Musculoskeletal Science and Practice 41 (2019) 55–63

Contents lists available at ScienceDirect

Musculoskeletal Science and Practice

journal homepage: www.elsevier.com/locate/msksp

Original article

Development of a topical menthol stimulus to evaluate cold hyperalgesia

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ARTICLE INFO

Keywords:
Cold hyperalgesia
Menthol
Sensory response
Clinical test

ABSTRACT

Purpose: Cold hyperalgesia is an indicator of widespread pain sensitivity and is associated with poor clinical outcomes. Menthol activates TRPM8, a cold-sensing receptor channel. This research evaluated topical menthol as a potential stimulus to be used in the clinical evaluation of cold hyperalgesia.

Methods: Participants were 59 pain free volunteers (17 male: 42 female). A blinded, repeated measures design was used. Participants received applications of menthol at different concentrations in a liquid (study 1) or gel (study 2) formulation with a 24-h interval between each application. Each menthol concentration was applied for 15 min and participants were asked to rate the sensation produced using a series of visual analogue scales and by selecting words from a descriptor list derived from the McGill pain questionnaire (MPQ). The menthol was applied to a site on the volar forearm. Participants also had their cold pain thresholds (CPT) evaluated at the same site using a contact thermode.

Results: There were significant concentration-dependent effects for intensity of cold, unpleasantness and pain VAS: cold $F_{(2,62)} = 8.67$, $p < 0.001$; unpleasantness $\chi^2_{(2)} = 14.14$, $p < 0.001$; $\chi^2_{(2)} = 11.74$, $p = 0.003$, with moderate effect sizes for unpleasantness and pain. There were also significant concentration dependent effects for descriptor indices, pain rating index (PRI) $F_{(2,62)} = 26.33$, $p < 0.001$; number of words chosen (NWC) $F_{(2,62)} = 19.62$, $p < 0.001$, with large effect sizes for 10–20% and 10–30% comparisons. Significant correlations were seen between measures of unpleasantness, pain, PRI, NWC and CPT dependent on menthol concentration.

Conclusion: Topical menthol has potential as a stimulus to evaluate cold hyperalgesia.

1. Introduction

The presence of cold hyperalgesia may be an indicator of widespread pain sensitivity (Berglund et al., 2002, Jorum et al., 2003, Stone et al., 2013) and an important prognostic indicator associated with poor outcomes in a number of conditions (Coombes et al., 2014, Goldsmith et al., 2012, O'Sullivan et al., 2014, Sterling, 2006, Wright et al., 2017). Conventional methods for assessing cold hyperalgesia require expensive laboratory equipment so there would be value in developing a clinical test that combines simplicity with good reliability and validity. This paper focuses on the development of a standardised topical stimulus for the evaluation of cold hyperalgesia in the clinical setting.

The most widely used approach to the assessment of cold hyperalgesia involves using a Peltier thermode to determine cold pain threshold (CPT). Previous studies have suggested that CPT values in excess of 12–13 °C (Maxwell and Sterling, 2013; Wright et al., 2017) may indicate the presence of cold hyperalgesia. Whilst measurement of CPT using a Peltier thermode is the accepted gold standard the test is limited to the determination of a single threshold value and does not reflect the qualitative change in sensation that is often associated with a hyperalgesic response. A number of psychophysical studies have reported that the response to cold temperature may be described as dysaesthetic (tingling, stinging or prickling) or as a paradoxical hot or burning sensation (Davis, 1998; Harrison and Davis, 1999b). Cold pain sensitivity therefore seems to be associated with a change in the quality of the perceived sensation as well as a change in the nociceptive threshold.

An alternative cold stimulus could be provided by the application of menthol to the skin since it has been shown to elicit a predominantly cold response (Green, 1992). Previous experimental pain studies in normal volunteers have used either low (Green and Schoen, 2007) or high (Binder et al., 2011, Wasner et al., 2004) concentrations of menthol. These studies indicate that low concentrations of menthol evoke weak sensations of non-noxious cold (Green and Schoen, 2007), whereas high concentrations evoke noxious cold sensations in a variable proportion of healthy subjects (Binder et al., 2011; Wasner et al., 2004). However, the exact range of normal sensory responses to different concentrations of menthol is unclear and no studies have

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https://doi.org/10.1016/j.msksp.2019.03.010
Received 24 January 2018; Received in revised form 25 March 2019; Accepted 27 March 2019

investigated the optimal concentration of menthol for differentiating individuals with cold hyperalgesia.

Basic science studies support the proposal that menthol may evoke a range of cold sensations, depending on concentration. TRPM8 cold receptors, expressed on Aδ thermo-fibres, are activated by menthol, evoking action potentials *in vitro* and behavioural responses in animals similar to that produced by cold stimulation (Belmonte et al., 2009). Each TRPM8 channel comprises four menthol binding sites so that receptor activation increases as the concentration of menthol increases and the open state stabilises (Janssens and Voets, 2011). TRPM8 receptors are expressed on cold and menthol sensitive low-threshold Aδ nociceptors (Campero et al., 2009).

The objective of the current study was to evaluate topical menthol as a potential stimulus to evoke a cold hyperalgesic response in the clinical setting. We sought to determine in healthy individuals whether different concentrations of menthol evoke only a cold sensation or whether a range of other sensations are also experienced. The primary aim was to evaluate differences in intensity and quality of sensation between different concentrations of menthol applied topically as either a liquid or a gel formulation. The two-part study also aimed to assess internal construct validity by examining associations between the intensity and quality of the sensory responses.

2. Methods

2.1. Study design

The study was completed in two parts. In the first study (Study 1) menthol at a range of concentrations was administered in liquid form and in the second study (Study 2) menthol was administered at a range of concentrations in a more practical gel formulation. In both cases a blinded, randomised repeated measures design was used, with each participant experiencing each of the menthol concentrations. A computer-generated randomization table was applied (https://www.randomizer.org/).

