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Research Submission

The Effects of Oral Contraceptives on Detection and Pain Thresholds As Well As Headache Intensity During Menstrual Cycle in Migraine

Michael Teepker, MD; Mira Peters, PhD; Bernd Kundermann, PhD; Helmut Vedder, MD; Karsten Schepelmann, MD; Stefan Lautenbacher, PhD

Background.—Clinically, oral contraceptives (OC) can influence pain in both migraine headache and temporomandibular pain disorders. Estrogen as an ingredient of OC might be a responsible factor for these observations. We conducted the present study to test whether OC are able to alter the severity of headache attacks as well as the detection or pain thresholds over the course of the menstrual cycle in patients with migraine.

Methods.—Thirteen healthy and regularly menstruating women and 26 migraineurs (13 using OC and 13 not using OC) were studied on the days 1, 4, 14, and 22 of their menstrual cycle. In all participants, saliva was collected first for determination of estrogen on each study day. Then, detection thresholds (warmth, cold, electrical current) and pain thresholds (cold, heat, pressure, electrical current) were assessed. Migraineurs were asked for headache attacks occurring in a period of 24 hours before testing and to estimate pain intensity on a verbal rating scale.

Results.—On day 4 of the menstrual cycle, migraineurs using OC suffered significantly more from severe migraine attacks than migraineurs not taking OC. With respect to detection and pain thresholds, no effects of OC could be observed as concerning the differences between migraineurs with or without OC medication. On day 22, the severity of migraine headache was significantly related with the pain thresholds for pressure and electrical current, suggesting paradoxically more severe headache attacks in patients presenting with higher pain thresholds. Healthy volunteers disclosed higher salivary estrogen levels than migraineurs and migraineurs not using OC higher concentrations than migraineurs using OC throughout the menstrual cycle.

Conclusions.—In this study, the use of OC intensified migraine (however only at the end of menstruation) however had no influence on detection and pain thresholds in migraineurs. Possible reasons for this dissociation will be discussed.

Key words: experimental pain, pain and detection threshold, menstrual cycle, migraine, headache intensity, oral contraceptive

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INTRODUCTION

Migraine is a trigemino-vascular headache disorder characterized by severe headache attacks with vegetative symptoms. Clinically, it can be divided into migraine with or without aura.

In spite of their pathophysiological and clinical differences, migraine[1,2] as well as temporomandibular pain disorders (TMD)[3] exhibit similarity by varying menstrually and by showing an onset after puberty with a higher prevalence for women than for men.[4-6]

From the Department of Neurology, Philipps-University of Marburg, Marburg, Germany (M. Teepker and K. Schepelmann); Department of Physiological Psychology, University of Bamberg, Bamberg, Germany (M. Peters and S. Lautenbacher); Department of Psychiatry and Psychotherapy, Philipps-University of Marburg, Marburg, Germany (M. Peters, B. Kundermann, and H. Vedder).

Address all correspondence to M. Teepker, Department of Neurology, Philipps-University Marburg, Rudolf-Bultmann-Str. 8, 35039 Marburg, Germany.

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Conflict of Interest: None.

Among women – including those suffering from migraine or TMD – oral contraceptives (OC) are commonly used. OC contain female reproductive hormones such as estrogens and are able to influence frequency and character of migraine attacks[7] and pain in TMD.[3,8] In migraineurs, attacks can both worsen or improve by the intake of OC.[7] In women with TMD, pain levels rated daily appeared to be more constant under OC medication than in women not using OC (NOC).[8] Conducting experimental pain assessment in TMD, the intake of OC was associated with higher pressure pain thresholds.[9] Bragdon et al[10] tested heat pain as well as ischemic arm pain and tolerance thresholds in women or men who were pain-free or suffered from TMD. In pain-free women using OC, thresholds for ischemic pain were significantly lower than in pain-free women without OC intake.

