Does Habituation Affect Conditioned Pain Modulation?

Michael J Oehler

University of North Carolina at Chapel Hill

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Faculty Advisor_____________________________

Dr. Mark Hollins
Abstract

Describing endogenous pain modulation (EPM) phenomena as occurring at “higher” or “lower” neural processing levels may help to better characterize the interactions between these mechanisms. Eleven undergraduate students took part in this experiment designed to assess whether conditioned pain modulation (CPM), a lower-level EPM process in which a painful \textit{conditioning stimulus} inhibits a less painful \textit{test stimulus}, can be altered by habituation, a higher-level process. The experiment was composed of three separate sessions. In the first session, participants’ thermal thresholds were determined, they rated the painfulness of the thermal grill illusion, and they rated pain evoked by a CPM-invoking paradigm. In the second session participants were randomly divided into two groups. The habituation group was repeatedly exposed to the conditioning stimulus of the CPM paradigm, painfully cold water. The control group was repeatedly exposed to innocuous water. In the third and final session, thermal thresholds, and painfulness of the thermal grill illusion and CPM-paradigm were reassessed. Results indicated that the experimental manipulation failed to produce more habituation in the experimental group than the control group. However, data combined across groups displayed a general trend toward habituation for nearly all pain measures in this study, though most were not statistically significant. Nevertheless, there was no trend towards a reduction in CPM in session three, though perhaps this was a reflection of a low sample size. The nature of the habituation observed in this study is theorized, and implications of the independence of CPM and pain habituation are discussed.

\textit{Keywords}: conditioned pain modulation, diffuse noxious inhibitory controls, habituation, thermal grill, endogenous analgesia, pain
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Does Habituation Affect Conditioned Pain Modulation?

Chronic pain is a prevalent and economically burdensome problem in the United States. It is estimated that more than 116 million Americans experience chronic pain each year (Institute of Medicine [IOM], 2011). Furthermore, chronic pain treatment is costly to the US economy. In 2011, the combined cost of chronic pain treatment and lost productivity due to chronic pain was around $635 billion (IOM, 2011). Clinical pain in general is so prevalent that hydrocodone, an analgesic pharmaceutical, was the most commonly prescribed drug in the U.S. during 2011 (Herper, 2010). Given this high prevalence and cost there is a need to find novel, affordable, and effective prevention and treatment methods for chronic pain. Endogenous pain modulation (EPM) research may prove to be a fruitful approach for making these discoveries.

Endogenous pain modulation is a term used to describe any of several phenomena in which the perception of a painful stimulus is altered through neural processing. For example, placebo analgesia is an EPM process in which expectations that a (otherwise inert) substance or activity will reduce pain leads to an actual pain reduction. Given that EPM processes are naturally occurring in the human body, it is hoped that an understanding of how they function may lead researchers to discover novel treatments and prevention methods for both chronic and acute pain.

One possibly productive avenue for EPM research is to identify the level of the nervous system at which different EPM phenomena take place. For example, temporal sensitization, the increase in pain that occurs during several short, repeated exposures to a noxious stimulus, is thought to be a “low-level process” (Hollins, Harper, & Maixner, 2011). In other words, during temporal sensitization, it seems that ascending nociceptive signals are modulated before the signals reach the cortex. Temporal sensitization and other low-level processes stand in contrast to
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“high-level processes,” those in which neural processing takes place predominantly in the cortex. Dividing EPM processes into high and low levels of processing may be important because such categorization allows researchers to determine which sensory modulations may occur prior to others in overall perceptual processing of pain. This type of knowledge can potentially be useful in diagnosing the points of malfunction in different chronic pain states and focusing pharmacological research for future pain treatments (Harper, 2012). Additionally, if EPM processes are shown to interact, knowledge of their respective levels of processing can help determine details such as the directionality of this interaction.

This study will attempt to build on knowledge regarding the levels of processing of two EPM paradigms: habituation and conditioned pain modulation (CPM), in order to determine whether the higher-level process, habituation, has a descending, modulatory influence on the lower-level process, CPM. In addition, this study will examine whether habituation alters the thermal grill illusion, a perceptual illusion whereby interlaced areas of innocuous warm and cool stimuli produce the sensation of heat pain (Craig & Bushnell, 1994). More fundamentally, this study will investigate whether higher-level processes, such as habituation, do, in fact, have descending influences to lower-level EPM processes.

**Research Avenues for Endogenous Pain Modulation**

EPM processes can be generally divided into hyperalgesic (pain increasing) or analgesic (pain reducing) effects. This study will focus on two processes of endogenous analgesia: conditioned pain modulation (CPM) and habituation. To a lesser extent, the thermal grill illusion, a hyperalgesic process, will also be examined. The endogenous analgesic phenomena are sometimes collectively referred to as comprising the body’s “antinociceptive system” (Bingel, Schoell, Herken, Buchel, & May, 2007; LeBars, Dickenson, & Besson, 1979b), implying that
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they use at least some of the same neuronal networks. Given this assumption, it is worthwhile to investigate how multiple types of endogenous analgesia may be related through interdependent networks.

One way to narrow down the potentially multitudinous theoretical interactions in this antinociceptive system is to characterize certain processes as “higher-level” or “lower-level.” Higher-level processes are EPM processes that occur primarily in the cortex. In contrast, lower-level processes are located primarily in the brain-stem and/or spinal cord. Such characterization can help determine possible interactions because higher-level processes can only alter lower-level processing through descending, modulatory pathways, and lower-level processes can only influence higher-level processes through ascending modulation.

**Conditioned Pain Modulation**

One of the most extensively studied forms of endogenous pain modulation is conditioned pain modulation (CPM). CPM occurs when the presence of a noxious, “conditioning” stimulus reduces the perceived pain caused by a “test” stimulus applied to a separate area of the body.

CPM is a particularly important form of endogenous pain modulation because it has been shown to be related to the development of chronic pain and clinical pain conditions. For example, Yarnitsky et al. (2008) found that CPM magnitude was negatively correlated with the likelihood of developing post-operative chronic pain following thoracic surgery. Additionally, CPM has been shown to be impaired in fibromyalgia patients (Kosek & Hansson, 1997). Together, these results suggest that the system mediating CPM may have some preventative role in the development of chronic pain.