2.2. Participants

Pain-free healthy adult volunteers were recruited from the Curtin University Bentley campus to participate in both studies, using posters and word of mouth. Participants were included if they were aged between 18 and 70 and were able to read and understand English. Exclusion criteria included any current pain or history of chronic pain, currently taking any analgesic or anti-inflammatory medication, the presence of any neurological disorder and any history of skin allergies or allergy to menthol.

All participants provided written informed consent before participating in the study. Ethical approval was provided by Curtin University Human Research Ethics Committee (Approval numbers PT058/2006 and PT067/2009).

2.3. Preparation of menthol solutions and gels

All menthol formulations were prepared under pharmacy laboratory conditions, following standardised procedures. Three solutions of different menthol concentration were prepared, plus a solvent only control solution (0%, 10%, 20% and 30% menthol). For the second study, gels were produced by adding 2% hydroxypropylcellulose (PCCA, Australia) to each of the solutions used and stirring with a magnetic stirring device for 1.5 h. All solutions and gels were prepared by an experienced pharmacist, stored in opaque jars to prevent photo degradation and identified with a coded label to facilitate participant and assessor blinding.

2.4. Cold pain threshold testing

Cold Pain Threshold was measured at the start of the first test session, using a Peltier thermode (Somedic AB, Sweden) (Rolke et al., 2006) which has a minimum achievable temperature of 5 °C. The 2 × 3cm contact probe was attached to the forearm test site 5 cm proximal to the dominant arm wrist crease with a Velcro® strap. Participants were given several minutes to adapt to the baseline temperature of 32 °C. The thermode temperature was decreased at a rate of 1 °C/s using the standard method of limits (Fruhstorfer et al., 1976, Rolke et al., 2006). Participants were instructed to press the control switch as soon as the cooling sensation changed to one of painful cold. This temperature was recorded (°C) and the thermode returned to baseline. One practice was followed by 3 trials, each trial separated by a randomly assigned pause of between 3 and 6 s. The mean of the 3 trials was calculated for analysis.

2.5. Assessment of menthol sensation

For both studies, 2 ml of solution or gel was brought to room temperature and applied to a 2 × 3cm area at the same site as for CPT measurement and immediately occluded by a Tegaderm (3M, United States) dressing. At one-minute intervals during the 15-min menthol application participants were asked to rate the intensity and quality of the sensation they were experiencing:

a *Intensity* of cold, unpleasantness and pain was measured by the participant marking each of three VAS scales on a single sheet of paper, one scale designated for each sensation of cold, unpleasantness and pain. Each VAS scale comprised a 100 mm line with endpoints marked “minimum [sensation] imaginable” and “maximum [sensation] imaginable”. A new sheet with the three VAS scales was presented each minute. Since some participants in study 1 indicated that they were perceiving warmth, in study 2 an additional VAS was added to assess heat sensation.

b *Quality* of sensation was measured by verbal selection of words (no minimum or maximum limit) from the McGill Pain Questionnaire (MPQ) descriptor list, provided on a large print laminated sheet. Participants were given 5 min to read through the words before the start of the menthol application. The investigator recorded word choices on a data collection sheet after each minute of application. The MPQ descriptor list has been widely used to characterise both spontaneous and evoked pain (Klepac et al., 1981). It is valid and reliable in characterising pain states and shows sensitivity to change in clinical pain (Strand et al., 2008).

2.6. Procedure

Participants attended for four test sessions in study 1 or two test sessions in study 2, as shown in Fig. 1. Each test session was separated by at least 24 h. For study 1, CPT was assessed at the start of the first test session, followed by a 10-min rest period and then by the first of the randomly allocated liquid menthol formulations. The remaining 3 liquid formulations were applied in sessions 2 to 4.

For all menthol testing, the randomly allocated formulation was applied to a test site on the volar surface of the forearm for 15 min. This application time replicated that used in previous studies (Hatem et al., 2006, Wasner et al., 2004). Cold pain threshold testing was carried out at the same test site. The volar forearm site was the preferred test site due to the lower variability between individuals in epidermal thickness and hairiness (Harrison and Davis, 1999a) at this location and because it would provide a convenient site for clinical testing. The dominant arm was used for all participants.

Participants were seated with their forearm positioned in full supination resting on a pillow. Before each test the forearm site was marked, gently cleaned with tepid water and dried with a paper towel.

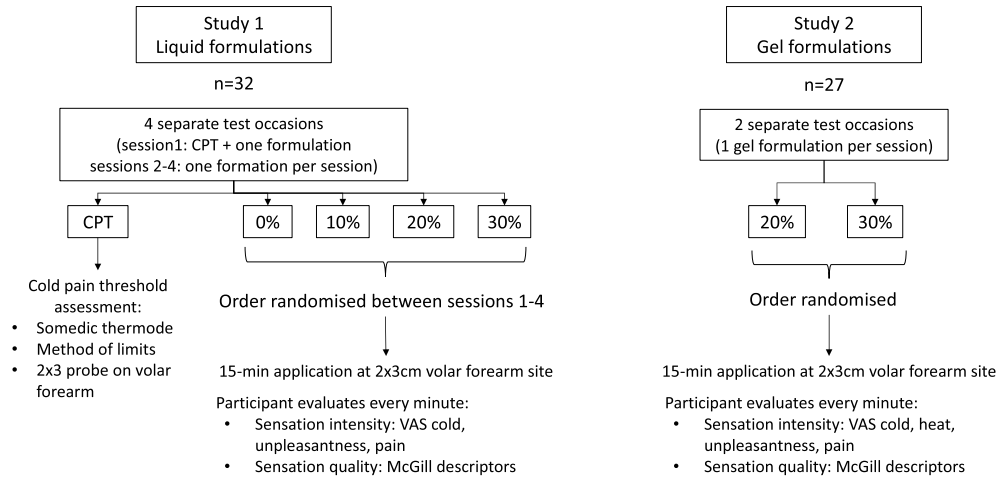


Fig. 1. Flow diagram illustrating key procedures for study 1 (liquid formulations) and study 2 (gel formulations).