The evidence of OC effects on experimental pain in healthy subjects is contradictory and depends strongly on the type of physical stressor used. Aversion thresholds for electrical shock, for example, were higher in OC users than in subjects without OC intake.[11] In contrast to this, pressure pain and tactile thresholds of the temporalis and masseter muscle were lower under OC medication.[12] Veith et al[13] found no differences between OC users and OC-free subjects in paradigm with electric shock and cold pressor task. The same was true for the study of Hapidou et al[14] using pressure pain stimuli.

It is worth speculating what might be the mechanisms of the putative action of OC? OC include the female gonadal hormone estrogen, which is able to influence nociceptive pathways via a modulation of the endogenous opioid[15] or serotonin metabolism.[16] Serotonin especially plays a crucial role in excitatory and inhibitory nociceptive networks as well as within the development and relief of headache.[17] OC are able to enhance serotonin metabolism, for example, by the enzymatic metabolism by monoamine oxidase.[16] Regarding migraine, estrogens are able to display regulating effects as well: in cultured trigeminal neurons of female rats, estrogens influence gene expression and intracellular signaling known to be important in hormonal regulation of pain systems in migraine.[18] Furthermore, estrogen is able to release nitric oxide (NO),[19,20] which can trigger migraine.[21,22] NO release might be mediated in turn by serotonergic pathways (ie, the receptor subunit 5-HT_{2A}).[23] MacGregor et al[24] found that a perimenstrual estrogen withdrawal is able to trigger migraine attacks whereas rising estrogen levels are able protect against migraine headache. Further OC-dependent mechanisms potentially involved in the pathophysiology of migraine include the up-regulation of N-methyl-D-aspartate and the decrease of inhibitory activity of γ-aminobutyric acid-ergic neurons (for review see[17]) leading to cortical hyperexcitability suggested to be evident in migraine.[25]

In migraine, pain thresholds are decreased during headache attacks leading to cutaneous allodynia. Burstein et al proposed an explanation based around a central sensitization of trigeminal neurons.[26]

We hypothesized that the intake of OC alters migraine attacks clinically and affects experimental detection and pain thresholds via estrogen-dependent parts of the pain system. Therefore, we examined the detection threshold for warmth and cold as well as pain thresholds for heat, cold, pressure, and electrical pain stimuli in female migraineurs using OC (M-OC) and not taking OC (M-NOC) and compared their results with a control group of healthy and normally menstruating women. The examinations were conducted on days 1, 4, 14, and 22 to include different phases of the physiological menstrual cycle (ie, menstrual/follicular phase – ovulatory phase – luteal phase). Each patient was asked for migraine attacks in a period of 24 hours before testing to assess severity of migraine pain as well as its relationship with pain thresholds. In order to estimate the influence of migraine attacks on pain thresholds, we tested the strength of relationship by computing regression and co-variance (headache intensity as co-variate) analyses. Furthermore, we collected saliva in order to determine the concentrations of estrogen over the course of the menstrual cycle.

METHODS

Subjects.—Thirteen healthy and pain-free women (for the control group; mean age = 32.23 ± 7.40 years) as well as 26 female migraineurs (13 OC users; mean age = 28.15 ± 6.96 years) and 13 non-users; mean age = 34.77 ± 9.44 years) participated in this