CPM can be viewed as the human analog of diffuse noxious inhibitory controls (DNIC), a neurophysiological phenomenon studied in animals. DNIC was first documented by recording
from rat spinal cord dorsal horn neurons that receive input from both tactile Aβ-fibers and nociceptive C-fibers while a noxious stimulus was applied to their receptive fields. By applying further noxious stimulation (pinch, heat, electrical stimulation, and bradykinin injection) to locations outside of these polymodal neurons’ receptive fields, the researchers were able to document a robust reduction in both the spontaneous and stimulated firing rates of these neurons (LeBars, Dickenson, & Besson, 1979a). These reductions were abolished after severing of the spinal cord just below the brain stem. This result suggests that DNIC - the observed reduction in firing rate - must reflect descending modulation from supra-spinal areas that are activated by ascending nociceptive signals from the conditioning stimulus (LeBars Dickenson, & Besson, 1979b).

In humans, CPM is believed to function through a similar spinal cord-midbrain-spinal cord loop. This descending modulation may originate in areas activated by the ascending nociceptive signal from the conditioning stimulus, such as the periaqueductal gray (PAG). The PAG, in turn, may activate other mid-brain structures, including the nucleus raphe magnus, which send descending signal to inhibit incoming pain signals at the spinal level (Basbaum & Fields, 1978; Heinricher, Tavares, Leith, & Lumb, 2009). Additionally, DNIC seems to involve the serotonergic system (Chitour, Dickenson, & LeBars, 1981), which further implicates descending influence from the nucleus raphe magnus because this structure releases serotonin in a descending circuit from the mid-brain to the spinal cord (Basbaum & Fields, 1978). Most importantly, it appears that CPM does not depend on higher-level, cortical structures. In other words, all evidence suggests that it is a low-level process.

Recent studies have also shown that descending modulation initiated by cognitive processes can affect CPM magnitude. In one study (Defrin et al., 2010), subjects were asked to
rate the pain elicited by a thermode test stimulus applied to the left, ventral wrist, and then provide additional pain ratings after another thermode, the conditioning stimulus, was applied to different locations on the body. The two thermodes were physically identical and were heated to a temperature that elicited a painfulness rating of five out of ten for each participant. The researchers were interested in identifying whether the distance between application sites of the thermodes and instructions given to direct participants’ attention had an interaction effect on pain perception.

The results indicated that when the thermodes were applied only 5 cm apart, participants’ pain ratings summated after the introduction of the conditioning stimulus, regardless of whether they were asked to pay attention to the pain elicited by one or both thermodes. In contrast, when the thermodes were applied to separate appendages, pain ratings did not summate for either condition, and ratings even declined when participants were asked to attend to the pain caused by only one thermode. However, when the conditioning stimulus was applied 30 cm from the test stimulus on the left arm participants reported a marked reduction in pain when asked to attend to only one thermode. Yet in this same condition participants reported a marked increase in pain when asked to attend to both thermodes. Furthermore, the observed reduction in pain was greater when participants attended to the thermode-induced pain that they were not rating, than when they were attending to the pain that they were rating. These results suggest that attention plays a role in whether and to what extent CPM-mediated pain reduction occurs (Defrin et al., 2010).

However, it should be noted that the procedure of this experiment diverged slightly from typical CPM experiments because the “conditioning” and “test” stimuli were identical.

In another study, Nir, Yarnitsky, Honigman, and Granot (2012) asked whether placebo analgesia and hyperalgesia could modulate CPM magnitude. First, CPM was measured by
having subjects rate a painfully hot stimulus to one arm while their opposite hand was in painfully hot water. Next, participants were instructed that a (placebo) cream would increase, decrease, or have no effect on the pain elicited by the hot water. Then CPM was reassessed using slightly hotter or cooler water, according to whether participants expected more or less pain. By comparing each participant’s first and second CPM measurements, the researchers were able to examine the effect of placebo expectations on CPM.

The researchers found that expectations of increases or decreases in pain due to the placebo did, in fact, increase or decrease the perceived pain of the hot water, relative to what was experienced by controls. Additionally, they found that this increase and decrease in perceived pain of the water corresponded to an increase or decrease, respectively, in CPM magnitude, relative to what was experienced by controls (Nir et al., 2012).

The results of these two studies show that CPM is capable of being modulated by higher-level, cognitive processes such as attention and placebo analgesia (Defrin et al., 2010; Nir et al., 2012). Such cognitive influence on CPM is significant because it suggests that CPM, known to be primarily determined at the midbrain and spinal levels, is subject to modulation from cortical processes, presumably through descending neural pathways.

**Habituation**

In contrast to CPM, the neural processes underlying pain habituation, a higher-level process defined as a reduction in perceived pain after repeated exposures to a noxious stimulus, have only recently begun to be explored. Before proceeding, a few clarifying points about habituation should be discussed.

First, it should be noted that habituation can refer to a variety of distinct, yet related phenomena. Many sensory modalities exhibit reduction in perceptual intensity after prolonged or
repetitive exposure to a stimulus. To be clear, the word “habituation” in this article always refers to the habituation of pain.

Second, the change in pain perception associated with habituation is correlated with, but distinct from, non-psychological habituation. Zbrozyna and Westwood (1990) found that after six to ten daily sessions of exposure to a repetitive, painfully cold water stimulus both physiological responses, such as vasoconstriction and vasodilation of blood vessels near the skin, and psychological measures, such as pain intensity ratings, habituated to the stimulus. However, when participants were subsequently retested once a week for several weeks, the researchers found that the physiological measures still displayed some habituation, but pain intensity ratings typically returned to or even surpassed baseline.

Finally, habituation can be both short-term and long-term (Hollins, Harper, & Maixner, 2011). As a short-term process, “habituation” refers to the reduction in perceived stimulus intensity that occurs after seconds or minutes of continuous or repetitive exposure. This phenomenon may primarily reflect neuronal fatigue (sometimes called “adaptation”) at one or more stages of sensory processing. In contrast, long-term habituation occurs when perceived stimulus intensity is attenuated after several temporally separated episodes of exposure (May et al., 2011). This type of habituation may reflect a more enduring change within the neuronal processing circuit. This experiment is concerned only with long-term habituation.