In study 1 (liquid formulation) a 2 × 3cm piece of cotton gauze was then applied to the test site and 2 ml of menthol solution dispersed across the gauze with a syringe. The test site was immediately occluded with a transparent adhesive dressing (Tegaderm, 3M, United States). In the second study (Study 2) 2 ml of menthol gel was applied to the test site using a 5 ml syringe. An occlusive dressing with a 2 × 3cm window was immediately placed over the gel and the gel was gently spread so that it filled the window. In both cases, once the occlusive dressing had been applied a timer was started for the 15-min application period.

At 1-min intervals throughout the 15-min test period participants were asked to rate the intensity of sensation using the VAS scales described above, and to select words from the MPQ descriptor list to describe the sensations they were experiencing. After 15 min, the dressing was removed, skin checked for erythema and the menthol solution or gel immediately washed off. Further recordings of VAS intensity ratings and sensory descriptors were taken at 5 and 10 min after formulation removal (20 and 25 min).

2.7. Data management and analysis

Data from intensity and quality ratings were managed as follows:

2.7.1. Intensity

VAS values (0–100 mm) for intensity of cold, heat, unpleasantness and pain every minute for the 15-min application were each quantified as:

- Area under the VAS-time curve (AUC) for 1–15 min, as a measure of total intensity experienced during application;
- Maximum VAS value, as a measure of the greatest intensity experienced during the 15-min application;

2.7.1. Quality

MPQ descriptor list choices for quality of sensation experienced every minute for 15 min were quantified using two standard MPQ indices:

- Pain Rating Index (PRI): sum of MPQ ranking values for each different word selected. No single word was included more than once.
- Number of Words Chosen (NWC): the total number of different words selected by an individual during the 15-min application.

Normality testing was carried out using the Shapiro-Wilk test. Differences between concentrations were evaluated using repeated measure ANOVA or t-tests. Where data were not normally distributed, non-parametric analyses (Friedman ANOVA by ranks or Mann Witney U tests) were performed. Effect sizes were calculated with Cohen's-d. Associations between measures were analysed using Pearson's or Spearman's Correlation Coefficients as appropriate. A series of linear regression models were applied iteratively to determine which menthol response measures were most predictive of CPT. Data were analysed using the SPSS statistical package, version 22. Alpha was set at $p < 0.05$.

3. Results

3.1. Study 1

There were 32 participants (7 males: 25 females) in study 1. The mean age of the group was 29 years (range 19–61). The solvent-only control solution did not evoke any sensations of cold, unpleasantness or pain in any participant and so was excluded from further analyses.

All three liquid applications containing menthol (10%,20%, 30%) evoked cold, unpleasantness and pain sensations greater than 0/10 VAS in a proportion of participants (cold VAS > 0, 94–100%; unpleasantness VAS > 0, 66–84%; pain VAS > 0, 35–47%) indicating that the menthol stimulus evokes a range of sensations in addition to cold in healthy individuals (Fig. 2). However, max VAS intensity ratings were low, ranging from 30.0 to 39.9/100 for cold to 11.3–25.1/100 for

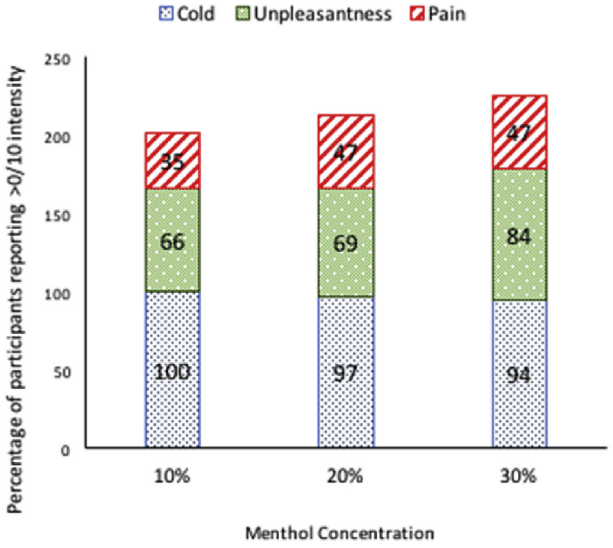
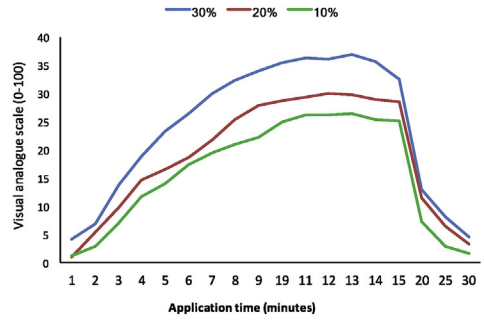
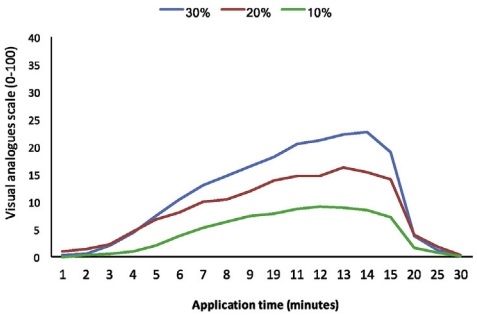


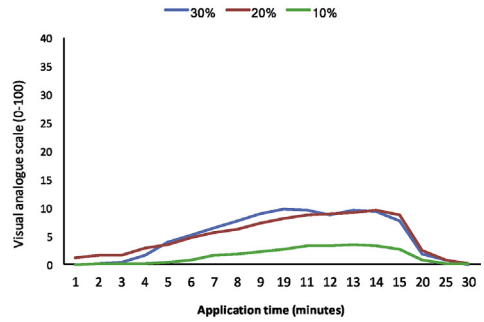
Fig. 2. Percentage of participants reporting > 0/10 intensity of cold, unpleasantness or pain at each liquid menthol concentration.



a): Cold VAS values for 30%, 20% and 10% menthol concentrations during 15-minute application and 15-minute post-application periods.



b): Unpleasantness VAS values for 30%, 20% and 10% menthol concentrations during 15-minute application and 15-minute post-application periods.



c): Pain VAS values for 30%, 20% and 10% menthol concentrations during 15-minute application and 15-minute post-application periods.

