13.3

Descriptive values (e.g., mean, standard deviation, 95% confidence interval, proportions) were calculated in order to characterize CEP parameters.

A general linear mixed model was used to analyze main effects of (1) the different stimulation sites; (2) sex and; (3) height on CEP parameters, which were determined as dependent factors in the model.

Test-retest analyses were only performed for the clinically relevant parameters N2/P2 amplitude, and N2 latency.

Test-retest reliability was explored using intraclass correlation coefficients (ICC, single measures, two-way mixed effects model) and Bland-Altman plots [3] for the analysis of limits of agreement between the two time points. ICCs were characterized as ''poor'' (<0.40), ''fair'' (0.41—0.60), ''moderate'' (0.61—0.80), and ''substantial'' (0.81—1.00) [43]. The Bland-Altman analysis was set-up by a one-sample *t*-test revealing whether the mean differences of CEP parameters in the test and retest were significantly different from zero. Then reliability was analyzed with a Bland-Altman plot incorporating the limit of agreement (coefficient of repeatability; mean ± 1.96*standard deviation).

Results

Subjects

All subjects passed the clinical screening assessments (anamnesis, inclusion and exclusion criteria) and the sensory

examination (mechanoreception, nociception, cool perception). Out of the 20 recruited subjects, 3 had to be excluded due to technical problems with the set-up or non-compliance (too many artefacts). To summarize, 17 subjects were included (7 females, 10 males, mean age 29.3 ± 7.7 years, mean height 174.9 ± 6.6 cm), whereof 15 subjects were retested (6 females, 9 males, mean age 27.9 ± 4.1 years, mean height 174.2 ± 6.0 cm). Men were not significantly taller than women ($P > 0.05$). The time period between test and retest was 31.7 ± 20.8 days. All participants were right-handed.

Proportion of CEPs

An overview of the data is given in Fig. 1, incorporating averaged traces of all subjects into a grand average.

The proportions of detectable CEPs (i.e., number of recorded EPs in comparison to absent, non-recordable EPs) are shown in Table 1. Regardless of stimulation site and time point, the examination of averaged traces led to potential detection in approximately 90% of all subjects. Within glabrous skin, overall persistence of EPs was the lowest.

Effects of stimulation site on CEP parameters

CEP parameters (i.e., N2 latency, N2P2 amplitude and cold rating) for all tested dermatomes are illustrated in Fig. 2.

The general linear mixed model revealed a main effect of stimulation site only on N2 latency ($F = 76.49$, $df = 1$, $P < 0.001$). Contact cold application yielded longer N2 latencies after stimulation of glabrous skin compared to both hairy skin within the same dermatome and the C4 dermatome. In contrast, neither sex nor height showed any significant effect on CEP parameters.

Test-retest reliability

Test-retest reliability analyses were based on data from 15 subjects that had been recruited for the second measurement. The proportions of EPs for both time points (i.e., test and retest) are summarized in Table 1. Exact McNemar's test did not indicate any significant difference between test and retest in terms of EP proportions ($P > 0.05$).

CEP parameters (i.e., N2 latency, N2P2 amplitude and cold rating) of all three stimulation sites are presented in Table 2.

A synopsis of ICCs and Bland-Altman coefficients for all three tested sites is given in Table 3. Cold rating was excluded from these analyses due to observed floor effects in the NRS. For C6 glabrous skin, the *t*-test of the mean difference of the N2 latency indicated a significant difference to zero ($P < 0.05$), suggesting there is no agreement between the two measurements. For all parameters Bland-Altman plots are shown in Fig. 3. The N2 latency of C6 hairy skin appeared to be the most persistent parameter, considering that test-retest differences with regard to upper and lower limits of agreement are closer to zero and to the bias (i.e., mean difference) than those of the N2P2 amplitudes.