A recent study (Bingel, Schoell, Herken, Buchel, & May, 2007) suggests that habituation is a higher-level process primarily mediated by the rostral anterior cingulate cortex (rACC). In this study, healthy participants underwent a program of 60 six-second exposures to noxious (48º C) heat on the left, volar forearm every day for eight days. fMRI was performed during this pain program on days 1 and 8, with an additional study session 14 days after the last pain application
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session (22 days from the beginning of the study). The results indicated that pain thresholds increased and pain intensity decreased between days 1 and 8, but not between days 8 and 22. Analysis of fMRI data revealed decreased pain-evoked activity between days 1 and 8 in many cortical areas associated with pain processing, including the primary somatosensory cortex, cingulate cortex, and medial temporal gyrus.

Only one area, the rACC displayed increased activation between days 1-8, suggesting that this structure mediates the reduction in pain perception observed in habituation (Bingel et al., 2007). That is, since this structure was activated more during an analgesic effect, whereas other areas associated with pain processing showed decreased activation, the data suggests that the rACC plays an integral role in attenuating pain during habituation. Additionally, follow-up testing at one year showed that, although pain thresholds and ratings had returned to baseline, rACC activity was still increased during the pain paradigm, suggesting that these cortical changes are long-term and do not necessarily imply changes in perception of pain intensity (Bingel, Herken, Teutsch, & May, 2008).

In addition to cortical changes, the lack of involvement of the opioid system suggests that habituation is a higher-level process. Rennefeld, Wiech, Schoell, Lorenz, and Bingel (2010) exposed subjects to a pain program identical to that described for Bingel et al. (2007) every day for eight days. In addition to this pain program, on days 1 and 8 pain thresholds were obtained on the contralateral arm and ipsilateral leg and the pain program was repeated on the contralateral arm. On days 1 and 8, half of the participants received the μ-opioid receptor antagonist naloxone before the pain exposure session began.

The researchers found that between days 1 and 8 pain thresholds increased at all three sites tested, and pain ratings decreased at both sites that were subjected to the habituation
stimulus. These results indicate that habituation occurred and its effects diffused to distal areas of the body. However, there were no differences between the naloxone and control groups in terms of how their pain changed across sessions, suggesting that habituation does not depend on the endogenous opioid system (Rennefeld, et al., 2010).

The finding that habituation is independent of the μ-opioid receptors inhibited by naloxone is significant because it suggests that, if habituation functions through descending modulatory pathways, then these pathways must not require the use of opioids. However, in a separate experiment, Benedetti, Arduino, and Amanzio (1999) found that naloxone infusion completely abolished the effects of placebo analgesia. Therefore, placebo analgesia, a higher-level process which is known to modulate the effects of lower-level CPM (Nir et al., 2012), requires opioids to function, but habituation, another higher-level process, does not. Since a major descending pain inhibitory pathway utilizes opioids at several stages (Basbaum & Fields, 1978), this discrepancy between placebo analgesia and habituation calls into question whether habituation is capable of modulating CPM, as placebo analgesia has been shown to do (Nir et al., 2012).

**Purpose of the Present Study**

This study is designed to investigate whether habituation has a descending, modulatory influence on CPM. I hypothesize that habituating to the conditioning stimulus of CPM will not affect CPM’s ability to reduce the pain perceived from the test stimulus. Such a result would imply that habituation does not have a descending influence on CPM. Such results are expected predicted because 1) CPM is a low-level phenomenon and 2) habituation, a high-level process (Bingel et al, 2007), does not seem to utilize the opioid system that is involved in many forms of descending inhibition (Basbaum & Fields, 1978; Rennefeld et al, 2010).
Method

Participants

Thirteen undergraduates enrolled in Psychology 101 at the University of North Carolina – Chapel Hill and between the ages of 18 and 20 participated in this three-session study. One participant withdrew from the experiment, and another was discontinued at the end session one after providing maximal pain ratings for some of the stimuli. Therefore, data from eleven participants (five female, mean age = 18.9 years) was utilized. Exclusion criteria were neurological impairments, peripheral vascular disease, and diagnosis of chronic pain conditions, diabetes, urticaria, and Raynaud’s disease. All participants provided written, informed consent. Participants received credit toward completion of their Psychology 101 research requirement for taking part in this study. This study was approved by the University’s Institutional Review Board.

Materials

Visual analog scale. Participants provided psychophysical ratings of the intensity of their sensations using several different computerized visual analog scales (VAS’s). Each VAS was presented as a horizontal gray bar with written anchors describing the least and most intense sensations over the left and right ends of the bar, respectively. By moving the mouse right or left, participants filled this bar more or less with a coloring in order to provide continuous ratings of their sensations. VAS data was recorded by computer every 0.1 second as a percentage of the VAS bar currently filled.
**Thermal stimuli.**

**Thermal grill.** The thermal grill was composed of 12 copper bars of 1 cm diameter placed horizontally on a flat, plastic base, and spaced 0.2 cm apart. Two 19-L water coolers were placed above the thermal grill so that gravity would facilitate the flow of water through the bars. Plastic tubing connected one cooler to the first copper bar and every odd-numbered bar thereafter, in series. The other cooler was similarly connected to the second bar and every even-numbered bar thereafter. Controlling the temperature of the water in each cooler allowed for the production of warm (42.0 +/- 0.5 °C) and cool (18.0 +/- 0.5 °C) interlaced bars, replicating temperatures which have previously produced illusory heat pain in our lab (Harper, Irvin, & Hollins, in preparation).

**Thermode.** Thermal stimuli were applied to participants’ left palm and left, lateral calf using a 16 x 16 mm computer-controlled thermode (Medoc, Ramat-Yishai, Israel: TSA-II).

