Headache
© 2013 American Headache Society

ISSN 0017-8748
doi: 10.1111/head.12117
Published by Wiley Periodicals, Inc.

Research Submission

Waning of "Conditioned Pain Modulation": A Novel Expression of Subtle Pronociception in Migraine

Hadas Nahman-Averbuch, MSc; Yelena Granovsky, PhD; Robert C. Coghill, PhD; David Yarnitsky, MD, PhD; Elliot Sprecher, PhD; Irit Weissman-Fogel, PhD

Objective.—To assess the decay of the conditioned pain modulation (CPM) response along repeated applications as a possible expression of subtle pronociception in migraine.

Background.—One of the most explored mechanisms underlying the pain modulation system is "diffuse noxious inhibitory controls," which is measured psychophysically in the lab by the CPM paradigm. There are contradicting reports on CPM response in migraine, questioning whether migraineurs express pronociceptive pain modulation.

Methods.—Migraineurs (n = 26) and healthy controls (n = 35), all females, underwent 3 stimulation series, consisting of repeated (1) "test-stimulus" (Ts) alone that was given first followed by (2) parallel CPM application (CPM-parallel), and (3) sequential CPM application (CPM-sequential), in which the Ts is delivered during or following the conditioning-stimulus, respectively. In all series, the Ts repeated 4 times (0-3). In the CPM series, repetition "0" consisted of the Ts-alone that was followed by 3 repetitions of the Ts with a conditioning-stimulus application.

Results.—Although there was no difference between migraineurs and controls for the first CPM response in each series, we found waning of CPM-parallel efficiency along the series for migraineurs ($P = .005$ for third vs first CPM), but not for controls. Further, greater CPM waning in the CPM-sequential series was correlated with less reported extent of pain reduction by episodic medication ($r = 0.493$, $P = .028$).

Conclusions.—Migraineurs have subtle deficits in endogenous pain modulation which requires a more challenging test protocol than the commonly used single CPM. Waning of CPM response seems to reveal this pronociceptive state. The clinical relevance of the CPM waning effect is highlighted by its association with clinical parameters of migraine.

Key words: migraine, endogenous analgesia, diffuse noxious inhibitory control, conditioned pain modulation, waning effect

Abbreviations: ANCOVA analysis of covariance, ANOVA analysis of variance, COVAS computerized visual analog scale, CPM conditioned pain modulation, Cs conditioning-stimulus, MOA migraine without aura, MWA migraine with aura, NPS numerical pain scale, PCS Pain Catastrophizing Scale, RM-ANOVA repeated measures analysis of variance, Ts test-stimulus

(*Headache* 2013;53:1104-1115)

From the Laboratory of Clinical Neurophysiology, Technion Faculty of Medicine, Haifa, Israel (H. Nahman-Averbuch, Y. Granovsky, D. Yarnitsky, and E. Sprecher); Department of Neurology, Rambam Health Care Campus, Haifa, Israel (Y. Granovsky and D. Yarnitsky); Department of Neurobiology and Anatomy, Wake Forest University School of Medicine, Winston Salem, NC, USA (R.C. Coghill); Department of Physical Therapy Faculty of Social Welfare and Health Sciences, University of Haifa, Haifa, Israel (I. Weissman-Fogel).

Address all correspondence to I. Weissman-Fogel, Department of Physical Therapy Faculty of Social Welfare and Health Sciences, University of Haifa, 31905 Mount Carmel, Haifa, Israel, email: ifogel@univ.haifa.ac.il

Accepted for publication March 6, 2013.

Conflict of Interest: There are no conflicts of interest to report for any of the authors.

Pain inhibition during endogenous analgesia may be elicited by several mechanisms. One of the most explored mechanisms underlying the pain inhibitory system is "diffuse noxious inhibitory controls," which is mediated by activation of a spino-bulbo-spinal loop.[1] This mechanism is based on the "pain inhibits pain" phenomenon and can be measured psychophysically in the lab by the conditioned pain modulation (CPM) paradigm.[2] CPM is manifested as a decrease in the perceived pain generated by a noxious stimulus (test-stimulus; Ts) during or following application of another noxious stimulus (conditioning-stimulus; Cs).

There are different ways to induce CPM. The most widely used method is one in which the Cs is delivered in parallel with the Ts (CPM-parallel) in a temporally overlapping fashion. Alternatively, CPM can also be assessed during the sequential delivery of the Cs and Ts, such that the Ts is initiated after the termination of the Cs (CPM-sequential).[3,4] However, the CPM-sequential paradigm yields a lower CPM response than CPM-parallel,[5] suggesting that the 2 protocols represent different aspects of the CPM process. The CPM-parallel represents the peak of inhibitory capacity, while the CPM-sequential represents the temporal profile of this inhibitory capacity, examining its extent during waning down from its peak.

Patients with various pain disorders, such as temporomandibular disorder,[6-8] irritable bowel syndrome,[7,9] fibromyalgia,[10-12] and tension-type headache,[13] exhibit less efficient CPM than healthy controls. However, contradicting results exist in the literature regarding CPM efficiency in migraine patients. For example, migraine patients have been reported to have marked alterations of CPM in that they facilitate rather than inhibit the nociceptive flexion reflex during and after using the cold pressor test as a Cs.[14] Similarly, they have been reported to have no significant difference in laser-evoked potentials, before, during, and after the use of capsaicin as a Cs.[15] In contrast, Coppola et al[16] demonstrated no difference between migraine patients and healthy controls in the recovery curve of the nociceptive-specific blink reflex during an electrical Cs. Similarly, Perrotta et al,[17] using temporal summation threshold as Ts, found a similar CPM response during and after the Cs, in both migraine patients and healthy subjects. In these studies that examined both males and females, CPM was assessed in paradigms using a single application of Cs, thus, no information is available regarding the ability of migraineurs to sustain pain inhibitory activity across repeated activations of CPM.

