Original Research Articles

Pain Perception in Healthy Young Men Is Modified by Time-Of-Day and Is Modality Dependent

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Abstract

Objective. Several physiological processes exhibit 24-hour oscillations termed circadian rhythms. Despite numerous investigations on the circadian dynamics of pain perception, findings related to this issue remain inconsistent. This study aimed to assess the effect of time-of-day on multimodal experimental pain perception in healthy males, including “static” and “dynamic” quantitative sensory tests.

Design. A random order tests were performed in the morning, afternoon and evening.

Subjects. Forty-eight healthy males (25.9 ± 4.7 years old).

Methods. Three different pain modalities i) mechanical (pain threshold, tolerance, and intensity), ii) heat (pain threshold and intensity), iii) cold (pain threshold measured in °C and in seconds and cold pain tolerance and intensity) utilizing nine “static” pain parameters, and two “dynamic” pain paradigms i) temporal summation and ii) conditioned pain modulation were assessed in each session.

Results. Pain scores varied significantly in six pain parameters during the day. Specifically, lower pain scores were found in the morning for cold pain threshold (in seconds and in °C), cold pain intensity, cold pain tolerance, heat pain threshold and intensity. There were no significant diurnal differences in the mechanical evoked pain parameters or in either of the “dynamic” pain paradigms.

Conclusions. Thermal pain scores varies during the day and morning seems to be the time-of-day most insensitive to pain. Also, dynamic tests and the mechanical pain model are not appropriate for detecting diurnal variability in pain. The results of this study may be partially explained by a potential analgesic effect of some hormones known to have diurnal variation (e.g., melatonin and cortisol).

Key Words. Diurnal Variation; Healthy Volunteers; Quantitative Sensory Test
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Introduction

Several physiological and behavioral processes exhibit 24-hour oscillations termed circadian rhythms. These include circadian rhythms in sleep and activity patterns [1]; body temperature [2]; serum levels of thyroid stimulating hormone (TSH) [3] and prolactin [4]; the production of cytokines in rheumatoid arthritis (RA) [5]; and plasma melatonin and cortisol levels [6–8]. The mechanisms of the time-dependent variations of these processes are complex and multifaceted.

Despite numerous investigations on the circadian dynamics of pain perception, findings related to this issue remain inconsistent either in clinical pain patients or in healthy volunteer participants. Studies on clinical populations have shown peak pain levels in the morning in patients with different chronic pain conditions, such as back pain [9]; migraine [10]; fibromyalgia [11]; osteoarthritis [12]; and RA [13]. Other studies have demonstrated peak pain levels at noon in patients with osteoarthritis [14] or in the evening in patients with cancer and back pain [15,16]. Studies in healthy subjects exposed to experimental pain models have also reported conflicting results. While some studies have shown diurnal variation [17–21], others have not [22–28]. However, as those studies were methodologically diverse by means of study settings, pain models used and the exact time points of measurements, the ability to compare findings and to generalize conclusions from one study to another is limited. Hence, diurnal variation in pain perception is still an open question and warrants further investigation.

This study aimed to assess the effect of time-of-day on experimental pain perception in healthy males exposed to multimodal pain tests, including “static” and “dynamic” quantitative sensory tests (QST).

The importance of this study is in its potential to identify the time-of-day when individuals are prone to be more painful, as well as to choose the most adequate pain tests to be used for exploring this issue. Such information may shed light on the normal pain system and may assist when setting up an experimental pain study to avoid a potential source of bias.

Materials and Methods

Sample

The required sample size was calculated by G* Power analysis [29]. Accordingly, 40 subjects were required for a within-subjects, repeated measures design, with a medium effect size (0.25), \( \alpha \leq 0.05 \), power of 0.80. Forty-eight healthy male subjects (25.9 ± 4.7 years old) were recruited by advertisements at the University of Haifa.

Inclusion criteria were: 1) healthy young men over 18 years of age; 2) Hebrew speakers; 3) free of any type of chronic or acute pain; 4) not taking any chronic medication; and 5) with the ability to communicate and understand the purposes and instructions of the study. The subjects were instructed to avoid any activity that may influence their pain perception at least four hours before the experiment began (i.e., heavy meal, tobacco and alcohol consumption, and physical exercise).

Pain Tests

The study included exposure to three different modalities of noxious stimulations (i.e., mechanical, cold, heat), which produced eleven outcome measures.