**Water bath.** A plastic cooler (23.0 x 23.5 x 23.5 cm) partitioned diagonally into two triangular prisms was filled with enough water (7 +/- 2 L) that participants could submerge their hand up to the wrist. At different times during the experiment, the cooler was filled with either neutral (32.0 +/- 0.5° C), or painfully cold (6.5 +/- 0.5° C) water.

**Questionnaires.** Participants completed two separate questionnaires: 1) A Demographics Questionnaire asking their age, gender, race, and ethnicity (see Appendix A), and 2) a Physical Activity and Cold Therapies questionnaire, designed for this study, which inquired about their current physical activity level and personal history with using cold therapies, such as ice-baths (see Appendix B). This questionnaire contained a question that utilized The Rating of Perceived
Exertion Scale, a psychophysical scale created by Borg (1982) that quantifies perceived athletic exertion.

**Design**

This study was divided into three experimental sessions. In the first session, baseline measurements of thermal thresholds, thermal grill pain intensity, water bath pain intensity, and CPM were assessed. Prior to the second session, participants were divided into two groups (control and experimental) using a random number generator that was constrained to produce counterbalanced groups. In this session, participants in the control group repeatedly placed their left hand into a neutral water bath, and participants in the experimental group placed the same hand into a cold water bath. Session two was administered by another investigator (Daniel Harper, M.A.), so that the experimenter in sessions two and three (the author), would be blinded to each participant’s group. In the third session, thermal thresholds, thermal grill pain intensity, water bath pain intensity, and CPM were reassessed.

**Procedure**

Each participant took part in three separate, one-hour experimental sessions. In almost all cases, these sessions were separated by 1-7 days each. However for one participant, sessions one and two were separated by 20 days.

**Session One.** After completing a brief Demographics Questionnaire (reproduced in Appendix A), participants progressed through the following procedure:

**VAS training.** Participants were trained to provide continuous psychophysical ratings of the intensity of their sensations by listening to a piece of classical music while providing ratings
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using a VAS, with “No loudness” and “Loudest music imaginable” as the left and right anchors, respectively.

*Thermal grill illusion.* Participants sat in a chair and placed their left palm on four of the thermal grill’s temperature-controlled copper bars for 15 seconds. While their hand was on the bars, participants provided continuous ratings of pain intensity on a VAS scale with anchors of “No pain” and “Most intense pain imaginable.” The thermal grill illusion was administered by Daniel Harper.

*Thermal threshold determinations.* To determine each participant’s warm difference, cool difference, heat pain, and cold pain thresholds, the thermal contactor was applied to participants’ left palms at a baseline temperature of 32.0°C and either increased or decreased at a rate of either 0.5°C/sec (for warm and cool difference thresholds) or 1.0°C/sec (for heat and cold pain thresholds). The trial ended when participants clicked the computer mouse to indicate they felt either a temperature change (for the warm and cool difference thresholds) or pain (for the heat and cold pain thresholds). Eight separate trials were administered, two for each threshold. The two trials for each thermal threshold were always administered contiguously. Between each trial, the thermal contactor was moved to an unused location of the palm. For each participant, the threshold determination trials proceeded according to one of two randomly assigned orders.*

*CPM protocol.* CPM was measured by comparing ratings of test pain (Medoc) during neutral and cold water runs. In the neutral water run, participants placed their left hand, up to

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*Order 1 proceeded: cool, cool, warm, warm, cold, cold, hot, hot. Order 2 proceeded: warm, warm, cool, cool, hot, hot, cold, cold. Therefore, the warm and cool thresholds were always determined before the cold and heat pain thresholds. This was done to avoid the more extreme pain threshold temperatures from interfering with warm and cool threshold trials by significantly increasing or decreasing participants’ skin.*

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their wrist, in a neutral water bath. In the cold water run, they placed their left hand in a cold water bath. The order of the runs was counterbalanced between subjects. Participants provided continuous VAS ratings of the painfulness of the water for 50 sec after submerging their hand. For the next ten seconds, the VAS bar was reset to zero and was not under the participants’ control. Next a five-second countdown appeared on the computer monitor. Following the countdown, a ten-second heat stimulus was applied to participants’ left, lateral calf using the thermal contactor. Next, another five-second countdown was displayed. After the countdown, another ten-second heat stimulus was applied to a different area of the lateral calf. Throughout these ten-second intervals, participants provided VAS ratings of the intensity of pain caused by these heat stimuli. Two test stimulus temperatures, 47°C and 49°C, were applied, one during each trial, in random order.

Session Two. Participants submerged their left hand into either neutral or painfully cold water, depending on their group assignment, for three 90-sec runs. During each run, they provided continuous VAS ratings of pain intensity. Runs were separated by 15-min breaks.

Session Three. Session three proceeded identically to session one, except that participants did not take part in the VAS training exercise, and instead of completing a demographics form at the beginning of the session, they completed the “Physical Activity and Cold Therapies Questionnaire” (reproduced in Appendix B) at the end of the session.

Data Analysis

VAS ratings of both the thermal grill illusion and Medoc stimuli were averaged over the last 5.0 seconds of each trial to compute average VAS scores for each participant. VAS ratings of water baths were similarly averaged over the last 10.0 seconds of each run. Only these last few
seconds of VAS data were analyzed because thermal pain ratings typically stabilize over time, and therefore the last few seconds provide the most stabilized data. However, data collected during session two was averaged over seconds 40.0-50.0 in order to better compare these average VAS scores from those obtained in other sessions.

Lastly, thermal thresholds were computed as the average of the two trials for each threshold and participant. Difference threshold are reported as temperature change from 32° C at which subjects indicated feeling a temperature change. In three cases, participants clicked the mouse, indicating perception of a change in temperature, before the Medoc had begun changing temperatures during the warm or cool trials. In these cases, the other (good) trial for that threshold was reported as the participant’s difference threshold for that session.

CPM scores were calculated by subtracting average scores of cold water runs from neutral water runs for each combination of participant, session, and Medoc temperature. A large CPM score indicates that pain of the test stimulus (Medoc) was much lower in the cold water run than the neutral water run, i.e. a robust CPM effect.