In view of the contradicting results on efficiency of CPM in migraineurs, as demonstrated for single CPM series, our reasoning for this work was that repeated CPM series might uncover a mild CPM dysfunction, not expressed in a single trial which might be expressed as gradual decrease in efficacy across series of trials. Thus, our aim was to examine the temporal changes in the CPM efficacy along several repetitions during 1 session, in healthy volunteers and migraine patients, across 2 types of CPM paradigms.

METHODS

Subjects.—Twenty-nine migraine patients and 38 healthy female volunteers were enrolled in the study. Three migraineurs and 3 healthy subjects could not tolerate the stimuli and therefore were excluded. Thus, 26 female migraine patients (age 35.3 ± 11.6 years, mean ± standard deviation; 12 without aura (MOA) and 14 with aura (MWA)) who met the International Headache Society criteria[18] and 35 healthy female volunteers (29.3 ± 9.3 years) participated in the study. None of the patients received any preventive medication or episodic analgesic medication within 24 hours before the testing. The tests were performed at least 24 hours after the last migraine attack termination. For healthy subjects, the exclusion criteria were: acute or chronic pain, neurological or psychiatric diseases, or use of medications related to these fields on a regular basis, inability to communicate or to understand the instructions of the study, and inability to tolerate the stimuli. The study was approved by the local Ethics Committee of Rambam health care in Haifa, and informed consent was obtained from all of subjects prior to the experiment.

Ts.—The Ts was a tonic heat pain delivered to the lower left leg for 30 seconds at an intensity of 47.5°C. The Ts was tested in the leg area and not in the face because in previous studies, allodynia in the trigemi-

nal nerve territory was found in migraine patients in-between attacks and therefore we preferred to choose a stimulation area that is outside the nerve territory. In this way, the results would not be contaminated by the existence of allodynia. Moreover, as we aimed to assess the central pain modulation process, we applied the Ts on a remote body area. The intensity of the Ts was determined based on the results of a pilot study (data are not presented) indicating that this was the optimal tolerable noxious temperature along several stimulus repetitions. Stimuli were delivered using the thermode $1.6 \times 1.6\ cm^2$ of the Thermal Sensory Analyzer (TSA2001, Medoc, Ramat Yishai, Israel). The temperature increase and decrease rate was 5°C/second from a baseline temperature of 32°C. The pain intensity of the Ts was rated continuously during the exposure to the contact heat stimuli using computerized visual analog scale (COVAS, Medoc) ranging between "no pain" and "worst pain imaginable," and later converted to a 0-100 scale. The average of the COVAS ratings along each Ts application was taken as the Ts pain rating.

Cs.—The Cs consisted of immersion of the right foot into a cold water bath for 60 seconds. The water temperature was set to 10°C using cold water and ice cubes, and measured by a thermometer before and immediately after each CPM series. However, as each CPM series comprised repeated immersions of the foot in the bath, the temperature at the end of the series increased up to 12°C. Subjects rated the pain intensity from the Cs after 20 and 60 seconds verbally using numerical pain scale with same 0-100 scale. The average of the 2 ratings was taken as the Cs pain rating.

Psychological Questionnaires.—Anxiety level was assessed by Spielberger's State-Trait Anxiety Inventory,[19] using the validated Hebrew version.[20] This questionnaire has 2 parts, anxiety state and anxiety trait. Each part includes 20 items and the subjects were asked to rate their feelings about each statement on a 4-point scale (1-4).

Pain catastrophizing level was assessed by the Pain Catastrophizing Scale (PCS)[21] using the validated Hebrew version.[22] This questionnaire contains 13 items representing the 3 components of rumination, magnification, and helplessness. Subjects were asked to complete the questionnaire and relate to previous pain events.

Study Design.—Prior to sensory testing, subjects completed the psychological questionnaires, rated their tiredness level (range of 0 – not tired at all, to 100 – very tired), and reported the number of days from their last menstrual period. In addition, migraine patients were interviewed regarding their clinical characteristics including frequency, duration, and pain intensity of their headache episodes and their regular medication routine during the last month episodes. Then, subjects were familiarized with the study stimuli and with pain ratings procedure. The familiarization included delivery of 3 heat stimuli to the lower left leg (45, 47, and 49 degrees for 5 seconds each) and 15 seconds of the Cs. Ten-minute break was kept between the familiarization and the experiment.

For sensory testing, all subjects underwent 3 stimulation series (1) Ts-alone – the Ts was repeated 4 times with 40 seconds break between stimuli. This series was always given first. (2) CPM-parallel – a single administration of Ts, followed by 3 Ts administrations simultaneously with the Cs, with Ts given during the last 30 seconds of the 60-second-long Cs. A break of 40 seconds was kept between each repetition. (3) CPM-sequential – single Ts followed by 3 administrations of Cs, with Ts starting 15 second after the termination of the Cs. Similarly, a break of 40 seconds was kept between each repetition (Fig. 1). CPM-parallel and CPM-sequential were given in a randomized order. Each series was delivered one time with an 8-minute interval between series.

Statistical Analysis.—Statistical analyses were performed using SAS (SAS Institute, Cary, NC, USA). Ts pain was calculated as the average of the COVAS ratings along each 30-second stimulus. The "*CPM response"* was determined as the difference between pain rating of the Ts obtained during the CPM series and the match pain rating of the Ts obtained in the Ts-alone series. An efficient CPM response is represented as negative value. In addition, the difference between the last and the first CPM responses within each series was calculated and defined as "*CPM change"* value. A reduction of the CPM response (ie, waning effect) is represented as positive value. Pain