Mechanical Pain by Pressure Algometer

The computerized pressure algometer, AlgoMed (Medoc, Ramat Yishai, Israel), was used for mechanical pain stimulation. This device is a software-based computerized Algometer that produces real-time visual and auditory feedback to control and monitor applied pressure rates. The device has a rubber hand grip held by a lever and a 1 cm² rubber tip placed on the tested region. A constant rate of pressure is delivered by the examiner, and the pressure is recorded in Kilonpascal (kPa). In this study, the stimulation was applied between the medial and distal joints of the phalanges of the dominant hand. Each stimulus was applied to finger two, three, or four in consecutive order during each stimulation.

Subjects were instructed to press a button at the exact point in time when the pressure stimulation began to elicit pain. This time point is defined as the pressure pain threshold (kPa). Three such stimulations are averaged to create a pressure pain threshold (PT) score. The point until the subject cannot tolerate the pressure pain stimulation is also recorded and is defined as the pressure pain tolerance (kPa). Likewise, records of the three tolerance trials are averaged to create a tolerance score. In addition, immediately after the mechanical pain tolerance time point, subjects are asked to numerically rate the pressure pain intensity produced by the pressure stimulation from 0 to 100 on a Numeric Pain Scale (NPS).

Cold Pain by Cold Pressor Test

The cold pressor test (CPT) apparatus (Heto CBN 8–30 Lab equipment, Allerod, Denmark) is a temperature-controlled water bath with a maximum temperature variance of ±0.5°C, and is continuously stirred by a pump. In accordance with the standard protocol, subjects were asked to place their right hand in the CPT (1°C) in a still position with their fingers spread wide apart. A stopwatch was simultaneously activated, and the subjects were requested to keep their hand in the cold water for as long as possible. A cut-off time of 180
seconds was set for safety reasons. Subjects were instructed to indicate the exact point in time when the cold sensation began to elicit pain. This time point was defined as the cold PT and was measured in seconds (CT-Sec). The time (in seconds) until spontaneous hand withdrawal was also recorded and was defined as the cold pain tolerance. In addition, for those subjects who could tolerate the cold stimulation for over 15 seconds, numerical pain ratings (NPS 0–100) were obtained at the specific time point of 15 seconds following the hand immersion. Notably, as nine subjects could not tolerate the cold stimulus for at least 15 seconds, the analysis of this parameter was based on 39 subjects only.

**Thermal PTs by Thermal Testing Analyzer**

A thermal testing analyzer (TSA) thermode of 30 × 30 mm (Medoc TSA-2001 device, Ramat Yishai, Israel) was attached to the skin of the dominant hand on the thenar part of the palm. Starting from 32°C, the temperature was increased or decreased at a rate of 1°C/sec up to 50°C to measure the heat threshold (HT- °C) and down to 0°C to measure the cold threshold (CT- °C). Subjects were instructed to push a button when the cold/hot sensation began to elicit pain. Three consecutive tests with interstimulus interval of 10 seconds between them were conducted for determining arithmetical mean for each thermal (heat/cold) threshold.

**Conditioned Pain Modulation**

To induce conditioned pain modulation (CPM), the heat stimulation (46.5°C) was considered as the “test stimulation,” whereas the noxious cold stimulation was used as a “conditioning” stimulation. After the first heat stimulus provided the baseline heat pain rating, subjects were asked to immerse their right hand into the CPT at 12°C. Following 30 seconds of immersion, while the hand was still in the CPT, the second heat pain stimulus was delivered and pain intensity was recorded (Test 1). Subjects were then asked to remove their hand from the CPT. NPS (0–100) was used by subjects to rapidly rate the pain intensity experienced during the heat stimulus. The NPS was used verbally as both hands were occupied by the two different devices (TSA and CPT). The magnitude of CPM was calculated by the subtraction of pain scores obtained in Test 1 from the baseline pain scores.

**Temporal Summation**

Tonic noxious heat stimulation was applied by the TSA to the dominant volar part of the hand using a ramp and hold method [30]. The baseline temperature was set at 32°C, was increased at a rate of 1°C/sec up to a destination temperature of 46.5°C, and then remained constant for 120 sec. During the entire test (a total duration of 135 seconds), subjects continuously rated the magnitude of the perceived pain using a computerized visual analogue scale (COVAS, 0–100), which was automatically recorded every 0.1 seconds. The recorded data were averaged each 5 seconds, leading to 29 readings for the entire test period (i.e., time 0 seconds to 135 seconds). The magnitude of temporal summation (TS) was calculated individually for each subject by subtracting the lowest (nadir) reading from the maximal pain level at the end of the stimulus pain reading. In addition, another calculation was made to identify the individual rate of peak pain intensity induced by the heat stimulation (ranging from 15–35 seconds after stimuli induction). This was defined as the heat pain intensity.