The data were analyzed using a series of mixed model, repeated measures analyses of variance (ANOVA’s). Average VAS scores of the water baths were analyzed using a 2x2x2 ANOVA. The three factors were Water Temperature (neutral or cold), Session (first or third), and Group (control or experimental). Average CPM scores were analyzed using a 2x2x2 ANOVA, with factors being Session, Group, and Medoc Temperature (45° or 47° C). Two further 2x2 ANOVA’s – factors Session and Group – were also performed for average VAS ratings of the test stimulus during neutral runs and of the thermal grill illusion. Student’s t-tests were used for post-hoc analyses and to test certain a priori hypotheses, as needed.
Results

Water Bath

Table 1 displays the means and standard deviations of participants’ VAS ratings of the neutral and cold water baths for sessions one and three. These statistics are displayed both overall, and for each group.

The results of the 2x2x2 ANOVA are displayed in Table 2. They indicate a significant main effect of Water Temperature, such that overall the cold water bath was perceived to be much more painful than the neutral water bath. There was also a significant main effect of Group, such that the control group found the water baths to be more painful than the experimental group. When analyzing only VAS ratings of cold water, this group difference remained significant, $F(1, 10) = 5.868, p = .04$, especially in session three (see Table 1).

There was no interaction effect of session and group for ratings of the cold water bath only. Therefore, the results do not indicate that the experimental group habituated to the cold water more than the control group, which was the expected effect of the habituation paradigm. However, there was a trend toward a significant main effect of session, such that the water stimuli were rated more painful in session one than in session three, regardless of group. Therefore, it seems that though neither group displayed significantly more habituation than the other, there was an overall trend toward habituation (see Figure 1).

CPM

Table 3 displays the average CPM scores for each session and Medoc temperature both for each group and overall. Figure 2 displays these group values graphically. Table 4 shows this data in more detail: As the average VAS rating of the final 1.0 second for each Medoc stimulus for each participant.
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The results of the ANOVA analyzing the CPM data are displayed in Table 5. Importantly, the average CPM value (“Intercept” in the ANOVA) for the entire data set was significantly greater than zero, $F(1,10) = 13.3, p = .005$. This result indicates that participants rated the Medoc stimulus as more painful when their hands were in Neutral water than when they were in the Cold water. In other words, significant CPM was observed in this experiment.

Although visual examination of Table 3 seems to show that CPM scores were lower in session three than in session one, the effect of session did not approach significance. Therefore, unlike the result observed for VAS ratings of water, CPM scores did not trend towards differing between sessions one and three.

No other results of this analysis were significant.

Thermal Grill Illusion

Table 6 displays participants’ average VAS ratings of the thermal grill illusion according to session and group. The results of 2x2 ANOVA are displayed in Table 7. There were no significant differences in the data, although ratings for both groups did tend to be lower in session three than in session one, suggesting at least some habituation to the illusion. Figure 3 displays this trend graphically.

Thermal Thresholds

Participants did not indicate sensing heat pain for a total of two trials during session one, and they did not indicate sensing cold pain for nine trials during session one and eight trials during session three. The most extreme temperature reached by the Medoc was recorded as participants’ pain thresholds during these trials. Tables 8 and 9 display the observed means and standard deviations for participants’ difference and pain thresholds, respectively. In general,
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group thresholds changed little between sessions one and three. This is especially true for overall average thresholds, which are displayed in Figure 4.

Subject Variables

Only one participant had received an ice bath in the last twelve months. This subject had received them on her contralateral foot, which has been shown to have the least significant effect on CPM efficacy for the hand (Defrin et al., 2010).

Nine participants reported having ever received other cold therapies. Six of these participants had received such therapies within the last twelve months. No participant had received more than four such therapies within the last twelve months, and none had received any on their left hand or arm.

Participants’ ratings of cold water as well as their CPM values for session one were analyzed for correspondence with a number of subject variables. Notably, the initial (session one) VAS rating of cold water was significantly correlated with the amount of time since subjects had last used a cold therapy, \( r(8) = .704, p = .02 \). In other words, participants who had used cold therapies more recently tended to rate the cold water as less painful during session one. However, participants’ initial CPM values were not correlated to this or any other subject variable.

Participants’ initial cold water VAS ratings were also found to have a significant relationship with the order of the water bath runs. In particular, participants who first rated the neutral water first found the cold water to be less painful than those who first rated the cold water, \( t(10) = 3.33, p = .008 \).
Discussion

This study was designed to investigate whether habituation exerts a descending, modulatory influence on CPM. Recent research has shown that CPM can be influenced through descending modulation from higher-level processes, such as attention and placebo analgesia (Defrin et al., 2010; Nir et al., 2012). However, it is unknown whether habituation, another higher-level process (Bingel et al. 2008), may also influence CPM processing. For this reason, this study sought to experimentally produce both CPM and habituation, and to ascertain whether habituation to the conditioning stimulus affects CPM magnitude. In this study, CPM and other psychophysical quantities were measured both before and after an experimental manipulation (session two) designed to produce habituation in the experimental group, but not the control group. Ideally, any observed differences between these two groups in session three would reflect perceptual differences caused by habituating to the CPM conditioning stimulus.

If habituation does descend and modulate CPM, the experimental group should have shown less CPM in the third session than the control group. Intuitively, this is because habituation reduces the conditioning stimulus’ ability to “mask” the test stimulus via its ascending signals. However, it is hypothesized that habituation does not have a descending, modulatory influence on CPM, in which case no difference would be observable between the session three CPM scores of the control and experimental groups. In this case, the modulation leading to habituation of the conditioning stimulus would occur after the modulation that causes CPM in the ascending perceptual processing. So the magnitude of CPM would be unaltered in spite of the conditioning stimulus being less painful.
In order to answer whether or not habituation exerts a descending influence, it is first necessary to establish the presence of both CPM and habituation in the study. CPM was clearly observed. On the other hand, the evidence for habituation is more complex.