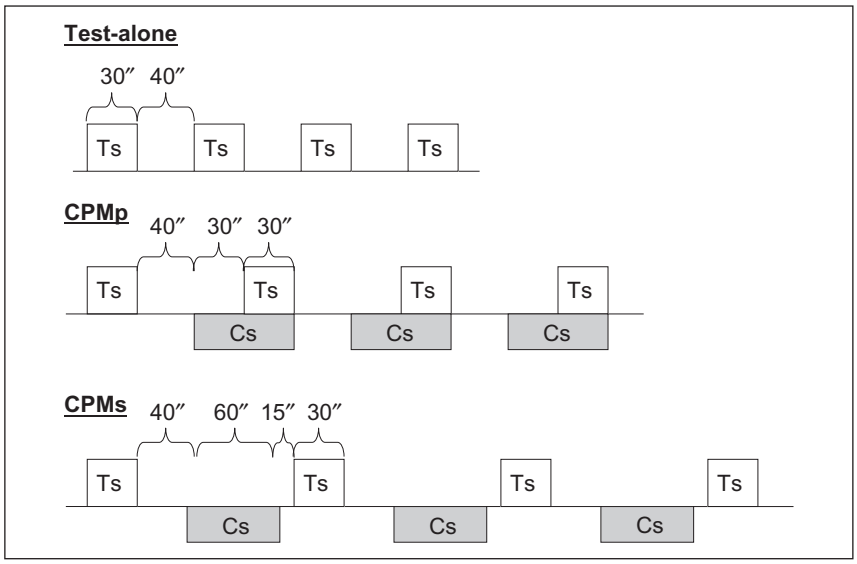

Fig 1.—Study design. The time-course of the Ts-alone, CPM-parallel (CPMp), and CPM-sequential (CPMs) series. In order to examine the CPM response, heat stimuli were presented alone (test-alone), concurrently (CPMp), and following (CPMs) foot immersion into cold water. CPM = conditioned pain modulation; Cs = conditioning-stimulus; Ts = test-stimulus.

diminution following episodic medication was calculated as the difference between pain rating after episodic medication and pain rating before episodic medication. A negative value indicates greater pain reduction following medication.

Several analysis of variance (ANOVA) or analysis of covariance (ANCOVA) models were used as appropriate, depending on whether or not covariates were included. All of these were repeated measures analyses, implemented as mixed models using SAS PROC MIXED, with subject as the random factor, appropriate nesting as required, and optimum covariance structure chosen among several candidates based on inspection of statistical information criteria and other ANOVA diagnostics. Post-hoc Tukey–Kramer tests were employed as appropriate.

1. To test the order or carryover effects of the CPM, we examined the first reading in each of the 3 stimulation series (Ts-alone, CPM-parallel, and CPM-sequential), as this reading is produced under equivalent stimulus conditions in all 3 series. In the first analysis, we modeled group (migraines vs controls), the order of the series (first, second, or third), and their interaction, using mixed-model ANOVA. The second analysis was similar, except that we modeled the stimulus series rather than the order (in both cases, the Ts was always the first one administered).
2. To evaluate the CPM responses, repeated measures ANOVA (RM-ANOVA) model was utilized and included group (migraines vs controls), series (CPM-parallel and CPM-sequential), and repetition (first, second, and third repetitions) as independent variables and the interactions between them, taking the CPM response as a dependent variable.
3. To examine the habituation of the Ts pain ratings in the Ts-alone series, an RM-ANOVA model including group (migraines vs controls), repetition (4 repetitions: zero, first, second, and third), and the interaction between them was utilized, taking the pain rating of the Ts as a dependent variable.
4. To assess differences in the habituation of the Cs, an RM-ANOVA was applied including group (migraines vs controls), series (CPM-parallel and CPM-sequential), repetition (first, second and third repetitions), and the interaction between them, taking the pain ratings of the Cs during the CPM-parallel and CPM-sequential series as a dependent variable.

Table 1.—Characteristics of Migraine Patients

	P value	Without Aura	Aura
Duration (years)	.261	14.4 ± 10.9	19.4 ± 11.0
Time from last episode (days)	.686	16.7 ± 19.9	11.7 ± 10.5
Mean # of episodes/month	.215	5.9 ± 6.6	3.3 ± 1.8
Episode duration (hours, no medication taken)	.972	27.3 ± 31.5	26.8 ± 19.7
Episode duration (hours, after medication taken)	.587	1.8 ± 1.3	8.7 ± 12.8
Mean pain (0-100 NPS, no medication)	.877	83.1 ± 16.2	83.7 ± 16.2
Max pain (NPS, no medication)	.605	90.6 ± 9.4	94.0 ± 11.4
Mean pain (NPS, after medication)	**.011**	30.7 ± 22.1	57.5 ± 25.7
Max pain (NPS, after medication)	.529	70.0 ± 25.5	75.6 ± 27.9

Data are presented as mean ± standard deviation. The boldfaced value is for a significant *P* value.
Max = maximal; NPS = numerical pain scale.

In the last 2 tests (habituation of the Ts and the Cs), the model residuals were not normally distributed until an arcsine-square-root transformation was applied.

Differences in age and personality variables between the patients and controls and within the migraine group were examined using *t*-test. Pearson correlations were conducted between clinical and personality characteristics, and the CPM responses as well as the CPM change value. The correlations were corrected for multiple comparisons using the Bonferroni correction. Statistical significance was defined as $P < 0.05$.

RESULTS

Subjects.—Clinical characteristics of migraine patients (MWA and MOA) are presented in Table 1. As can be seen from the table, there was no difference between MWA and MOA in any of the clinical measures except for the mean pain rating during migraine attacks after taking pain relief medication therefore, they were considered as 1 group.

Migraine patients differed from healthy subjects in years of age (35.3 ± 11.6 and 29.3 ± 9.3, respectively, $P = .036$) and PCS score (30.6 ± 11.7 and 23.4 ± 9.4, respectively, $P = .015$). No significant group difference was observed between migraine patients and healthy subjects in the number of days from last menstrual period (12.0 ± 10.6 and 18.6 ± 20.0, respectively, $P = .140$), tiredness (27.0 ± 24.9 and 25.8 ± 18.9, respectively, $P = .847$), state anxiety (29.1 ± 5.3 and 27.7 ± 6.7, respectively, $P = .405$), and trait anxiety (36.9 ± 10.2 and 34.8 ± 9.4, respectively, $P = .412$) scores.

CPM Responses Differ Between Migraine Patients and Controls.—Analyses of the pain readings in response to single administration of the Ts, in each series, indicated that no order or carryover effects of the CPM were present. For the first analysis, neither the group × order interaction nor the order effect was significant ($P = .5$ for each of them). Also for the second analysis, neither the group × series nor the series effect was significant ($P = .4$ and $P = .7$, respectively). Both analyses indicate that the "washout" delay procedure of 8 minutes interval between series was effective.