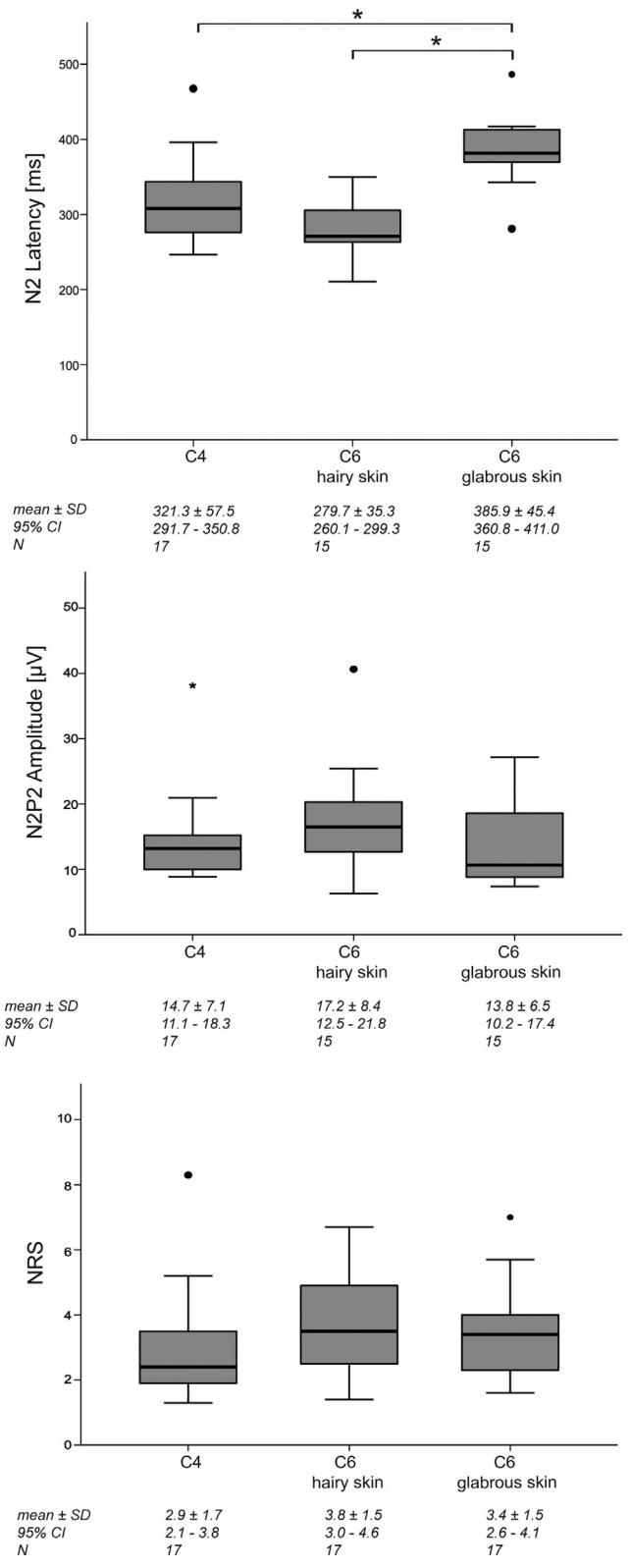

Figure 2 Boxplots and descriptive statistics of N2 latency, N2P2 amplitude and cold rating for all three stimulation sites based on data from the first test. NRS: numeric rating scale.

Table 2 Summary (mean ± SD) of N2 latency, N2P2 amplitude and cold rating (NRS) for both stimulation sites as well as for test and retest.