**Patterns of Habituation**

It was expected that habituation would be observed as an interaction effect between Session and Group for the VAS ratings of the cold water. This pattern of results was expected because it was believed that additional exposure to the cold water in session two would cause the experimental group to habituate to that stimulus more than the control group. However, THIS habituation was instead observed as a trend toward a main effect of Session. That is, both groups combined, but neither group individually, showed a trend toward habituation to the cold water stimulus. This pattern of habituation was also illustrated by the lower average VAS ratings of the thermal grill illusion and Medoc stimuli in session three than session one (see Tables 4 and 7). Therefore, habituation, as observed in this study, seems to encompass many painful stimuli, and does not even depend on the amount of exposure to the cold water stimulus.

These observations of habituation are notable because the experimental paradigms often used to produce habituation administer dozens, if not hundreds, of identical stimuli over the course of several days or weeks (Bingel et al., 2007; LeBlanc & Potvin, 1965; Rennefeld et al., 2010), and such repetitive paradigms have not always produced significant pain habituation (Zbrozyna & Westwood, 1990). Therefore, given these precedents set by the literature, it is very surprising that noticeable, albeit insignificant, reductions in VAS ratings were observed during only the second exposure of so many painful stimuli.

Little is known of the cognitive and neural underpinnings of pain habituation. Bingel and colleagues (2007) proposed that this analgesic phenomenon serves to help individuals ignore
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unavoidable, painful situations. If this is truly the function of pain habituation, it seems likely that its mechanism may become activated after as little as one exposure because that may be all that is required for an organism to decide that a certain painful stimulus is unavoidable. In fact, given that the experimental group displayed a significant amount of habituation in their VAS ratings of their first and second exposure to the cold water stimulus, it seems that such rapid habituation is possible. In light of this model, the relatively quick and generalized habituation observed in this study may reflect an active cognitive decision that participants made regarding the unavoidability of painful stimuli used in this study.

In summary, though no definitive measure of habituation was observed in this study, the pattern of results observed suggests that several of the measured variables (water bath VAS, CPM scores, thermal grill ratings) were trending towards significantly habituating. Interestingly, only pain threshold measurements clearly contrasted this general trend. The fact that thresholds were not altered but other measurements of pain perception were suggests that while subjects’ ability to distinguish between thermal sensations was not altered, their perceptions of the intensity of pain may have habituated.

Does Habituation Affect CPM?

Although the results of this study suggest that participants’ ratings of water stimuli were trending toward significant habituation, changes in participants’ CPM ratings between sessions one and three did not approach statistical significance. Since CPM was not altered by the changing perceptions of the water stimuli, it appears that this study supports the conclusion that habituating to the conditioning stimulus does not alter CPM magnitude. Therefore, the evidence does not suggest that habituation has a descending, modulatory influence on CPM.
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However, this conclusion must be made tentatively. Close examination of Table 3 reveals that every measure of CPM declined between sessions one and three. Perhaps with a larger sample size, this reduction would have reached statistical significance. Nevertheless, given the data currently available, it is more prudent to withhold rejection of the null hypothesis.

This tentative conclusion can be viewed as evidence for a more sophisticated model of the physiological basis of endogenous analgesia phenomena. The seeming lack of influence from habituation on CPM detracts from the notion of a single unified “antinociceptive system” implied by previous research (Bingel et al., 2007). If only one such “system” were responsible for the many endogenous analgesia phenomena, then it seems unlikely that it involves aspects such as CPM, known to involve the endogenous opioid system (Niesters et al., 2011) and habituation, which does not utilize endogenous opioids (Rennefeld et al., 2010) and does not seem to exert descending influence on CPM. It seems more likely that several distinct endogenous analgesia modules function and exert influence within a complex network of interconnections. For example, in the present study participants tended to rate the thermal grill illusion as less painful during the final session than the first session. Therefore it is plausible that individuals can habituate to the illusory pain of the thermal grill. Combining this assertion with previous research, it seems that the thermal grill illusion can be acted upon by both CPM (Irvin, 2011) and habituation, yet habituation does not exert a modulatory influence on CPM. Taken together, this body of evidence suggests that CPM and habituation are at least partially independent processes.

Limitations

The main limitation of the present study is its small sample size of only 11 participants. Such a small sample is likely to have greater random sampling error, which reduces the
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capabilities of drawing valid inferences from data patterns. For instance, in this study those who had used cold therapies in the last 12 months were disproportionately assigned to rate the neutral water run first, though random assignment was used to decide this order. These same individuals tended to rate the cold water as less painful than other participants. Given this random error in assignment of run order, it cannot be known whether run order, and effect of recent cold therapies, or a combination of the two is responsible for the lower VAS ratings of the cold water bath given by these participants. Greater participation would have also increased statistical power, allowing for the assertion of implications with greater confidence.

Conclusion

This paper has described the observed lack of descending, modulatory influence from habituation to CPM. In addition to clarifying the results of this study through a larger sample size, more investigation will be needed to accurately identify and characterize the network of interactions between distinct endogenous pain modulation processes, particularly those known to function at “high” and “low” levels of processing. For instance, a complimentary study to this experiment that would further this aim would be to investigate the effects of habituation to the test stimulus in CPM.
References


CPM, Habituation


CPM, Habituation

Table 1.

*Means and Standard Deviations for VAS Results of Water Ratings*

<table>
<thead>
<tr>
<th>Group</th>
<th>Neutral Water Session 1</th>
<th>Neutral Water Session 3</th>
<th>Cold Water Session 1</th>
<th>Cold Water Session 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>Control</td>
<td>5.3</td>
<td>6.9</td>
<td>0.7</td>
<td>1.0</td>
</tr>
<tr>
<td>Experimental</td>
<td>0.8</td>
<td>1.4</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Overall</td>
<td>2.8</td>
<td>5.0</td>
<td>0.5</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Table 2.