Table 1.—Basic Characteristics of Migraineurs Without and With OC Intake

Patient Number	Diagnosis		Number of Migraine Attacks Per Month
Without OC intake			
#1	Migraine without aura	No menstrual association	1.2
#2	Migraine without aura	No menstrual association	2
#3	Migraine without aura	No menstrual association	0
#4	Migraine with aura	Menstrual associated migraine	3
#5	Migraine without aura	Menstrual migraine	1
#6	Migraine without aura	Menstrual associated migraine	1.78
#7	Migraine without aura	Menstrual associated migraine	1.33
#8	Migraine without aura	Menstrual associated migraine	1.45
#9	Migraine without aura	Menstrual migraine	1
#10	Migraine without aura	No menstrual association	1
#11	Migraine without aura	No menstrual association	1.6
#12	Migraine without aura	Menstrual migraine	1.33
#13	Migraine without aura	Menstrual associated migraine	0.57
With OC intake			
#1	Migraine with aura	No menstrual association	0.67
#2	Migraine without aura	Menstrual associated migraine	1.33
#3	Migraine without aura	No menstrual association	1.6
#4	Migraine without aura	Menstrual associated migraine	1.5
#5	Migraine with aura	No menstrual association	0.75
#6	Migraine with aura	No menstrual association	2.86
#7	Migraine with aura	No menstrual association	0.8
#8	Migraine without aura	No menstrual association	1.6
#9	Migraine without aura	Menstrual associated migraine	2
#10	Migraine without aura	Menstrual associated migraine	2
#11	Migraine without aura	No menstrual association	1.2
#12	Migraine without aura	No menstrual association	1.33
#13	Migraine with aura	No menstrual association	2

OC = oral contraceptives.

study. Groups were matched for age (analysis of variance [ANOVA] for age differences between groups: $F[2/36] = 2.26$; $P = .12$).

Participants were recruited via wall posters and advertisements in local newspapers. In cases of interest, the respective subjects were examined both by a headache-specialized neurologist and a psychologist. Diagnosis of migraine was conducted according to the guidelines of the International Headache Society.[27] The 13 OC migraineurs used a low-dose combination of estrogen and progesterone in a cycle of 21 or 22 days followed by 6 or 7 hormone-free days. Except for one subject taking a biphasic OC, all women received monophasic OC.

Menstrual cycles had to be regular (28 days plus/minus 1 day) for all participants. This was indicated by self reports and questionnaires in healthy volunteers. Migraineurs had to keep a headache diary for at least 6 weeks in order to indicate menstrual cycle, headache attacks, and pain intensity. The onset of the menstrual bleeding was defined as the first day of the menstrual cycle as documented by self reports and questionnaires (in the control group) or by headache diaries (in the migraine groups). Basic characteristics of migraineurs are demonstrated in Table 1. The exclusion criteria for all subjects were pregnancy, hypertension, acute and chronic pain other than migraine, endocrine disorders, gynecological diseases, psychiatric disorders, peripheral and central neuropathy as well as dermatosis at the site of the pain stimulation. In addition, healthy controls were not allowed to suffer from migraine. All women were not allowed to take drugs for the prophylactic treatment of pain syndromes

nor to consume any kind of analgesics regularly. The "Mini-DIPS" (the German version of the Interview of Mental Disorders short version handbook)[28] was used to screen for psychiatric disorders. All subjects were free of any analgesics and sedatives for at least the 24 hours preceding each test session. They were instructed to be well rested and not having exercised for at least 1 hour before investigation.

Subjects were paid for participation and gave written informed consent before participating in the study. The experimental protocol was approved by the ethics committee of the medical school of the University of Marburg.

General Procedure.—After the initial screening for inclusion and exclusion criteria, the experimental investigations (all separately run by 1 of 2 female investigators) took place in a sound-attenuated laboratory of the University of Marburg and lasted approximately 1.5 hours each. Time of day for investigation was kept constant for each single subject over the 4 sessions. The sessions were run on the days 1, 4, 14, and 22 (plus/minus 1 day) of the menstrual cycle according to the self-reported usual cycle phase and length. They started with saliva sampling (see "Collecting Saliva and Measuring Hormones" section) and evaluation of headache intensity in migraineurs (see "Evaluation of Headache Intensity" section). Thereafter, subjects were carefully familiarized with the sensory tests and tests were run.

Evaluation of Headache Intensity.—All participants – healthy volunteers as well as migraineurs – were asked for headache symptoms that might have occurred during the preceding 24 hours including the test session by use of a 4-point Likert scale, which consisted of the categories "0 = no headache," "1 = mild headache," "2 = moderate headache," and "3 = severe headache." All healthy subjects did not report headache.