Stimulation site	CEP variable	Test	Retest	*n*
C4 dermatome	N2 Latency [ms]	313.6 ± 58.3	314.3 ± 60.3	14
	P2 Latency [ms]	456.9 ± 64.9	459.6 ± 63.1	14
	N2P2 Amplitude [μV]	15.0 ± 7.7	14.2 ± 8.4	14
	NRS	2.7 ± 1.1	2.4 ± 1.3	15
C6 dermatome: hairy skin	N2 Latency [ms]	274.7 ± 35.4	297.6 ± 38.3	13
	P2 Latency [ms]	433.7 ± 64.7	441.5 ± 52.7	13
	N2P2 Amplitude [μV]	18.6 ± 8.1	17.0 ± 6.5	15
	NRS	3.7 ± 1.3	3.5 ± 1.5	15
C6 dermatome: glabrous skin	N2 Latency [ms]	380.2 ± 40.2	314.6 ± 52.8	12
	P2 Latency [ms]	514.7 ± 67.8	497.1 ± 55.1	12
	N2P2 Amplitude [μV]	14.5 ± 6.7	12.7 ± 3.7	12
	NRS	3.2 ± 1.1	2.8 ± 1.3	15

n refers to the number of individuals with EPs for both test and retest.

Table 3 Test-retest analyses: Bland-Altman and intraclass correlation coefficients for all stimulation sites.

Stimulation site	CEP variable	Bland-Altman confidents (mean ± 1.96 SD)	Intraclass correlation coefficient (95% CI)
C4 dermatome	N2 Latency [ms]	0.6 ± 136.4	0.476 (−0.633 to 0.832)
	N2P2 Amplitude [μV]	−0.8 ± 9.8	0.892 (0.665 to 0.965)
C6 dermatome: hairy skin	N2 Latency [ms]	22.9 ± 108.8	−0.305 (−3.278 to 0.602)
	N2P2 Amplitude [μV]	−1.6 ± 13.7	0.711 (0.052 to 0.912)
C6 dermatome: glabrous skin	N2 Latency [ms]	−65.6 ± 121.3[a]	0.231 (−1.672 to 0.779)
	N2P2 Amplitude [μV]	−1.8 ± 10.5	0.678 (−0.119 to 0.907)

CI: confidence interval; SD: standard deviation, mean ± 1.96 SD, limit of agreement.
[a] $P < 0.05$, independent, one-sample *t*-test.

Discussion

CEPs — feasibility and reliability

In the present study, CEPs could be reliably recorded from cervical dermatomes. Feasibility was best for the C4 dermatome and stimulation within hairy skin. ICCs were only comparable for amplitudes with contact heat stimulation of the C4 dermatome [31], while latencies were less reliable compared to contact heat stimulation. This might be explained in terms of increased variability of receptor activation after contact cooling due to a less robust stimulus transduction. Skin texture and vascularization may attenuate the temperature waveform of the cold stimulus more profoundly than the contact heat pulse. This in turn results in a variable time-to-threshold and ultimately less consistent latency measures. The large difference in nominal ramps between CHEPs and CEPs, 70°C/s and 20°C/s respectively, may also translate into a different recruitment pattern of primary afferents. It is fair to assume that due to the different ramp, the CEP stimulation elicits a far less synchronized afferent volley.

Furthermore, CEP latencies are markedly shorter compared to CHEP latencies [26,31]. A likely explanation for this is the shorter time-to-activation-threshold after cold stimulation. As the baseline temperature for CEP stimulation lies close to the activation threshold of cold-sensitive A-delta fibers [41], the latter are activated almost instantly after stimulus onset. Contact heat stimuli, on the other hand, are usually delivered from a baseline temperature of 35°C. Consequently, the stimulus has to pass through lower temperatures before the activation threshold of type II AMH nociceptors (at ≥ ~42°C) is reached [44].

In contrast with previous studies using contact heat or laser stimulation in a different cohort of subjects [31,32,46], amplitudes were more robust than latencies in terms of test-retest reliability. Overall, amplitudes showed less variability and were markedly smaller than for contact heat stimulation [26]. The small decrement of cooling never evoked any noxious percepts, and the nature of the stimulus is likely to be far less salient than noxious heat [38]. Consequently, CEP amplitudes display a floor effect with inherently less variability and a seemingly good reproducibility over time. Larger amplitudes were obtained by other groups using a rapid-cooling protocol with faster stimulus onset [11].