Fig. 3. a–c: Cold, unpleasantness and pain VAS values for 10%, 20% and 30% menthol concentrations plotted over time.

unpleasantness and 4.0–12.2/100 for pain.

VAS data for mean cold, unpleasantness and pain at each measured time-point for each concentration of menthol were plotted on VAS-time graphs (Fig. 3a–c). These graphs show that cold intensity built quickly after initial application and maximum was reached at around 11 or 12 min, regardless of concentration. Unpleasantness intensity took slightly longer to build and reached maximum at around 12–14 min although it was also similar between concentrations. Pain intensity was slower again to build, reached maximum around 10–11 min for each concentration, but then plateaued until menthol removal.

Since cold VAS data were normally distributed (Shapiro-Wilk $p = 0.147$) parametric statistics were applied. However, unpleasantness and pain VAS data were not normally distributed, and so non-parametric analyses were used. A significant effect of liquid menthol concentration on total VAS intensity (AUC) was found for each of the measures (Table 1), with a gradual increase seen in perceived intensity as concentration increased. Post hoc comparisons showed that there was a significant difference between all menthol concentrations for cold VAS intensity (10–20% $p = 0.017$; 20–30% $p = 0.019$; 10–30% $p < 0.001$). Effect sizes ranged from small for 10–20% comparisons to medium for 10–30% comparisons (Table 2). For both unpleasantness and pain VAS there were significant differences between the lower

Table 2
Effect sizes (Cohen's-d) for intensity and quality descriptor values.

	10%–20%	20%–30%	10%–30%
VAS AUC			
Cold	0.262	0.408	0.614
Unpleasantness	0.501	0.281	0.856
Pain	0.619	0.000	0.520
VAS max			
Cold	0.120	0.400	0.499
Unpleasantness	0.437	0.373	0.829
Pain	0.644	0.055	0.514
PRI	1.280	0.485	1.735
NWC	1.743	0.359	2.003

Table 1
Mean (standard deviation (SD), 95% confidence interval (CI)) for area under the time-VAS curve (AUC) values and maximum VAS values for each sensation at each concentration (Study 1).

	10%			20%			30%			Statistic	p
	Mean	SD	95% CI	Mean	SD	95% CI	Mean	SD	95% CI		
VAS AUC											
Cold	273.8	204.7	207.6–349.9	337.9	184.9	263.5–392.6	428.5	257.3	327.1–504.4	$F_{(2,62)} = 11.31$	< 0.001
Unpleasantness	79.7	100.1	43.6–114.12	151.5	176.5	86.9–211.8	199.3	171.4	136.3–258.9	$\chi^2_{(2)} = 11.58$	0.003
Pain	26.7	59.4	5.0–47.7	91.7	135.7	42.2–139.3	91.6	166.3	31.4–150.2	$\chi^2_{(2)} = 16.44$	< 0.001
VAS max											
Cold	30.1	18.2	23.5–36.6	32.2	17.3	26.0–38.4	39.9	21.1	32.3–47.5	$F_{(2,62)} = 8.67$	< 0.001
Unpleasantness	11.3	12.9	6.6–16.0	18.1	17.8	11.7–24.5	25.1	19.7	18.0–32.2	$\chi^2_{(2)} = 14.14$	< 0.001
Pain	4.0	8.2	1.1–7.0	12.2	15.9	6.5–17.9	11.3	18.1	4.7–17.8	$\chi^2_{(2)} = 11.74$	0.003

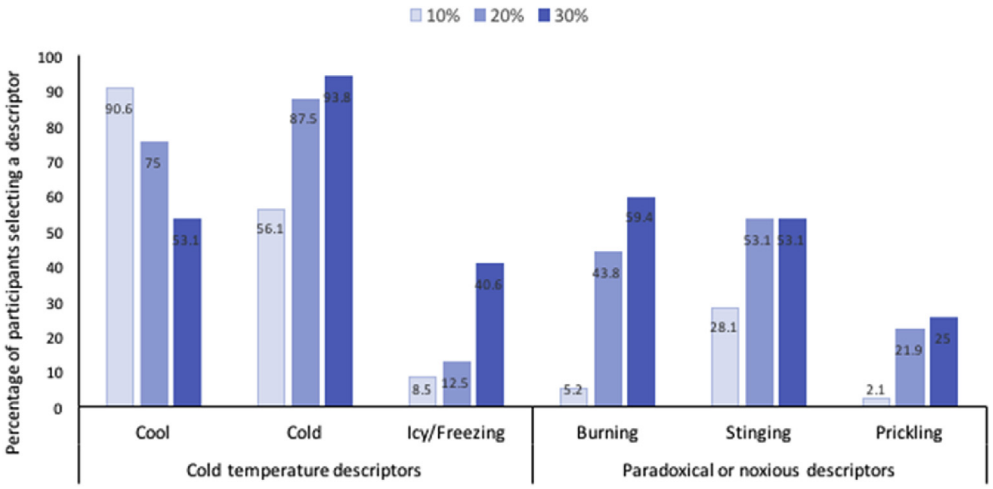


Fig. 4. Percentage of participants selecting cold and paradoxical or dysaesthetic sensory descriptors for each menthol concentration.

concentrations of 10 and 20% menthol (unpleasantness p = 0.012; pain p = 0.002, moderate effect sizes (Table 2), and between 10 and 30% menthol (unpleasantness p < 0.001; pain p = 0.003, moderate to large effect sizes), but not between 20 and 30% menthol (unpleasantness p = 0.108; pain p = 0.717).