The CPM responses of the migraine patients and healthy subjects across time are presented in Table 2.

Table 2.—The CPM Responses in the CPM-Parallel and CPM-Sequential Series in Migraine Patients and Healthy Subjects (Mean ± Standard Deviation)

Repetition Number	1	2	3
Migraine			
CPM response			
CPM-parallel	–16.3 ± 20.3	–11.2 ± 17.6	–2.2 ± 20.1
CPM-sequential	–9.5 ± 19.6	–1.2 ± 12.3	–4.5 ± 18.8
Healthy			
CPM response			
CPM-parallel	–10.1 ± 14.0	–10.8 ± 14.9	–7.5 ± 11.7
CPM-sequential	–3.9 ± 10.8	–9.3 ± 12.8	–1.6 ± 13.0

CPM = conditioned pain modulation.

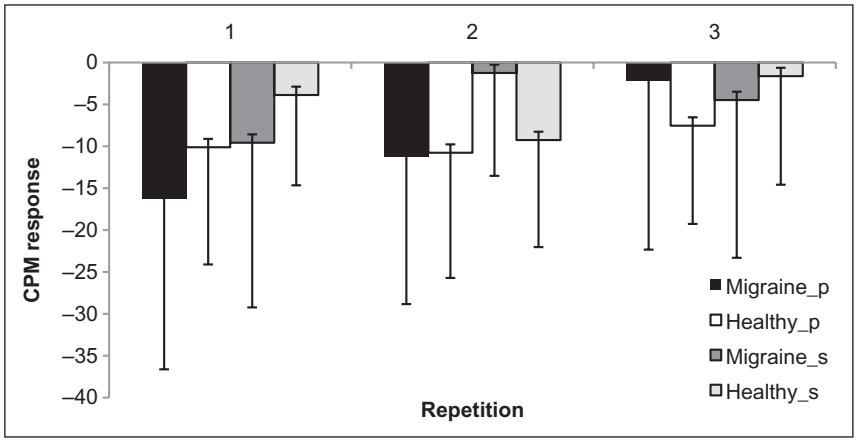

Fig 2.—Magnitude of conditioned pain modulation (CPM) in migraine patients and healthy controls. A significant decrease ($P = .005$) in the CPM response, from the first to the third repetition, was demonstrated in the migraine group during the CPM-parallel series. Additionally, a greater CPM response was shown in the migraine group during the second CPM repetition in the CPM-parallel compared with the CPM-sequential series ($P = .033$). p = parallel series; s = sequential series. Data are presented as mean ± standard deviation.

We originally ran an ANCOVA including Cs pain rating as a covariate; however, as this covariate was not significant, it was dropped in subsequent models. Three outlier observations (representing <0.9% [3/336] of the data) were identified based on inspection of the mixed-model ANOVA residuals diagnostics (Q-Q plots and distributions, as well as appropriate outlier box plots) and were removed from the analysis. There was no effect of group. However, a triple interaction of group, series, and repetition was demonstrated ($P = .002$). Post-hoc analysis revealed a change in CPM efficiency along the repetitions within series. For migraine patients, the first CPM response during the CPM-parallel series was more efficient than the third CPM response (–16.32 ± 20.3 and –2.21 ± 20.1, respectively, $P = .005$), while no such change was observed for the controls. Thus, the CPM change values during the CPM-parallel series and the sequential series were significantly different in the migraine patients ($P = .039$), but not in the healthy subjects ($P = .857$). This suggests a waning process of the CPM response for the migraineurs as opposed to control subjects. In addition, a significant difference was found in the migraine group between the second CPM responses in CPM-parallel compared with the CPM-sequential (–11.2 ± 17.6 and –1.2 ± 12.3, respectively; $P = .033$, Fig. 2). The CPM response was related to the series ($P = .006$) with more efficient CPM response for CPM-parallel than CPM-sequential.

The Pain Ratings for Ts-Alone Series.—The average pain ratings of each repetition of the Ts-alone series are depicted in Table 3. ANOVA revealed no effect of repetition ($P = .104$) and no group by repetition interaction ($P = .427$) indicating absence of temporal changes of the Ts-alone (eg, habituation or sensitization) neither in the controls nor in the migraine patients. However, a trend was found for difference in pain ratings between migraineurs and healthy subjects ($P = .053$), indicating that patients might have perceived higher pain from the Ts.

Healthy Subjects Habituated to the Cs.—The pain ratings of the Cs are depicted in Table 3. There was no effect of group ($P = .105$), but a trend was found for the series effect ($P = .056$), ie, higher pain ratings were observed during CPM-parallel compared with CPM-sequential. In addition, there was a group by repetition interaction ($P = .009$) such that for controls, pain rating of the last Cs was significantly lower than the first ($P = .023$) and the second Cs ($P < .001$). For migraine patients, the pain rating to second Cs was significantly higher compared with the first ($P < .001$) and the third Cs ($P < .001$, Table 3).

Correlations Between CPM Responses and Clinical as Well as Personality Characteristics.—Pain reduction following episodic medication use was

Table 3.—Ratings of Test and Conditioning Stimuli in the Test-Alone, CPM-Parallel, and CPM-Sequential Series in Migraine Patients and Healthy Subjects