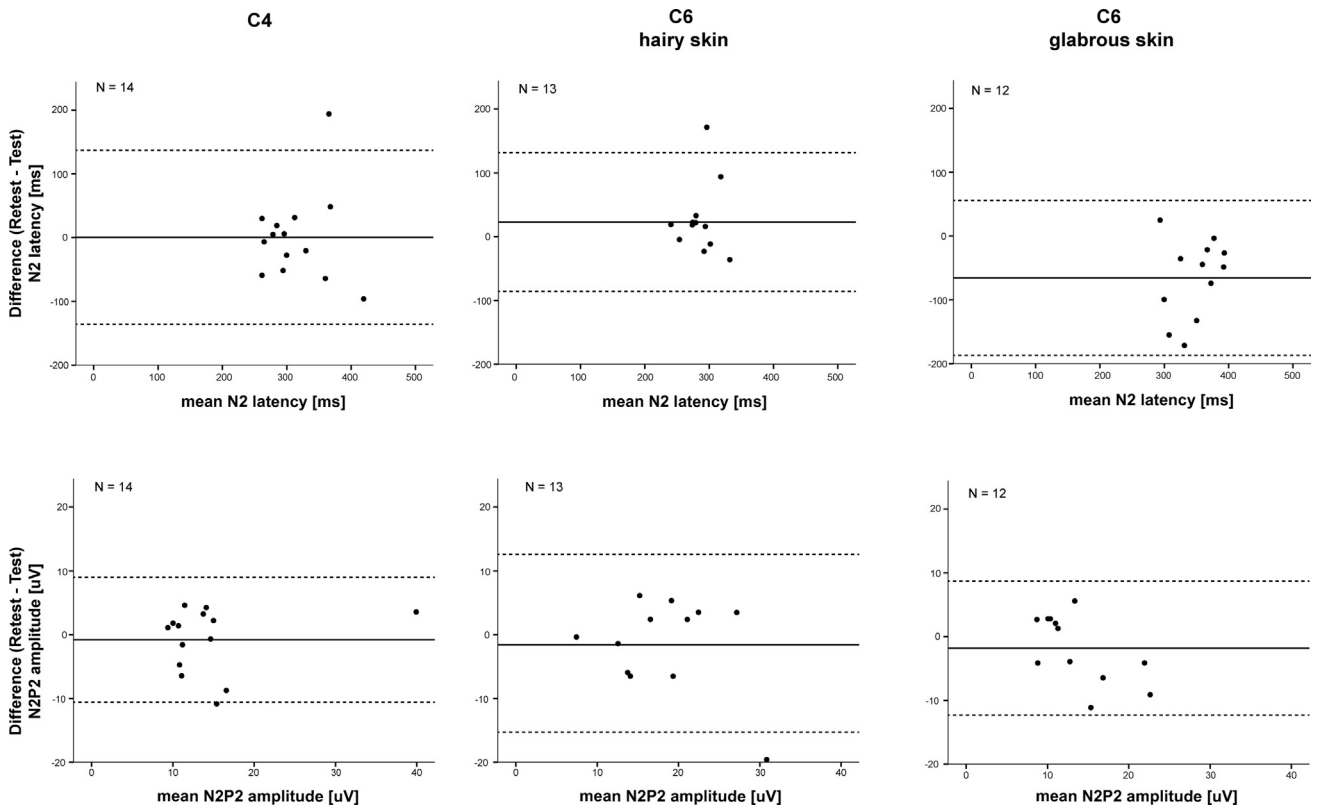

Figure 3 Bland-Altman plots for N2 latency and N2P2 amplitude of the three dermatomes. Mean difference of Retest-Test (bold line) and limits of agreements (dashed line).