Maximum VAS intensity experienced during the 15-min application period also showed a significant concentration-dependent effect for each sensation (Table 1). Post-hoc comparisons and effect size calculations showed a similar pattern as for AUC values, with significant differences for all sensations between the highest and lowest menthol concentrations. Significant differences in unpleasantness and pain were found between 10 and 20% menthol, but not between the higher concentrations of 20 and 30% menthol (unpleasantness p = 0.086; pain p = 0.710). Maximum cold VAS however showed a significant difference between 20 and 30% menthol, but not between 10 and 20% menthol (p = 0.322).

Analysis of sensory quality descriptors selected by participants from the McGill list supported VAS intensity findings by indicating that menthol evokes a complex sensory response that is concentration-dependent (Fig. 4). 10% menthol application resulted in a predominantly cold and relatively mild response. In contrast, the word icy or freezing and the dysaesthetic words burning, stinging and prickly were more often chosen at higher concentrations (Fig. 4).

When MPQ index values were calculated for the descriptors (PRI and NWC indices), a significant concentration-dependent difference was also found for both (PRI $F_{(2,62)} = 26.33$, $p < 0.001$; NWC $F_{(2,62)} = 19.62$, $p < 0.001$), with higher scores recorded at higher menthol concentrations (Fig. 5). Post-hoc comparisons showed that the difference was significant between the lowest concentrations of 10 and 20% menthol (PRI: $t_{(31)} = -7.01$, $p < 0.001$; NWC: $t_{(31)} = -5.80$, $p < 0.001$), and also between 10 and 30% menthol (PRI: $t_{(31)} = -5.56$, $p < 0.001$; NWC: $t_{(31)} = -5.40$, $p < 0.001$). Large effect sizes, from 1.28 to 2.003, were seen (Table 2). However, in a similar manner to VAS intensity ratings, there was no significant difference in sensory quality scores between the two highest concentrations of 20 and 30% menthol (PRI: $t_{(31)} = -1.14$, $p = 0.265$; NWC: $t_{(31)} = -1.13$, $p = 0.266$; effect sizes 0.485 and 0.359 respectively).

Mean forearm CPT was 8.95 °C (standard deviation 6.4, range 0–21.3 °C). There were significant moderate positive correlations between CPT and scores for PRI and NWC for 10 and 20% menthol, but no correlation between CPT and PRI or NWC for 30% menthol (Table 2). There was also a moderate positive correlation between CPT and 30% menthol pain VAS. The only strong correlation between CPT and cold intensity rating was for 10% menthol with no correlations apparent at other concentrations. Unpleasantness ratings and pain ratings were moderately correlated at all menthol concentrations. Unpleasantness also correlated with PRI and NWC scores at the higher concentrations of 20 and 30% menthol. Cold intensity ratings correlated with PRI and unpleasantness and pain ratings correlated with PRI and NWC scores, also at the higher concentrations (Table 3).

A series of linear regression models were applied to determine the 3 most significant menthol response predictors of CPT. Menthol response variables were initially entered into linear regression models grouped according to concentration. Variables with the highest r^2 value

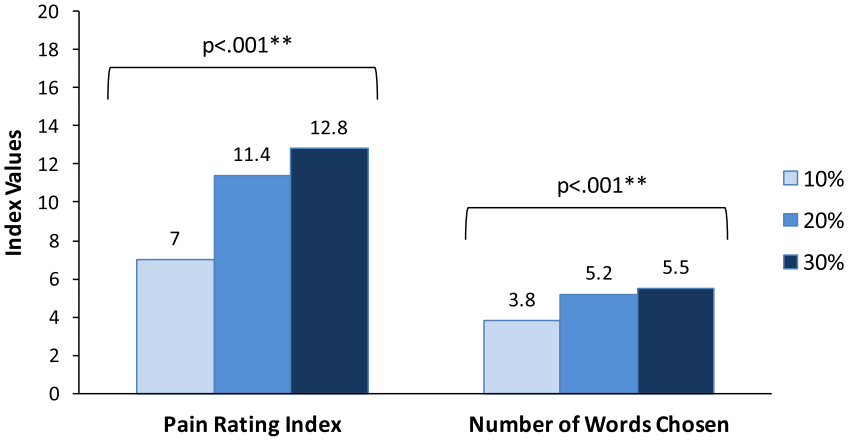


Fig. 5. Mean quality descriptor index scores for each menthol concentration.

Table 3
Correlations between CPT, VAS ratings, PRI and NWC scores for all menthol concentrations (Study 1).