Repetition Number	0	1	2	3
Migraine				
Test stimulus ratings (COVAS ratings)				
Test alone	29.6 (20.6-39.4)	35.0 (24.8-45.9)	33.8 (23.9-44.4)	34.8 (24.9-45.4)
CPM-parallel	29.6 (20.9-39.2)	18.7 (11.5-27.2)	21.5 (13.8-30.3)	31.0 (22.1-40.8)
CPM-sequential	29.8 (21.1-39.4)	26.0 (17.7-35.2)	30.5 (21.7-40.1)	29.5 (20.7-39.0)
Conditioning-stimulus ratings (NPS ratings)				
CPM-parallel	—	55.4 (40.5-69.8)	70.0 (55.7-82.6)	55.6 (40.6-70.1)
CPM-sequential	—	51.4 (36.2-66.6)	62.6 (47.4-76.6)	51.3 (37.2-65.4)
Healthy				
Test stimulus ratings (COVAS ratings)				
Test alone	18.1 (11.8-25.5)	20.4 (13.3-28.7)	24.3 (16.7-32.8)	23.0 (15.6-31.2)
CPM-parallel	18.4 (12.2-25.6)	9.6 (5.1-15.3)	13.1 (7.8-19.5)	15.2 (9.5-21.9)
CPM-sequential	15.6 (9.8-22.3)	12.4 (7.3-18.7)	14.6 (9.0-21.2)	20.7 (14.2-28.1)
Conditioning-stimulus ratings (NPS ratings)				
CPM-parallel	—	45.4 (32.8-58.2)	52.3 (39.5-64.9)	37.7 (25.8-50.4)
CPM-sequential	—	43.0 (30.2-56.3)	43.9 (31.2-57.1)	32.8 (21.9-44.8)

The values are back transformed and data are presented as average (95% confidence limit – based on standard error).
— = conditioning stimulus was not applied at time 0; COVAS = computerized visual analog scale; CPM = conditioned pain modulation; NPS = numerical pain scale.

correlated with the CPM change value along the CPM-sequential series (r = 0.493, *P* = .028, Fig. 3) and in trend for CPM change value along the CPM-parallel series. Thus, maintaining of CPM response is associated with better response to episodic medications use, and vice versa. This finding points toward waning of CPM efficiency as indicator for a more pronociceptive profile of the migraine.

Overall, there were no correlations between the personality parameters (anxiety trait and state and PCS score) and the CPM responses or the CPM change values for both healthy and migraine patients. However, ratings of the average and maximal pain (without medication) during migraine attacks were positively correlated with PCS scores (r = 0.655, *P* = .016 and r = 0.605, *P* = .056, respectively). The r

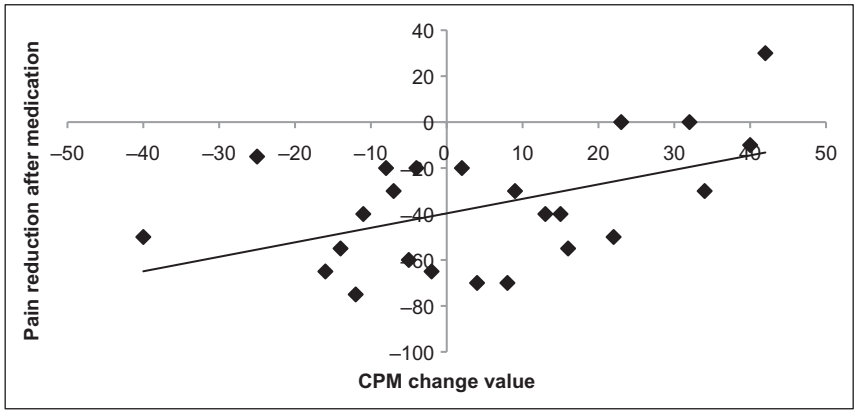

Fig 3.—Correlation between clinical characteristics of the migraine attack and the conditioned pain modulation (CPM) change value. Pain reduction following episodic medication (a negative value indicates greater pain reduction) was positively correlated with the CPM change value (a positive value indicates decrease of the CPM response) along the CPM-sequential series.

and *P* values for all correlations are presented in Tables S1 and S2.

DISCUSSION

The results of our study indicate that in-between attacks, migraine patients demonstrate a waning in efficiency of pain modulation along repeated CPM administrations, suggesting a subtle pronociceptive pain modulation pattern.

Previous studies indicated that migraine patients demonstrate enhanced pain sensitivity to nociceptive stimulation in-between attacks as expressed by increased temporal summation and reduced pain thresholds,[23-25] pointing toward a possible state of pronociception in their pain modulation. Nevertheless, when looking at reports on efficiency of CPM, another essential test for detection of pronociception, the literature is inconsistent. Several studies demonstrated less efficient CPM in migraineurs compared with healthy subjects,[14,15] while others found no difference.[16,17] Although the aforementioned CPM studies examined both male and females, in the current study, we tested only females, which do not allow a direct comparison with these studies. The existence of sex differences in pain perception and modulation is still controversial;[26,27] therefore, by examining one sex, we avoided a possible contamination of our results. Moreover, as migraine is more prevalent in females,[28,29] we studied only females.

In the present study, migraineurs had a CPM response that was not different from controls during the first CPM stimulus of each series. Yet, they failed to maintain their CPM response along repetitions during CPM-parallel series, the more robust CPM paradigm. This finding indicates that the efficacy of their endogenous analgesia waned over time in a manner different from that of healthy controls. We, therefore, suggest that migraineurs have a partial deficiency in their endogenous pain inhibition, which is not strong enough to be expressed in a standard, single administration of a CPM paradigm, in the way seen for patients with idiopathic pain syndromes. Rather, a more demanding CPM paradigm is required in order to uncover this more subtle deficit. Challenging the endogenous analgesia system to inhibit pain along a series of CPM applications shows the reduced ability of migraineurs to maintain the inhibitory capacity as compared with controls.

A second finding of our study is the less efficient CPM response shown by migraineurs for the second CPM-sequential block within series as compared with the CPM-parallel. This sequential paradigm is considered more challenging and demanding for the pain inhibitory system than the parallel one, in part because it may be related to the duration of CPM. Taken together with the waning observed with the CPM-parallel series, this finding raises the possibility that the effective duration of CPM is shorter in migraineurs than healthy subjects.