**Study Design**

The study was a within-subjects randomized crossover design. Following approval of the Haifa University ethics committee (no. 122/12), subjects were recruited by advertisements on campus. Those who met the inclusion criteria signed a consent form. All participants underwent experimentally evoked pain tests during three times in a 24 hours period: morning (9–11 AM), early afternoon (1–3 PM), and evening (5–7 PM). To avoid an order effect, each participant was randomized to begin testing at a different time-of-day (morning, afternoon, or evening) with subsequent testing following a clockwise sequence (e.g., those randomized to begin in the afternoon were scheduled for subsequent evening and morning testing sessions). A training session was provided 10 minutes prior to the beginning of the experiment to familiarize the subjects with the pain tests. All tests were conducted with an interval of at least 10 minutes between tests; each session lasted approximately 1 hour.

**Statistical Analyses**

All analyses were conducted using the SPSS for Windows Version 20 statistical package (SPSS, Inc., Chicago, IL). Descriptive statistics were generated for all eleven pain outcomes. As the pain measures were not normally distributed, a nonparametric test for dependent samples (Friedman Chi-square test) was used to assess variability between the three time points (morning, afternoon and evening). Wilcoxon signed-rank test post hoc analyses were computed to identify the significant differences between two specific time points for each pain measure. Results were considered significant at the 0.05 level. All results are given as mean ± SEM and median.

**Results**

All recruited subjects completed the protocol. No order effects were found for any of the 11 pain tests in the three time points (P > 0.05).

Significant diurnal variations in five of the pain parameters and a trend toward significance in one parameter were found. 1) CT-Sec (χ² = 8.8, P = 0.012); CT-Sec was significantly higher (i.e., less sensitive) in the
morning (mean ± SEM: 5.0 ± 0.5, median: 4.2 seconds) than in the evening (mean ± SEM: 3.9 ± 0.4, median: 3.5 seconds) ($Z = -2.47, P = 0.013$, Figure 1A). 2) For cold pain tolerance, although there was only a trend toward significance between the three time points ($\chi^2 (2) = 5.8, P = 0.055$), it was significantly higher in the morning (mean ± SEM: 45.0 ± 6.9, median 26.0 seconds; i.e., less sensitive) than in the evening (mean ± SEM: 34.0 ± 5.7, median 22.0 second; $Z = -2.14, P = 0.032$, Figure 1B). 3) NPS ($\chi^2 (2) = 6.5, P = 0.038$): cold pain intensity was significantly lower (i.e., less sensitive) in the morning (mean ± SEM: 67.7 ± 3.6, median 70.0) than in the afternoon (mean ± SEM: 70.8 ± 3.7, median 80.0) and in the evening (mean ± SEM: 74.5 ± 3.3, median 80.0) ($Z = -1.98, P = 0.047$ and $Z = -2.51, P = 0.012$, respectively, Figure 1C). 4) CT-°C ($\chi^2 (2) = 6.7, P = 0.035$): Cold PT in °C was significantly lower (i.e., less sensitive) in the morning (mean ± SEM: 13.9 ± 1.1, median 16.3°C) than in the afternoon (mean ± SEM: 15.7 ± 1.1, median 18.3°C; $Z = -2.34, P = 0.019$, Figure 1D). 5) HT-°C ($\chi^2 (2) = 8.6, P = 0.013$): heat PT in °C was significantly higher in the morning (i.e., less sensitive; mean ± SEM: 44.6 ± 0.4, median 44.7°C), and in the evening (mean ± SEM: 44.3 ± 0.4, median 44.1°C) compared to the afternoon (mean ± SEM: 43.7 ± 0.5, median 43.4°C; $Z = -2.03, P = 0.042$ and $Z = -2.46, P = 0.014$, respectively; Figure 1E). 6) Heat pain intensity ($\chi^2 (2) = 6.4, P = 0.041$) was significantly lower in the morning (i.e., less sensitive; mean ± SEM: 68.2 ± 4.3, median 79.0; $Z = -2.15, P = 0.031$) compared to the evening (mean ± SEM: 74.3 ± 4.3, median 82.0) and lower in the afternoon (mean ± SEM: 69.1 ± 4.3, median: 80.0) compared to evening ($Z = -2.77, P = 0.006$; Figure 1F).