*Results of ANOVA for VAS Ratings of Painfulness of Water Stimuli*

<table>
<thead>
<tr>
<th>Effect</th>
<th>F</th>
<th>η²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Session</td>
<td>3.338</td>
<td>.011</td>
<td>.10</td>
</tr>
<tr>
<td>Water Temp</td>
<td>41.393</td>
<td>.683</td>
<td>&lt;.001**</td>
</tr>
<tr>
<td>Group</td>
<td>6.145</td>
<td>.406</td>
<td>.04*</td>
</tr>
<tr>
<td>Session x Water Temp</td>
<td>1.125</td>
<td>.004</td>
<td>.3</td>
</tr>
<tr>
<td>Session x Group</td>
<td>0.452</td>
<td>.001</td>
<td>.5</td>
</tr>
<tr>
<td>Water Temp x Group</td>
<td>5.451</td>
<td>.090</td>
<td>.04*</td>
</tr>
<tr>
<td>Session x Water Temp x Group</td>
<td>1.812</td>
<td>.006</td>
<td>.2</td>
</tr>
</tbody>
</table>

*Note: * p < .05, ** p < .001
Table 3.

**Means and Standard Deviations for CPM Scores**

<table>
<thead>
<tr>
<th>Group</th>
<th>45° Stimulus</th>
<th></th>
<th>47° Stimulus</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Session 1</td>
<td>Session 3</td>
<td>Session 1</td>
<td>Session 3</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>Control</td>
<td>12.1</td>
<td>16.5</td>
<td>8.8</td>
<td>11.9</td>
</tr>
<tr>
<td>Experimental</td>
<td>17.2</td>
<td>20.8</td>
<td>7.3</td>
<td>9.9</td>
</tr>
<tr>
<td>Overall</td>
<td>14.9</td>
<td>18.3</td>
<td>8.0</td>
<td>10.3</td>
</tr>
</tbody>
</table>

Table 4.

**Medoc Ratings by Participant and Stimulus Averaged over Last Second**

<table>
<thead>
<tr>
<th>Participant</th>
<th>45° C</th>
<th>Cold</th>
<th>47° C</th>
<th>Cold</th>
<th>45° C</th>
<th>Cold</th>
<th>47° C</th>
<th>Cold</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neutral</td>
<td>Cold</td>
<td>Neutral</td>
<td>Cold</td>
<td>Neutral</td>
<td>Cold</td>
<td>Neutral</td>
<td>Cold</td>
</tr>
<tr>
<td>1</td>
<td>16.32</td>
<td>0.00</td>
<td>49.46</td>
<td>50.58</td>
<td>3.94</td>
<td>2.08</td>
<td>1.99</td>
<td>4.02</td>
</tr>
<tr>
<td>3</td>
<td>6.90</td>
<td>0.00</td>
<td>11.05</td>
<td>2.44</td>
<td>6.45</td>
<td>0.00</td>
<td>3.91</td>
<td>4.70</td>
</tr>
<tr>
<td>4</td>
<td>15.74</td>
<td>0.68</td>
<td>18.96</td>
<td>2.54</td>
<td>2.35</td>
<td>0.00</td>
<td>3.42</td>
<td>1.96</td>
</tr>
<tr>
<td>5</td>
<td>26.33</td>
<td>38.08</td>
<td>87.18</td>
<td>81.42</td>
<td>3.91</td>
<td>3.49</td>
<td>41.82</td>
<td>49.61</td>
</tr>
<tr>
<td>6</td>
<td>51.66</td>
<td>33.72</td>
<td>80.18</td>
<td>59.68</td>
<td>46.62</td>
<td>22.69</td>
<td>73.70</td>
<td>58.43</td>
</tr>
<tr>
<td>7</td>
<td>1.56</td>
<td>2.54</td>
<td>75.87</td>
<td>87.10</td>
<td>67.78</td>
<td>32.91</td>
<td>78.45</td>
<td>43.40</td>
</tr>
<tr>
<td>8</td>
<td>25.39</td>
<td>1.18</td>
<td>25.71</td>
<td>18.43</td>
<td>11.65</td>
<td>5.18</td>
<td>30.42</td>
<td>20.35</td>
</tr>
<tr>
<td>9</td>
<td>57.07</td>
<td>0.00</td>
<td>67.79</td>
<td>0.00</td>
<td>19.68</td>
<td>19.55</td>
<td>50.65</td>
<td>55.62</td>
</tr>
<tr>
<td>10</td>
<td>53.25</td>
<td>7.63</td>
<td>49.51</td>
<td>0.00</td>
<td>24.93</td>
<td>12.56</td>
<td>46.60</td>
<td>36.36</td>
</tr>
<tr>
<td>12</td>
<td>0.00</td>
<td>5.18</td>
<td>5.77</td>
<td>0.00</td>
<td>5.23</td>
<td>5.18</td>
<td>5.62</td>
<td>3.76</td>
</tr>
<tr>
<td>13</td>
<td>27.47</td>
<td>6.65</td>
<td>34.29</td>
<td>33.29</td>
<td>25.00</td>
<td>5.28</td>
<td>40.42</td>
<td>19.79</td>
</tr>
</tbody>
</table>

*Note:* Red rows denote control participants. Blue rows denote experimental participants.
Table 5.

*Results of ANOVA for CPM Scores*

<table>
<thead>
<tr>
<th>Effect</th>
<th>F</th>
<th>η²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Session</td>
<td>0.855</td>
<td>.075</td>
<td>.4</td>
</tr>
<tr>
<td>Medoc Temp</td>
<td>1.566</td>
<td>.005</td>
<td>.2</td>
</tr>
<tr>
<td>Group</td>
<td>0.465</td>
<td>.049</td>
<td>.5</td>
</tr>
<tr>
<td>Session x Medoc Temp</td>
<td>0.037</td>
<td>.0003</td>
<td>.9</td>
</tr>
<tr>
<td>Session x Group</td>
<td>0.309</td>
<td>.027</td>
<td>.6</td>
</tr>
<tr>
<td>Medoc Temp x Group</td>
<td>2.179</td>
<td>.007</td>
<td>.17</td>
</tr>
<tr>
<td>Session x Medoc Temp x Group</td>
<td>0.152</td>
<td>.001</td>
<td>.7</td>
</tr>
</tbody>
</table>

Table 6.

*Means and Standard Deviations for VAS Ratings of the Thermal Grill Illusion*

<table>
<thead>
<tr>
<th>Group</th>
<th>Session 1</th>
<th>Session 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>Control</td>
<td>30.6</td>
<td>25.0</td>
</tr>
<tr>
<td>Experimental</td>
<td>17.5</td>
<td>13.1</td>
</tr>
<tr>
<td>Overall</td>
<td>22.8</td>
<td>18.7</td>
</tr>
</tbody>
</table>

Table 7.