These findings are made clinically relevant by the fact that a change in CPM efficiency along the repeated CPM-sequential series was associated with the severity of migraine attacks. This is in line with previous finding from our lab that increased summation was found to correspond with more severe clinical parameters of migraine patients.[25] It, therefore, might be suggested that endogenous pain inhibitory capacity is a contributing factor to pain severity in migraine, such that patients with better pain inhibitory capacity suffer less pain, and vice versa. However, one cannot rule out the reverse explanation, that severe migraine attacks, due to other factors, had "consumed" the inhibitory capacity of patient's pain processing system, rendering it less efficient. We, hence, suggest waning of CPM to be a novel test protocol for revealing the mild pronociceptive state of migraineurs. The lack of correlations between the CPM response and the clinical characteristics of the migraine patients in previous studies[14,30] might stem from their use of only one CPM response for the correlation.

Although the use of both CPM-parallel and CPM-sequential paradigms is acceptable,[31-33] a significant effect of series was found, despite using the same stimulation parameters in both paradigms. The CPM response in the sequential series was less efficient than the parallel series. This was also demonstrated in previous studies that investigated the efficiency of the endogenous analgesia mechanism using single repetition of CPM. Lewis et al[34] found in their study that in healthy subjects, pressure pain thresholds measured during and 1 minute after cold pressor test or

ischemic pain were higher compared with baseline, meaning that the CPM response was induced in both parallel and sequential paradigms. Treister et al[35] compared pain ratings from heat stimuli obtained during and 12 seconds after cold pressor test application in healthy subjects and found that the CPM response during the Cs was higher than after the Cs. Sandrini et al[14] examined the nociceptive flexion reflex in healthy and migraine subjects during and immediately after the cold pressor test. While healthy subjects showed significant inhibition of the nociceptive reflex during Cs, which decreased to baseline following Cs, migraine patients showed facilitation of the reflex, which was terminated with the end of the Cs such that no CPM response was observed in any type of paradigm.

The difference found between the 2 CPM series in the present study can stem from lower pain ratings of the Cs reported by our subjects, or less distraction of Cs on the Ts in the CPM-sequential series. In the latter, the Ts is delivered after the end of the Cs, drawing all of subject's attention, while in the parallel series, the 2 stimuli are delivered simultaneously and thus the attention is probably divided. Attention has an effect of pain perception and usually, attention to a painful stimulus leads to increase in pain intensity while distraction leads to its decrease.[36] Indeed, fibromyalgia female patients demonstrated reduced temporal summation pain ratings under CPM paradigm only when the stimuli were applied concurrently with a distraction (when the patients were instructed to attend to the Cs and not to the Ts).[37] However, for migraine patients, no decrease in pain ratings of suprathreshold stimuli was found during a mental arithmetic distraction task.[38] Therefore, we suggest that differences between CPM-parallel and CPM-sequential are not due to distraction effect. This is further supported by recent findings from our lab that CPM-parallel response has more than just distraction.[39,40] Thus, the CPM-sequential paradigm might be more sensitive to the temporal changes of CPM, while the CPM-parallel paradigm might be more sensitive to the maximal analgesic effect of CPM. Still, the difference in the extent of the CPM response should be taken into consideration when choosing the CPM paradigm.

Migraine patients show impaired habituation to repeated stimuli of various modalities.[41,42] In the present study, neither migraineurs nor controls exhibited habituation. Healthy subjects usually habituate to repeated painful stimuli,[43,44] and this is even more prominent in women than in men.[45,46] However, in accordance to our results showing no such habituation, Lev et al[47] showed no habituation to repeated painful heat stimuli in healthy subjects. Moreover, Hashmi and Davis[45] showed that the habituation was related to the temperature of the stimulus, and in higher temperatures (as was the case in our study), subjects do not habituate. Furthermore, the lack of difference in pain ratings in the test-alone series between the migraine and healthy subjects indicates that the CPM responses reflect different pain modulation capability which is not due to habituation of the Ts.

Accumulating evidence points to the key role of pain catastrophizing in pain perception and modulation, and higher pain catastrophizing levels were associated with lower CPM efficiency.[4,48,49] However, Granot et al[50] found no relation between pain catastrophizing and CPM efficiency. The lack of correlations between the pain catastrophizing scores and the CPM responses that were found in the current study may stem from difference in CPM methodology. Higher pain catastrophizing scores were found in chronic pain patients[51,52] and were associated with pain severity and sensitivity.[53] In line with these reports, in the current study, it was found that pain catastrophizing scores differed between patients and healthy subjects, and it was correlated with the clinical pain ratings of the migraine attacks.

A possible disadvantage to this study is the decreased pain ratings across the Cs repetitions that were demonstrated for both migraine and healthy subjects. The effect of the Cs painfulness on the CPM efficiency was previously addressed and it was suggested that the CPM efficiency is independent of the pain ratings of the Cs, as long as the Cs is perceived as painful.[50,54] The water temperature that was chosen in the current study was previously found to evoke CPM response in healthy controls.[50] Another study limitation is lack of control for menstrual cycle. The effect of menstrual cycle on pain perception is controversial. Some studies found no difference in experimen-

tal pain perception along the menstrual cycle in healthy females.[55-57] However, Tousignant-Laflamme and Marchand[56] found differences in the CPM efficiency across the menstrual cycle, and thus, it may affect our results. Nevertheless, we did not find differences between patients and controls in the number of days from last menstrual period. Another potential limitation is the different inter-Ts interval along the time-course of the different series. A 40-second break was maintained between successive noxious stimuli (whether they were Ts or Cs). However, since the timing of the Ts relative to the Cs varied across series, the interval between successive Ts was 40 seconds in the Ts-alone series, 70 seconds in the CPM-parallel series, and 115 seconds in the CPM-sequential series.

CONCLUSIONS

Migraineurs have a subtle dysfunction of their endogenous analgesia capacity, which can be revealed only by repeated CPM testing, and which is associated with the severity of their attacks, potentially in a pathophysiological relationship. We propose repeated CPM testing as a new member in the psychophysical tool box, allowing finer sensitivity in assessing patient's pain processing capacity.

Acknowledgment: This study was financed based on a United States–Israel Binational Science Foundation grant number 2009097.