	CPT	Cold max	Cold AUC	Unpleasantness max	Unpleasantness AUC	Pain max	Pain AUC	PRI	NWC
10% menthol									
CPT		**r = −0.377**	**r = 0.971**	r = 0.167	r = 0.210	r = 0.114	r = −0.107	**r = 0.494**	**r = 0.423**
		p = 0.044*	**p < 0.001****	p = 0.395	p = 0.284	p = 0.558	p = 0.587	**p = 0.004***	**p = 0.016***
Cold max				r = 0.084	r = 0.042	r = −0.013	r = 0.031	r = 0.329	r = 0.164
				p = 0.648	p = 0.819	p = 0.945	p = 0.866	p = 0.066	p = 0.371
Cold AUC				r = 0.217	r = 0.245	r = 0.214	r = 0.160	r = 0.315	r = 0.225
				p = 0.258	p = 0.200	p = 0.258	p = 0.408	p = 0.096	p = 0.241
Unpleasantness max						**r = 0.490**	**r = 0.447**	r = 0.276	r = 0.125
						p = 0.004*	**p = 0.010***	p = 0.126	p = 0.496
Unpleasantness AUC						**r = 0.604**	**r = 0.560**	r = 0.339	r = 0.183
						p < 0.001**	**p < 0.001****	p = 0.058	p = 0.315
Pain max								r = 0.261	r = 0.305
								p = 0.149	p = 0.09
Pain AUC								r = 0.174	r = 0.168
								p = 0.341	p = 0.358
20% menthol									
CPT		r = −0.006	r = 0.210	r = 0.224	r = 0.264	r = 0.180	r = 0.191	**r = 0.493**	**r = 0.358**
		p = 0.975	p = 0.284	p = 0.242	p = 0.175	p = 0.351	p = 0.332	**p = 0.004***	**p = 0.044***
Cold max				r = 0.205	r = 0.142	r = 0.196	r = 0.128	**r = 0.416**	r = 0.255
				p = 0.261	p = 0.438	p = 0.282	p = 0.484	**p = 0.018***	p = 0.159
Cold AUC				r = 0.324	r = 0.262	r = 0.296	r = 0.250	**r = 0.415**	r = 0.257
				p = 0.070	p = 0.148	p = 0.100	p = 0.167	**p = 0.018***	p = 0.156
Unpleasantness max						**r = 0.755**	**r = 0.689**	r = 0.282	**r = 0.368**
						p < 0.001**	**p < 0.001****	p = 0.118	**p = 0.038***
Unpleasantness AUC						**r = 0.728**	**r = 0.736**	**r = 0.403**	r = 0.306
						p < 0.001**	**p < 0.001****	**p = 0.022***	p = 0.089
Pain max								r = 0.272	r = 0.237
								p = 0.133	p = 0.191
Pain AUC								**r = 0.405**	r = 0.144
								p = 0.021*	p = 0.430
30% Menthol									
CPT		r = −0.041	r = 0.142	r = 0.053	r = 0.198	r = 0.482	**r = 0.475**	r = −0.028	r = −0.057
		p = 0.832	p = 0.470	p = 0.786	p = 0.236	p = 0.008	**p = 0.011***	p = 0.879	p = 0.755
Cold max				**r = 0.397**	r = 0.235	r = 0.215	r = 0.216	r = 0.217	r = 0.222
				p = 0.024*	p = 0.196	p = 0.236	p = 0.234	p = 0.234	p = 0.223
Cold AUC				**r = 0.471**	r = 0.369	r = 0.299	r = 0.324	r = 0.289	**r = 0.376**
				p = 0.007*	p = 0.038*	p = 0.096	p = 0.071	p = 0.109	**p = 0.034***
Unpleasantness max						**r = 0.456**	**r = 0.432**	**r = 0.577**	**r = 0.421**
						p = 0.009*	**p = 0.014***	**p = 0.001***	**p = 0.016***
Unpleasantness AUC						**r = 0.572**	**r = 0.588**	**r = 0.514**	**r = 0.476**
						p = 0.001*	**p < 0.001****	**p = 0.003***	**p = 0.006***
Pain max								r = 0.336	r = 0.321
								p = 0.06	p = 0.073
Pain AUC								**r = 0.520**	**r = 0.387**
								p = 0.002*	**p = 0.029***

Table 4
Linear regression analysis of values for predicting CPT.

	Unstandardized Coefficients		Standardised Coefficients	t	Sig.	95.0% Confidence Interval for B	
	B	Std. Error	Beta			Lower bound	Upper bound
Constant	7.121	1.9		3.747	0.001	3.222	11.02
AUC 10% Cold	−0.025	0.006	−0.788	−3.969	0.000	−0.038	−0.012
AUC 20% Cold	0.022	0.007	0.595	2.983	0.006	0.007	0.037
AUC 30% Pain	0.016	0.005	0.436	3.213	0.003	0.006	0.027

(Nagelkirke) and p-value were then entered into a further linear regression model. The final regression model with 3 predictors demonstrated an r^2 value of 0.509, and p-values of $p < 0.001$ for AUC 10% cold, $p = 0.006$ for AUC 20% cold and $p = 0.003$ for AUC 30% pain. (Table 4).

Comparisons between those participants who scored a VAS rating of pain (n = 8) and those who did not rate the menthol stimulus as painful (n = 21) showed a significant difference in CPT for 30% menthol but not for the lower menthol concentrations (Table 5).

Table 5
Mean CPT values for participants who rated the menthol stimulus as painful and those who did not rate it as painful according to VAS pain ratings.

	10% menthol			20% menthol			30% menthol		
	n	CPT (°C)		n	CPT (°C)		n	CPT (°C)	
		mean	SD		Mean	SD		Mean	SD
Pain > 0	8	10.15	3.73	14	10.55	5.16	12	11.58	4.56
No Pain	21	8.49	7.21	15	7.46	7.27	17	7.09	6.99
statistic	−0.804			−1.31			−2.09		
p-value	0.430			0.201			0.047		

Table 6
Mean (standard deviation (SD), 95% confidence interval (CI)) and effect size (Cohen's d) for area under the time-VAS curve (AUC) values and maximum VAS values for each sensation at each concentration (Study 1).