Assessment of Detection and Pain Thresholds.—*Thermal Detection and Pain Thresholds.*—Cold and heat stimuli were applied using a computer-controlled thermal stimulator (TSA-2001, Medoc Ltd., Ramat Yishai, Israel) with a Peltier thermode (contact area: 6 cm^2). The thermode was attached to the left forearm.

DETECTION THRESHOLDS FOR WARMTH AND COLD.—Beginning at a temperature of 32°C, thermode temperature increased or decreased at a rate of 1°C/second until the subjects felt the first change in temperature and responded by pressing a button. Thereupon, the temperature returned with a rate of 1°C/second to baseline temperature (32°C) and was held constant until the next trial. Inter-stimulus intervals were at minimum 5 seconds. Data are expressed in relation to the baseline temperature of 32°C.

PAIN THRESHOLDS FOR HEAT AND COLD.—Temperature increased or decreased from a baseline of 32°C at a rate of 1.5°C/second until subject felt the stimulus to be slightly painful and responded by pressing a button. Thereupon, temperature returned with a rate of 2°C/second to baseline temperature. The inter-stimulus intervals were at minimum 10 seconds. For safety reasons, the upper limit of temperature was set to 52°C and the lower limit to 0°C. For those subjects who did not respond within the safety limits, the pain thresholds were set to the maximum values allowed, which was only necessary in case of cold pain thresholds.

Five trials for each threshold (warmth detection threshold, cold detection threshold, cold pain threshold, and heat pain threshold) were conducted and averages of these 5 trials were used as the measure of the 4 thermal thresholds.

Pressure Pain Threshold.—A pressure algometer (Somedic Sales AB, Hörby, Sweden) was used to assess responses to painful mechanical pressure stimuli, which were applied with a surface area of 1 cm^2 on the center of the left volar forearm. To avoid local sensitization, the probe was moved slightly after each trial. The pressure gauge displayed the stimulation rate as kilo Pascal per second [kPa/second] and the slope to be reached was defined as 10 kPa/second. The investigator increased the pressure constantly while monitoring the display until the subject felt the stimulus to be slightly painful and responded by pressing a button. Four trials were conducted and the average over the last 3 trials was used as a measure of pressure pain threshold.

Electrical Detection and Pain Thresholds.—An electro stimulator (Erich Jäger GmbH & Co. KG,

Würzburg, Germany) delivered the stimuli to the right volar forearm. The skin was cleaned and abraded. Each stimulus consisted of a train of 15 monophasic square wave pulses (pulse duration: 4 milliseconds) with a stimulus onset asynchrony of 10 milliseconds (resulting repetition rate within train: 100.0 Hz, resulting duration of each electrical stimulus: 144 milliseconds). Two bipolar electrodes with a surface area of 0.3 cm^2 covered with a special cream (Abralyt 2000, FMS Falk Minow Services, Herrsching-Breitbrunn, Germany) were attached 2 cm from each other slightly to the left and to the right of the center of the right volar forearm. For assessment of electrical detection and pain thresholds, stimulation intensity increased in 0.15 mA steps. For safety reasons, the intensity of stimulation was limited to 10 mA. Subjects indicated their sensations by rating each stimulus as leading to "no sensation," "not painful sensation," or "painful sensation." Detection threshold intensity was defined as the first current intensity leading to a rating of "not painful sensation," pain threshold intensity as first current intensity leading to a rating of "painful sensation." Detection and pain thresholds were assessed in 4 trials. The average over the last 3 trials was used as measures of detection and pain threshold.