Taken together, out of 11 pain tests, six tests indicated lowest pain scores in the morning. Findings of the five pain parameters that did not significantly vary between the three different time points (morning, afternoon, and evening) are depicted in Table 1.
Temporal summation

Conditioned pain modulation (CPM, δ)

Pressure pain intensity (0-100, NPS)

Pressure pain tolerance (kPa)

Pressure pain threshold (kPa)

Table 1  Sensitivity to mechanical and “dynamic” QST during the day

<table>
<thead>
<tr>
<th></th>
<th>Morning mean±SEM (Median)</th>
<th>Afternoon mean±SEM (Median)</th>
<th>Evening mean±SEM (Median)</th>
<th>Friedman χ²(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure pain threshold (kPa)</td>
<td>335.6±27.0 (316.9)</td>
<td>324.8±22.7 (307.1)</td>
<td>341.1±26.6 (294.7)</td>
<td>3.5 (0.16)</td>
</tr>
<tr>
<td>Pressure pain tolerance (kPa)</td>
<td>863.9±35.2 (873.5)</td>
<td>855.7±35.0 (831.3)</td>
<td>826.2±39.0 (755.3)</td>
<td>3.5 (0.17)</td>
</tr>
<tr>
<td>Pressure pain intensity (0-100, NPS)</td>
<td>75.0±2.4 (80.0)</td>
<td>75.8±2.4 (80.6)</td>
<td>74.5±2.3 (80.0)</td>
<td>0.9 (0.62)</td>
</tr>
<tr>
<td>Conditioned pain modulation (CPM, δ)</td>
<td>25.3±2.6 (21.5)</td>
<td>20.4±3.0 (20.0)</td>
<td>22.6±2.3 (20.0)</td>
<td>1.9 (0.38)</td>
</tr>
<tr>
<td>Temporal summation (TS, δ)</td>
<td>39.9±3.8 (34.0)</td>
<td>35±4.0 (30.0)</td>
<td>37.8±3.9 (32.0)</td>
<td>0.06 (0.90)</td>
</tr>
</tbody>
</table>

kPa = kilopascal; NPS= Numerical pain scale.

Discussion

This study found significant variation in pain perception among healthy subjects during the day in most of the thermal “static” pain parameters. Specifically, it appears that in the morning, experimental pain perception is decreased. No such variation was found in the mechanical “static” pain parameters or in the “dynamic” psychophysical tests (i.e., CPM and TS).

Previous studies examining daily variation in experimental pain perception in healthy, pain free individuals showed conflicting findings [31]. Although most of the studies (some of them from the early 20th century) failed to demonstrate such variations using a wide range of pain models and pain parameters, a few studies did find diurnal variation using electrical [17,18] and ischemic [19,20] evoked pain. Notably, these studies differed in their study setting, evoked pain devices and also by the specific hours of measurement.

Our main finding indicating lower pain scores in the morning is supported by several previous studies among healthy volunteers. Davis et al. showed that subjects were significantly less sensitive to electrical evoked pain in the morning in comparison to the afternoon by means of decreased pain scores as well as decreased amplitude of EEG evoked potentials [17]. Koch and Raschka showed that following 1 minute of the tourniquet procedure, pain levels were significantly lower in the morning in comparison to three additional time points across a 24-hour period [20]. Bourdalle-Badie et al. found that the electrical pain ratings were lowest between 9 AM to 5 PM, as measured by pain intensity and nociceptive pain reflex threshold [21]. Yet this study was based on five subjects only, which makes it difficult to draw any solid conclusions. In contrast, Göbel and Cordes showed that the lowest ischemic pain scores were in the early afternoon (2 PM) and gradually increased until 2 AM, after which it consistently decreased again to a minimum at 2 PM [19].

In the clinical setting, the question of diurnal variation in pain perception also remains inconclusive. Some studies showed the highest pain ratings in the morning among patients with various chronic pain conditions [11,13,32], whereas one study found the highest pain ratings were in the afternoon [14] and a few others reported peak pain ratings in the evening [15,16].

Notably, these studies pointed toward the diurnal peak pain levels only, with no information regarding the nadir pain levels. Only two studies reported the nadir of pain during the day [12,33]. Bellamy et al. found the lowest pain ratings in the afternoon among osteoarthritis patients [12]. Jamison and Brown explored diurnal variation in clinical pain among 189 chronic pain patients with heterogeneous etiologies. Although they failed to show a consistent diurnal pattern of pain in this sample, the authors presented several profiles of pain fluctuations during the day. Specifically, majority of patients (55%) showed the lowest pain intensity in the morning, with a linear increase as the day progressed. An opposite profile (i.e., highest pain intensity in the morning with a gradual decline during the day) was found in a minority of the patients (8%). A reverse U-shape profile (i.e., lowest levels of pain in the morning with an increase in the afternoon and a decrease again in the evening) was demonstrated in 14% of the patients. Other pain profiles included a U-shape (7%), a poly-slope (8%), or no diurnal variation (28%) [33]. Taken together, while several diurnal pain profiles were identified by Jamison and Brown, approximately 50% of these patients reported least pain in the morning, which is in line with our findings.