	10%			20%			statistic	P	Cohen's d
	Mean	SD	95% CI	Mean	SD	95% CI			
VAS AUC									
Cold	227.9	209.5	145.0–310.8	260.2	278.0	150.2–370.1	$F_{(1,52)} = -0.88$	0.386	0.131
Heat	49.9	96.2	4.9–81.0	80.2	156.9	18.1–142.3	$F_{(1,52)} = -2.05$	0.050	0.233
Unpleasantness	85.4	130.9	33.6–137.2	155.4	178.7	84.7–226.1	$\chi^2_{(1)} = -2.76$	0.011	0.447
Pain	18.3	56.5	4.0–40.9	55.5	101.5	15.3–95.6	$\chi^2_{(1)} = -2.71$	0.005	0.453
VAS max									
Cold	27.8	22.7	18.4–36.4	35.0	25.3	25.8–45.7	$F_{(1,52)} = -2.65$	0.013	0.300
Heat	5.9	12.8	0.97–11.1	12.8	15.8	2.6–19.0	$F_{(1,52)} = -2.81$	0.004	0.480
Unpleasantness	11.9	19.1	5.9–21.0	18.7	21.6	9.5–26.6	$\chi^2_{(1)} = -2.86$	0.003	0.334
Pain	2.6	4.7	0.28–6.4	9.3	15.1	2.3–14.2	$\chi^2_{(2)} = -2.94$	0.002	0.599

3.2. Study 2

There were 27 participants (10 males: 17 females) in study 2. The mean age of the group was 34 years (range 18–52 years).

Because study 1 did not show significant differences in sensory response between 20% and 30% menthol, study 2 was limited to comparisons between the 10% and 20% menthol gel formulations. There were significant concentration-dependent differences in VAS intensity ratings, both as measured by area under the VAS-time curve and by maximum VAS for heat, unpleasantness and pain, although cold only showed a dose-dependent pattern with maximum VAS values (Table 6). Effect sizes for maximum VAS intensity ratings were generally small to medium (Table 6).

There was also a significant difference between concentrations in descriptor choice, with the higher concentration evoking higher scores for PRI and NWC: PRI $t_{(26)}$ = -3.53, p = 0.002; NWC $t_{(26)} = -3.00$, p = 0.006 (Fig. 6). Effect sizes were moderate for both indices (Cohen's d: PRI 0.571; NWC 0.537).

Study 2 also demonstrated significant correlations between VAS unpleasantness ratings and pain ratings (Table 7). These ratings were also strongly correlated with PRI and NWC ratings at the higher menthol concentration (20%), showing a clear association between intensity and unpleasant or painful sensation quality. Cold VAS was associated with unpleasantness ratings for 20% menthol but there were no other correlations between heat or cold ratings and other measures (Table 7).

4. Discussion

The objective of this research was to evaluate topical menthol as a potential stimulus to evoke a cold hyperalgesic response in the clinical setting. We sought to determine in healthy individuals whether different concentrations of menthol evoke only a cold sensation or whether a range of other sensations are also experienced. We also assessed internal construct validity by examining associations between the intensity and quality of the sensory responses. The two studies systematically investigated whether different concentrations of topical menthol evoked dose-dependent differences in intensity of sensation and/or quality of sensation. They clearly showed that different concentrations of menthol elicited a graded response in the sensory system and evoked a range of sensations.

The majority of participants reported that menthol at all concentrations evoked a predominantly cold sensation, which increased in intensity with increasing concentration. Participants reported higher intensity and clear differences in the quality of sensation at the higher menthol concentrations. Whilst the lowest concentration of 10% menthol elicited a predominantly cool or cold sensation, 20% and 30% menthol evoked more intense cold or icy sensations, associated with noxious sensations such as stinging, prickling or burning.

Findings from both studies also indicated a clearly graded VAS rating response. In study 1 the difference in VAS intensity ratings for unpleasantness and pain was most clearly shown between 10% and 20% menthol and between 10% and 30% menthol concentrations, but showed limited difference between 20% and 30%. The same pattern was shown for scores related to sensation quality (PRI and NWC). Clear distinctions between 10% and 20% menthol concentrations for VAS, PRI and NWC measures were confirmed in study 2. Overall this suggests that menthol has a concentration dependent effect but that there is a ceiling effect with relatively little change in either intensity or quality of sensory response at concentrations above 20%.

In both studies there were significant correlations between intensity ratings for unpleasantness and pain and between those ratings and PRI

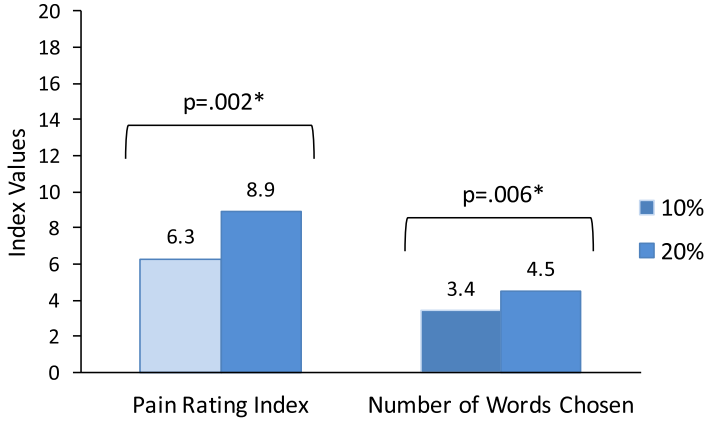


Figure 6: Mean quality descriptor index scores for each menthol concentration (study 2).

Fig. 6. Mean quality descriptor index scores for each menthol concentration (study 2).

Table 7
Correlations between CPT, VAS ratings, PRI and NWC scores for both menthol concentrations (Study 2).