Collecting Saliva and Measuring Hormones.— Salivary samples were collected each time on days 1, 4, 14, and 22 of the menstrual cycle in order to determine the course of levels of the physiological estrogen 17-β-estradiol. Subjects were instructed not to smoke, not to eat, and not to drink fruit juice 1 hour before collecting saliva. The volunteers had to chew a tasteless swab of cotton for 45 seconds, which were put thereupon into a plastic tube ("Salivette," Sarstedt, Nürnbrecht, Germany), frozen directly and stored at –20°C. For further analysis, the samples were thawed and centrifuged for 10 minutes at 3000 rpm to remove cellular debris. An ELISA assay (DRG Instruments GmbH, Marburg, Germany) was used to determine the hormonal concentrations following the producer's instructions. The intra-assay coefficient of variation was in the range of 2.59-6.92%, the inter-assay coefficient of variation in the range of 2.1-4.33%, and the lower detection level was 0.389 pg/mL (data from the user's manual; DRG Instruments GmbH).

There was one subject in each group of migraineurs (M-OC and M-NOC) who was not able to provide an adequate volume of saliva at all 4 sessions. Therefore, these women were excluded from further statistical evaluation of estrogen leading to a number of 12 subjects tested in each migraine group.

Statistics.—Descriptive data are given as mean ± standard deviation. Physical or chemical units were (pg/mL) for salivary estrogen concentration, (°C) for thermal thresholds, (kPa) for pressure pain thresholds, and (mA) for electrical thresholds. The primary outcomes measures of the study were somatosensory and pain thresholds as well as headache intensity, the secondary ones were salivary concentrations of estrogen. Differences in salivary concentration of estrogen, detection, and pain thresholds between the groups and over time were analyzed with an ANOVA for repeated measurements with one between-subject factor "group" (three-staged) and the four-staged within-subject factor "time point of testing" (ie "time") using SPSS 16.0 for Windows. Statistical significance was defined as $P < .05$. For fields of variables with multiple measures as somatosensory sensitivity (3 thresholds) and pain sensitivity (4 thresholds), we corrected the significance level according to the Bonferroni method by dividing the alpha level of 0.05 by the number of measures, resulting in corrected significance levels of $\alpha < 0.013$ (pain thresholds) and $\alpha < 0.017$ (detection thresholds), respectively. For assessment of headache intensity among migraineurs, an ANOVA with a 2-staged group factor was performed. Adjusting degrees of freedom with Greenhouse–Geisser correction was necessary when sphericity could not be observed. In case of significant results, *post hoc* tests (*t*-test) were used.

In order to determine whether concurrent headache during the day of measurement accounted for changes observed in the various pain thresholds, the measure of headache intensity (as assessed by the numerical rating scale) was used as co-variate. Because the co-variate itself varied over the menstrual cycle, a statistical approach taking this particularity into account was chosen. Linear regression analyses for each of the 4 time points of measurement were conducted for the subjects suffering from

migraine headaches, that is, the groups M-NOC and M-OC, with headache intensity as predictor for the various pain thresholds. In a second step, the resulting non-standardized residual scores were added to the corresponding mean of pain thresholds for each of the 4 times of measurement. These scores, which were now statistically freed from the influence of concurrent headache intensity, were finally entered in an ANOVA with the same factorial design as for the raw values. As stated, this approach was to be preferred to a classical analysis of co-variance because the co-variate "headache" also varied over time, leading to 4 different levels. Differences between the 2 ANOVAs (using raw values or residuals) would suggest critical impact of the headache pain on the pain thresholds.

RESULTS

Headache Intensity in Migraineurs.—Healthy volunteers did not report any headache bouts. Because there was no main effect of "group" in the analysis on only the 2 migraine groups as regards headache pain [F (1/24) < 0.001; $P = 1.0$], it appeared unlikely that there were general effects of OC on migraine pain. However, there was a significant interaction effect "time" × "group" [F (3/72) = 3.742, $P = .015$], which resulted from a significantly higher level of headache intensity in the M-OC group than in the M-NOC group on day 4 (*post hoc t*-test for independent samples: T = 2.162; df = 24, $P = .041$) (see Fig. 1). Additionally, a significant "time" effect was observed [F (3/72) = 3.717, $P = .015$]. Pain intensity was significantly different between day 1 vs day 14 (*t*-test: T = 2.301, df = 25, $P = .030$) and day 4 vs day 14 (T = 3.563, df = 25, $P = .02$) with the lowest scores on day 14.