REFERENCES

1. Le Bars D, Dickenson AH, Besson JM. Diffuse noxious inhibitory controls (DNIC). I. Effects on dorsal horn convergent neurones in the rat. *Pain*. 1979;6:283-304.
2. Yarnitsky D, Arendt-Nielsen L, Bouhassira D, et al. Conditioned Pain Modulation (CPM): Recommendations on terminology and practice of psychophysical DNIC testing. *Eur J Pain*. 2010;14:339.
3. Pud D, Granovsky Y, Yarnitsky D. The methodology of experimentally induced diffuse noxious inhibitory control (DNIC)-like effect in humans. *Pain*. 2009;144:16-19.
4. van Wijk G, Veldhuijzen DS. Perspective on diffuse noxious inhibitory controls as a model of endogenous pain modulation in clinical pain syndromes. *J Pain*. 2010;11:408-419.
5. Ram KC, Eisenberg E, Haddad M, Pud D. Oral opioid use alters DNIC but not cold pain perception in patients with chronic pain – new perspective of opioid-induced hyperalgesia. *Pain*. 2008;139:431-438.
6. Bragdon EE, Light KC, Costello NL, et al. Group differences in pain modulation: Pain-free women compared to pain-free men and to women with TMD. *Pain*. 2002;96:227-237.
7. King CD, Wong F, Currie T, Mauderli AP, Fillingim RB, Riley JL 3rd. Deficiency in endogenous modulation of prolonged heat pain in patients with irritable bowel syndrome and temporomandibular disorder. *Pain*. 2009;143:172-178.
8. Maixner W, Fillingim R, Sigurdsson A, Kincaid S, Silva S. Sensitivity of patients with painful temporomandibular disorders to experimentally evoked pain: Evidence for altered temporal summation of pain. *Pain*. 1998;76:71-81.
9. Wilder-Smith CH, Robert-Yap J. Abnormal endogenous pain modulation and somatic and visceral hypersensitivity in female patients with irritable bowel syndrome. *World J Gastroenterol*. 2007;13:3699-3704.
10. Kosek E, Hansson P. Modulatory influence on somatosensory perception from vibration and heterotopic noxious conditioning stimulation (HNCS) in fibromyalgia patients and healthy subjects. *Pain*. 1997;70:41-51.
11. Lautenbacher S, Rollman GB. Possible deficiencies of pain modulation in fibromyalgia. *Clin J Pain*. 1997;13:189-196.
12. Staud R. Abnormal pain modulation in patients with spatially distributed chronic pain: Fibromyalgia. *Rheum Dis Clin North Am*. 2009;35:263-274.
13. Pielsticker A, Haag G, Zaudig M, Lautenbacher S. Impairment of pain inhibition in chronic tension type headache. *Pain*. 2005;118:215-223.
14. Sandrini G, Rossi P, Milanov I, Serrao M, Cecchini AP, Nappi G. Abnormal modulatory influence of diffuse noxious inhibitory controls in migraine and chronic tension-type headache patients. *Cephalalgia*. 2006;26:782-789.
15. de Tommaso M, Difruscolo O, Sardaro M, et al. Effects of remote cutaneous pain on trigeminal laser-evoked potentials in migraine patients. *J Headache Pain*. 2007;8:167-174.
16. Coppola G, Di Clemente L, Fumal A, et al. Inhibition of the nociceptive R2 blink reflex after supraorbital or index finger stimulation is normal in

migraine without aura between attacks. *Cephalalgia*. 2007;27:803-808.
17. Perrotta A, Serrao M, Sandrini G, et al. Sensitisation of spinal cord pain processing in medication overuse headache involves supraspinal pain control. *Cephalalgia*. 2010;30:272-284.
18. Headache Classification Subcommittee of the International Headache Society. The International Classification of Headache Disorders, 2nd edn. *Cephalalgia*. 2004;24:1-160.
19. Spielberger CD, Gorsuch RL, Lushene R. *The State-Trait Anxiety Inventory*. Palo Alto: Consulting Psychologists Press; 1969.
20. Teichman Y, Malineck F. *Manual for the Hebrew State-Trait Anxiety Inventory*. Tel-Aviv Israel: Tel-Aviv University Press; 1978.
21. Sullivan MJL, Bishop S, Pivic J. The Pain Catastrophizing Scale: Development and validation. *Psychol Assess*. 1995;7:524-532.
22. Granot M, Goldstein Ferber S. Prediction of postoperative pain intensity by assessment of Pain Catastrophizing Scale and anxiety. *Clin J Pain*. 2005;21: 439-445.
23. Filatova E, Latysheva N, Kurenkov A. Evidence of persistent central sensitization in chronic headaches: A multi-method study. *J Headache Pain*. 2008;9:295-300.
24. Fusco BM, Colantoni O, Giacovazzo M. Alteration of central excitation circuits in chronic headache and analgesic misuse. *Headache*. 1997;37:486-491.
25. Weissman-Fogel I, Sprecher E, Granovsky Y, Yarnitsky D. Repeated noxious stimulation of the skin enhances cutaneous pain perception of migraine patients in-between attacks: Clinical evidence for continuous sub-threshold increase in membrane excitability of central trigeminovascular neurons. *Pain*. 2003;104:693-700.
26. Racine M, Tousignant-Laflamme Y, Kloda LA, Dion D, Dupuis G, Choinière M. A systematic literature review of 10 years of research on sex/gender and experimental pain perception – part 1: Are there really differences between women and men? *Pain*. 2012a;153:602-618.
27. Racine M, Tousignant-Laflamme Y, Kloda LA, Dion D, Dupuis G, Choinière M. A systematic literature review of 10 years of research on sex/gender and pain perception – part 2: Do biopsychosocial factors alter pain sensitivity differently in women and men? *Pain*. 2012b;153:619-635.
28. Lipton RB, Stewart WF, Diamond S, Diamond ML, Reed M. Prevalence and burden of migraine in the United States: Data from the American Migraine Study II. *Headache*. 2001;41:646-657.
29. Bigal ME, Lipton RB, Stewart WF. The epidemiology and impact of migraine. *Curr Neurol Neurosci Rep*. 2004;4:98-104.
30. de Tommaso M, Lo Sito L, Di Fruscolo O, et al. Lack of habituation of nociceptive evoked responses and pain sensitivity during migraine attack. *Clin Neurophysiol*. 2005;116:1254-1264.
31. Brock C, Olesen SS, Valeriani M, Arendt-Nielsen L, Drewes AM. Brain activity in rectosigmoid pain: Unravelling conditioning pain modulatory pathways. *Clin Neurophysiol*. 2012;123:829-837.
32. Reidler JS, Mendonca ME, Santana MB, et al. Effects of motor cortex modulation and descending inhibitory systems on pain thresholds in healthy subjects. *J Pain*. 2012;13:450-458.
33. Sprenger C, Bingel U, Büchel C. Treating pain with pain: Supraspinal mechanisms of endogenous analgesia elicited by heterotopic noxious conditioning stimulation. *Pain*. 2011;152:428-439.
34. Lewis GN, Heales L, Rice DA, Rome K, McNair PJ. Reliability of the conditioned pain modulation paradigm to assess endogenous inhibitory pain pathways. *Pain Res Manag*. 2012;17:98-102.
35. Treister R, Eisenberg E, Gershon E, Haddad M, Pud D. Factors affecting – and relationships between – different modes of endogenous pain modulation in healthy volunteers. *Eur J Pain*. 2010;14:608-614.
36. Villemure C, Bushnell MC. Cognitive modulation of pain: How do attention and emotion influence pain processing? *Pain*. 2002;95:195-199.
37. Staud R, Robinson ME, Vierck CJ Jr, Price DD. Diffuse noxious inhibitory controls (DNIC) attenuate temporal summation of second pain in normal males but not in normal females or fibromyalgia patients. *Pain*. 2003;101:167-174.
38. de Tommaso M, Baumgartner U, Sardaro M, Difruscolo O, Serpino C, Treede RD. Effects of distraction versus spatial discrimination on laser-evoked potentials in migraine. *Headache*. 2008;48: 408-416.
39. Moont R, Crispel Y, Lev R, Pud D, Yarnitsky D. Temporal changes in cortical activation during distraction from pain: A comparative LORETA study with conditioned pain modulation. *Brain Res*. 2012; 1435:105-117.