	Cold max	Cold AUC	Heat max	Heat AUC	Unpleasantness max	Unpleasantness AUC	Pain max	Pain AUC	PRI	NWC
10% menthol										
Cold max			r = −0.025 p = 0.903	r = −0.131 p = 0.514	r = 0.070 p = 0.728	r = −0.006 p = 0.977	r = 0.046 p = 0.821	r = −0.035 p = 0.862	r = 0.090 p = 0.655	r = −0.094 p = 0.741
Cold AUC			r = −0.169 p = 0.400	r = −0.255 p = 0.199	r = 0.221 p = 0.267	r = −0.147 p = 0.466	r = 0.142 p = 0.478	r = −0.004 p = 0.984	r = 0.197 p = 0.323	r = −0.017 p = 0.933
Heat max					r = −0.214 p = 0.284	r = −0.211 p = 0.291	r = −0.095 p = 0.639	r = −0.010 p = 0.961	r = −0.128 p = 0.524	r = −0.64 p = 0.749
Heat AUC					r = −0.181 p = 0.368	r = −0.161 p = 0.423	r = −0.014 p = 0.946	r = 0.087 p = 0.665	r = −0.059 p = 0.770	r = −0.006 p = 0.975
Unpleasantness max							**r = 0.519** **p = 0.005***	r = 0.273 p = 0.169	**r = 0.449** **p = 0.019***	r = 0.348 p = 0.075
Unpleasantness AUC							r = 0.421 p = 0.029*	r = 0.271 p = 0.171	r = 0.320 p = 0.103	r = 0.220 p = 0.269
Pain max									**r = 0.604** **p < 0.001****	**r = 0.529** **p = 0.005***
Pain AUC									r = 0.354 p = 0.070	r = 0.333 p = 0.089
20% menthol										
Cold max			r = −0.159 p = 0.429	r = −0.159 p = 0.429	**r = 0.486** **p = 0.010***	**r = 0.400** **p = 0.039***	r = 0.322 p = 0.102	r = 0.299 p = 0.130	r = 0.304 p = 0.123	r = 0.148 p = 0.461
Cold AUC			r = −0.1169 p = 0.401	r = −0.224 p = 0.261	**r = 0.454** **p = 0.016***	r = 0.360 p = 0.065	r = 0.272 p = 0.170	r = 0.252 p = 0.204	r = 0.293 p = 0.138	r = 0.144 p = 0.473
Heat max					r = −0.042 p = 0.836	r = −0.040 p = 0.845	r = 0.146 p = 0.467	r = 0.107 p = 0.597	r = 0.036 p = 0.859	r = 0.135 p = 0.501
Heat AUC					r = −0.046 p = 0.821	r = −0.046 p = 0.819	r = 0.166 p = 0.409	r = 0.127 p = 0.529	r = 0.012 p = 0.954	r = 0.102 p = 0.612
Unpleasantness max							**r = 0.697** **p < 0.001****	**r = 0.688** **p < 0.001****	**r = 0.643** **p < 0.001****	**r = 0.510** **p = 0.007***
Unpleasantness AUC							**r = 0.674** **p < 0.001****	**r = 0.679** **p < 0.001****	**r = 0.714** **p < 0.001****	**r = 0.594** **p = 0.001***
Pain max									**r = 0.699** **p < 0.001****	**r = 0.655** **p < 0.001****
Pain AUC									**r = 0.766** **p < 0.001****	**r = 0.722** **p < 0.001****

and NWC scores. There was also a positive association between CPT, pain ratings, PRI and NWC, suggesting a similar construct. In contrast, there were only limited correlations between cold and heat intensity and CPT. This suggests that while these measures are evaluating aspects of the sensory response to menthol they are not strongly associated with a noxious or hyperalgesic response. A measure combining unpleasantness, and pain intensity with PRI and NWC measures in response to topical menthol stimulation may have good construct validity as a means of evaluating cold hyperalgesia.

Linear regression analysis showed an association between measures of cold response and a measure of pain response to menthol and CPT. Cold intensity was most predictive at 10% and 20% menthol concentration and pain at 30%. This again highlights the potential importance of developing a combination measure to most accurately reflect the difference in perceived intensity and quality of sensation associated with cold hyperalgesia.

There is limited data with which to compare these results. Green and Schoen (2007) applied 10% menthol in a similar aqueous-ethanol vehicle to a slightly larger area on the volar forearm before assessing cold and nociceptive sensations. Baseline menthol effect was rated as 'between barely detectable and weak', with cold being felt marginally more than pain. Hatem et al. (2006) used a higher concentration of menthol as an experimental cold sensitising agent. The volume and area of application was very similar to the current study. However when participants were asked for spontaneous words to describe what they were feeling, 90% reported coolness after 10 min, 10% reported warmth and no-one reported pain. The higher reports of unpleasantness and pain from participants in the current study most likely reflect differences in methodology. Hatem et al. (2006) did not use a systematic questioning approach since the study focus was on the effects of cold temperature on pain perception. In contrast the current studies required participants to concentrate on the sensation experienced and evaluate it using specific VAS scales and descriptor lists. The current studies were therefore biased towards more detailed reporting of sensory responses.

There were some limitations to these studies. For study 1 approximately 40% of the participants experienced visible erythema after removal of the liquid formulation. In approximately 20% this extended beyond the area of gauze application and appeared to be associated with some pooling of the liquid. When the liquid was washed off the erythema dispersed within 15 min and no long term irritation occurred. The use of a gel formulation and a more even application in study 2 reduced these problems. Although pooling of the liquid appeared to result in more intense sensations such as stinging no reduction in the overall sensory response was seen with the gel formulation in study 2.

Cold hyperalgesia may be a useful early indicator of persistent pain development in a range of musculoskeletal conditions. Whilst measurement of CPT remains the gold standard method for differentiating individuals with cold hyperalgesia, a clinically applicable, low cost, low tech method has yet to be developed. Measurement of response to an ice block has been proposed (Maxwell and Sterling, 2013). However, topically-applied ice is likely to be noxious to a large number of individuals suggesting that a stimulus such as menthol that can more clearly differentiate an abnormal response may be more useful. When trying to identify the presence of cold hyperalgesia, having data for both sensation intensity and quality allows more comprehensive characterisation to occur than is possible with a single outcome variable, such as CPT temperature. The current studies provide evidence to support content and construct validity for topical menthol as a stimulus to evoke cold responses and so identify individuals experiencing cold hyperalgesia. The combination of topical menthol with a suitable response measure has considerable potential as a relatively simple way to evaluate cold hyperalgesia in the clinical environment.

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