*Results of ANOVA for VAS Ratings of the Thermal Grill Illusion*

<table>
<thead>
<tr>
<th>Effect</th>
<th>F</th>
<th>η²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Session</td>
<td>3.168</td>
<td>.259</td>
<td>.11</td>
</tr>
<tr>
<td>Group</td>
<td>0.872</td>
<td>.088</td>
<td>.4</td>
</tr>
<tr>
<td>Session x Group</td>
<td>0.045</td>
<td>.004</td>
<td>.8</td>
</tr>
</tbody>
</table>
CPM, Habituation

Table 8.

**Means and Standard Deviations for Difference Thresholds**

<table>
<thead>
<tr>
<th>Group</th>
<th>Cool Difference</th>
<th>Warm Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Session 1</td>
<td>M</td>
</tr>
<tr>
<td>Control</td>
<td>2.54</td>
<td>1.63</td>
</tr>
<tr>
<td>Experimental</td>
<td>3.40</td>
<td>2.15</td>
</tr>
<tr>
<td>Overall</td>
<td>3.01</td>
<td>1.89</td>
</tr>
</tbody>
</table>

*Note:* Temperatures are displayed as °C below or above baseline (32°C)

Table 9.

**Means and Standard Deviations for Pain Thresholds**

<table>
<thead>
<tr>
<th>Group</th>
<th>Cold Pain (°C)</th>
<th>Hot Pain (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Session 1</td>
<td>M</td>
</tr>
<tr>
<td>Control</td>
<td>8.97</td>
<td>6.54</td>
</tr>
<tr>
<td>Experimental</td>
<td>9.49</td>
<td>7.01</td>
</tr>
<tr>
<td>Overall</td>
<td>9.26</td>
<td>6.46</td>
</tr>
</tbody>
</table>
Figure 1. This graph displays VAS ratings of cold water as a function of exposure number for each group. Exposure 1 took place during session one. Exposures 2-4 occurred when participants placed their hand into cold water during the three runs of session two. Therefore, only the experimental group underwent these exposures. Exposure 5 took place during session three. Both groups display a general decrease in VAS ratings after exposure 1. The greatest decrease between VAS ratings was observed between exposures 1 and 2 for the experimental group. This decrease in ratings was statistically significant, $t(5) = 2.745, p = .04$. Subsequent exposures all displayed relatively constant VAS ratings. Additionally, the consistently higher VAS ratings given by participants in the control group are clearly seen in this graph. Error bars denote standard error.
Figure 2. CPM scores were higher in the experimental than the control group during the first session. Scores generally declined between session one and three, especially for the experimental group. However, none of these differences were significant.
Figure 3. The control group rated the thermal grill as more painful than the experimental group did. Both groups generally rated the thermal grill illusion as less painful in session three than in session one, though this trend was not significant. None of these differences were significant, however.
Figure 4. Thresholds changed negligibly from session one to session three. All thresholds are displayed as average temperatures at which participants indicated feeling either a temperature difference from 32° C (for ‘Cool’ and ‘Warm) or pain (for ‘Cold’ and ‘Hot’).
Appendix A

Participant ID: ________

Date: ________________

Demographic Information

Please fill out the following information and return it to the researcher when you are finished.

1. Your Age:

2. Gender (circle one):      male      female

3. Ethnic Category (circle one):
   Hispanic or Latino
   Not Hispanic or Latino

4. Racial Category (circle all that apply):
   American Indian/Alaskan Native
   Asian
   Native Hawaiian or Other Pacific Islander
   Black or African American
   White

5. Right / Left     Handed (circle one)
Physical Activity and Cold Therapies Questionnaire

Please fill out the following information and return it to the researcher when you are finished.

1. In a typical week, how many hours do you spend engaged in moderate to hard physical activity? ______

2. On average, how many days per week do you engage in moderate to hard physical activity lasting a half hour or more? ______

3. Using the scale from 6 to 20 at the end of this questionnaire, how hard or strenuous does a typical session of physical activity feel to you, overall? Your choice should reflect your total feeling of exertion during that activity. ______

The following three questions pertain only to your use of ice-baths:

4. Within the past 12 months, how many times have you used ice-baths as treatment for an injury, soreness, or other therapeutic purpose?

   Never             1-5 times           6-10 times           11 or more times

5. How long has it been since you last used an ice-bath? (excluding this experiment) ______

6. Within the past 12 months, how many times have you used ice-baths on each of the following areas?
   a. Left foot/leg: ______
   b. Right foot/leg: ______
   c. Left hand/arm: ______
   d. Right hand/arm: ______
   e. Other areas: ______
The following three questions pertain only to your use of ice-packs or other cold-therapies, such as Icy-Hot®:

7. Within the past 12 months, how many times have you used ice packs or other cold therapies, excluding ice-baths?

   Never          1-5 times          6-10 times          11 or more times

8. How long has it been since you last used ice packs or another cold-therapy, excluding ice-baths? __________

9. Within the past 12 months, how many times have you used ice packs or other cold-therapies on each of the following areas, excluding ice-baths?
   a. Left foot/leg: ______
   b. Right foot/leg: ______
   c. Left hand/arm: ______
   d. Right hand/arm: ______
   e. Other areas: ______
<table>
<thead>
<tr>
<th>rating</th>
<th>description</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>NO EXERTION AT ALL</td>
</tr>
<tr>
<td>7</td>
<td>EXTREMELY LIGHT</td>
</tr>
<tr>
<td>8</td>
<td>VERY LIGHT</td>
</tr>
<tr>
<td>9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>LIGHT</td>
</tr>
<tr>
<td>12</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>SOMEWHAT HARD</td>
</tr>
<tr>
<td>14</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>HARD (HEAVY)</td>
</tr>
<tr>
<td>16</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>VERY HARD</td>
</tr>
<tr>
<td>18</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>EXTREMELY HARD</td>
</tr>
<tr>
<td>20</td>
<td>MAXIMAL EXERTION</td>
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