The effect of skin type: glabrous vs. hairy skin

Cortical latencies after stimulation of the glabrous skin over the thenar eminence showed longer latencies than for stimulation of the dorsum of the hand. However, similar subjective ratings of coldness were reported for both stimulation sites. There are some inconsistencies in the literature about the precise location of cold-specific primary afferents within the skin. In the cat cold receptors are located at 100—150 μm deep within the skin, at the dermal-epidermal border [20]. Consequently, epidermal thickness directly impacts cold receptor activation. The stratum corneum of the glabrous skin of the palm is considerably thicker than in hairy skin [36,54]. Interestingly, fibers conveying cold pain are considered to be localized deep within the human skin as perivascular nociceptors [1,29]. These findings are supported by a study in humans by Bushnell and colleagues, identifying separate afferent channels for noxious and innocuous cold with receptors for noxious cold located deep within subdermal layers possibly in the vicinity of blood vessels [6]. The deep location of fibers conveying noxious cold sensation may thus preclude the elicitation of painful cold evoked potentials.

Latencies after stimulation of glabrous and hairy skin were significantly different. A similar finding was reported for laser stimulation at the palm of the hand [22], and contact cold stimulation at the sole of the foot [21]. Iannetti and colleagues explained this in terms of differences in skin thickness leading to a delay and attenuation of the temperature pulse [22]. Differences in skin thickness can also explain why amplitudes were most variable for glabrous skin stimulation. Furthermore, persistence of evoked potentials was the lowest after stimulation of glabrous skin. Overall, our data indicate that CEP from glabrous skin display the poorest test-retest reliability, and caution should be taken during a possible clinical application. Advances in stimulation paradigms, i.e., rapid-cooling protocols [11], may help overcome these limitations.

CEPs for clinical use — promise and potential limitations

Contact and radiant heat stimulation are widely considered useful in the examination of peripheral and central nociceptive pathways [26,27,45]. Particularly in SCI, a segmental acquisition of CHEPs has proven advantageous over clinical sensory testing and may even index afferent sparing where clinical testing reveals no sensation [18,30].

In this context, CEPs may also prove helpful and supplement clinical findings as indicated by previous reports [25,39]. The present study shows that segmental acquisition of CEPs is feasible and can be achieved with reasonable reliability.

So far, a relevant shortcoming of the CEP method is the relatively poor signal-to-noise ratio. Here, more advanced stimulation techniques like those recently published by Mouraux and colleagues [11] may further improve the acquisition and facilitate the implementation of CEPs as a clinical tool. Improved acquisition may also be achieved by slight

repositioning of the thermode between the stimuli in order to reduce receptor adaptation. In the case of CHEPs this has been shown to significantly improve signal acquisition [17], and others also reported enhanced signal-to-noise ratios for CEPs in doing so [11]. In the present study, repositioning of the thermode was not possible for methodological reasons as the subjects had to adapt to the baseline temperature of 30 °C before the stimulation protocol was started.

Conclusion

CEPs are gaining increasing importance as an objective test for cold-specific A-delta fibers. Clinically, this may close an important diagnostic gap and improve clinical practice in neurological disorders with altered cold processing. Acquisition from cervical dermatomes is feasible, however, conclusions about reliability are hampered due to a floor effect observed for amplitudes and a poor reliability for CEPs latencies. The relatively small signal — particularly compared to other methods like CHEPs and LEPs — renders CEPs prone to technical interferences. Rigorous control of the experimental condition, and standardization is required for a clinically meaningful outcome. We strongly recommend further optimizations of the set-up in order to improve the signal-to-noise ratio. First endeavors in this direction have been made by others in the field [11].

Study limitations

A more homogenous time period between test and retest condition would have been desirable in order to minimize confounding factors like seasonal differences in skin architecture/moisture [32]. In order to improve artefact correction, an electrooculogram and an additional cortical lead (e.g., a temporal derivation) should have been included following the recommendations for the clinical use of somatosensory-evoked potentials [10]. In addition to improving artefact correction, using a temporal derivation also allows the recording of the N1 component, which might be of additional value in the interpretation of the results.

Disclosure of interest

The authors declare that they have no competing interest.

Acknowledgements

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