Although our study population consisted of healthy pain free individuals, and our main findings support other previous studies but contradict others, the major added value of this study is that it includes multimodal pain tests which strengthen the findings.

Our finding demonstrating lowest pain scores in the morning deserves further consideration. In an attempt to
hypothesically interpret this finding, additional physiological processes that exhibit circadian rhythmicity and that have been associated with mechanisms of pain are being discussed.

Melatonin (melatonin-acetyl-5-methoxytryptamine) is a hormone produced during the hours of darkness by the pineal gland. From very low daytime levels, an increase in plasma melatonin occurs in the evening, peaking between 1 AM and 5 AM, and becoming less detectable again by about 9–10 AM [7,34,35]. Compelling evidence has shown that melatonin attenuates nociceptive responses in animals [36,37] as well as in humans [38,39]. A recently published review reported that the administration of exogenous melatonin has the potential to induce analgesia in healthy subjects exposed to experimental pain models [40].

Given its antinociceptive effect, the circadian rhythm of melatonin secretion may point toward a possible explanation in regard to the findings of this study. The lowest pain scores demonstrated in the morning may be partly related to the residual endogenous melatonin left in the serum at this time of the day. Despite the well-established evidence that melatonin is almost cleared by about 9–10 AM [41], some studies have shown a certain amount of serum melatonin in the late morning hours in humans [35,42,43]. These levels may nonetheless have some antinociceptive effect, which may support our findings. As serum melatonin levels were not measured in this study, this claim must be cautiously considered, pending further investigation.

Another hormone that exhibits circadian rhythmicity is cortisol [44], which reaches its peak level in the morning and its nadir level at night (i.e., inversely to melatonin) [43]. Cortisol has the potential to suppress pain, probably due to its involvement with endogenous opioids and activity of the proopiomelanocortin peptide, which enhance analgesia [45]. Consequently, cortisol has the potential to affect the diurnal variation of pain. However, only few studies have investigated this issue in healthy subjects. One study found a negative association between the level of cortisol and cold pain intensity [46]. Another study found an association between cortisol levels and pressure pain tolerance [47]. Based on our findings indicating the lowest level of pain in the morning, it may be hypothesized that peak morning cortisol levels contribute to reduced pain perception at that time of the day. Undoubtedly, future studies are warranted to verify our hypotheses regarding the diurnal variations of cortisol and melatonin as part of the underlying mechanisms affecting changes in pain perception during the day while testing these hormones levels among subjects.

Another notable point refers to the finding that the magnitude of CPM and TS were stable and did not significantly vary within the three time points. Both CPM and TS are “dynamic” psychophysical tests aimed to focus on the endogenous pain modulation process. In support of our finding regarding stability, previous studies have also demonstrated stability in the magnitude of inhibition/facilitation measured by these paradigms [48–50].

In addition, the finding that the mechanical pain scores did not vary across the day was surprising. Unfortunately, we are unable to provide any hypothetical explanation for this finding. However, this result suggests that using a mechanical modality may not provide a sensitive pain measure in the investigation of diurnal pain variation. Rather, it is suggested that thermal “static” QST have the potential to become useful in examining diurnal variation in pain perception.

There are notable limitations in this study. First, due to the exploratory nature of this study, multiple comparisons were not corrected (i.e., Bonferroni correction). Second, due to the vast variation in pain perception in females during different phases of the menstrual cycle [51,52], only men were recruited, thus limiting generalizability of the findings to females. Third, although this study provides novel results, it is limited by deducing the findings into clinical pain states. Hence, future research in clinical populations is required to verify our conclusions.

In conclusion, the diversity among laboratories in the implementation of psychophysical studies may lead to biases and limit the ability to generalize conclusions from one study to another. One way to minimize these biases is to utilize consistent methodological approaches to the extent possible. Time-of-day is one such vital feature which has thus far received only scant attention and should be considered more often when designing a psychophysical study. This study showed for the first time that by utilizing multipyschophysical tests, subjects perceive less thermal pain in the morning and that dynamic tests and mechanical pain are not appropriate for detecting diurnal variability in pain.

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