40. Moont R, Pud D, Sprecher E, Sharvit G, Yarnitsky D. "Pain inhibits pain" mechanisms: Is pain modulation simply due to distraction? *Pain*. 2010;150:113-120.
41. Brighina F, Palermo A, Fierro B. Cortical inhibition and habituation to evoked potentials: Relevance for pathophysiology of migraine. *J Headache Pain*. 2009; 10:77-84.
42. Coppola G, Pierelli F, Schoenen J. Habituation and migraine. *Neurobiol Learn Mem*. 2009;92:249-259.
43. De Marinis M, Pujia A, Natale L, D'arcangelo E, Accornero N. Decreased habituation of the R2 component of the blink reflex in migraine patients. *Clin Neurophysiol*. 2003;114:889-893.
44. Smith BW, Tooley EM, Montague EQ, Robinson AE, Cosper CJ, Mullins PG. Habituation and sensitization to heat and cold pain in women with fibromyalgia and healthy controls. *Pain*. 2008;140: 420-428.
45. Hashmi JA, Davis KD. Effects of temperature on heat pain adaptation and habituation in men and women. *Pain*. 2010;151:737-743.
46. Hashmi JA, Davis KD. Women experience greater heat pain adaptation and habituation than men. *Pain*. 2009;145:350-357.
47. Lev R, Granovsky Y, Yarnitsky D. Orbitofrontal disinhibition of pain in migraine with aura: An interictal EEG-mapping study. *Cephalalgia*. 2010;30:910-918.
48. Parr JJ, Borsa PA, Fillingim RB, et al. Pain-related fear and catastrophizing predict pain intensity and disability independently using an induced muscle injury model. *J Pain*. 2012;13:370-378.
49. Weissman-Fogel I, Sprecher E, Pud D. Effects of catastrophizing on pain perception and pain modulation. *Exp Brain Res*. 2008;186:79-85.
50. Granot M, Weissman-Fogel I, Crispel Y, et al. Determinants of endogenous analgesia magnitude in a diffuse noxious inhibitory control (DNIC) paradigm: Do conditioning stimulus painfulness, gender and personality variables matter? *Pain*. 2008;136: 142-149.
51. Theunissen M, Peters ML, Bruce J, Gramke HF, Marcus MA. Preoperative anxiety and catastrophizing: A systematic review and meta-analysis of the association with chronic postsurgical pain. *Clin J Pain*. 2012;28:819-841.
52. van Wijk RM, Geurts JW, Lousberg R, et al. Psychological predictors of substantial pain reduction after minimally invasive radiofrequency and injection treatments for chronic low back pain. *Pain Med*. 2008;9:212-221.
53. Edwards RR, Cahalan C, Mensing G, Smith M, Haythornthwaite JA. Pain, catastrophizing, and depression in the rheumatic diseases. *Nat Rev Rheumatol*. 2011;7:216-224.
54. Nir RR, Granovsky Y, Yarnitsky D, Sprecher E, Granot M. A psychophysical study of endogenous analgesia: The role of the conditioning pain in the induction and magnitude of conditioned pain modulation. *Eur J Pain*. 2011;15:491-497.
55. Söderberg K, Sundström Poromaa I, Nyberg S, Bäckström T, Nordh E. Psychophysically determined thresholds for thermal perception and pain perception in healthy women across the menstrual cycle. *Clin J Pain*. 2006;22:610-616.
56. Tousignant-Laflamme Y, Marchand S. Excitatory and inhibitory pain mechanisms during the menstrual cycle in healthy women. *Pain*. 2009;146:47-55.
57. Klatzkin RR, Mechlin B, Girdler SS. Menstrual cycle phase does not influence gender differences in experimental pain sensitivity. *Eur J Pain*. 2010;14:77-82.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Correlations between CPM responses and clinical as well as personality characteristics in the migraine patients (corrected for multiple comparisons using the Bonferroni correction).

Table S2. Correlations between CPM responses and personality characteristics in healthy subjects (corrected for multiple comparisons using the Bonferroni correction).