Pain Thresholds.—There were no significant results regarding the between-factor "group" (this time including all 3 groups) for all pain thresholds tested [cold: F (2/36) = 0.056, $P = .945$; heat: F (2/36) = 0.312, $P = .734$; pressure: F (2/36) = 0.363, $P = .698$, electrical current: F (2/36) = 0.047, $P = .954$] (see Fig. 2). In other words, patients suffering from migraine with and without use of OC did not differ from each other as well as from healthy women as regards their pain thresholds.

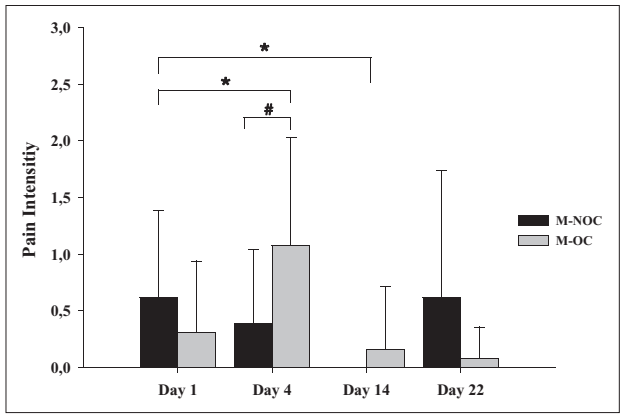

Fig 1.—Severity of migraine attacks (mean ± SD) on different days of menstrual cycle in (A) migraineurs without intake of OC (M-NOC) and (B) migraineurs with intake of OC (M-OC). Variations of headache intensity during menstrual cycle, as determined by ANOVA (main effect "time"), were statistically specified by *post hoc* paired *t*-tests (with pooled data) to detect differences between certain times of measurements and indicate by "*" in case of statistical significance. Furthermore, group differences at a certain time of measurement were evaluated with *post hoc t*-tests for independent samples and marked – if statistically significant (ie, $P < .05$) – by "#". Note that all patients of the M-NOC group reported zero headache on day 14.

Significant main effects for "time" were found for cold (see Fig. 2B) [F (2.467/88.806) = 5.273, $P = .004$; *t*-test: day 1 vs day 4: T = 2.802, df = 38, $P = .008$; day 1 vs day 14: T = 3.402, df = 38, $P = .002$; day 1 vs day 22: T = 2.408, df = 38, $P = .021$] and electrical pain thresholds (see Fig. 2D) [F (2.417/87.003) = 10.394, $P < .001$, *t*-test: day 1 vs day 4: T = –3.783, df = 38, $P = .001$; day 1 vs day 14: T = –4.280, df = 38, $P < .001$; day 1 vs day 22: T = –5.003, df = 38, $P < .001$] whereas no significance was evident for heat (see Fig. 2A) [F (3/108) = 0.794, $P = .500$] or pressure pain thresholds (see Fig. 2C) [F (1.988/71.581) = 0.832, $P = .439$]. Regarding the interaction effects between the factors "time" and "group" on pain thresholds, level of significance was never reached [cold: F (4.934/88.806) = 0.856, $P = .514$; heat: F (6/108) = 1.495, $P = .187$; pressure: F (3.977/71.581) = 2.350, $P = .063$, electrical current: F (4.834/87.003) = 1.066, $P = .384$].

Detection Thresholds.—No main effects could be observed regarding the between-subject factor "group" for the thresholds for warmth [F (2/36) = 0.938, $P = .401$], cold [F (2/36) = 0.198, $P = .821$], and electrical current [F (2/36) = 1.851, $P = .172$] (see

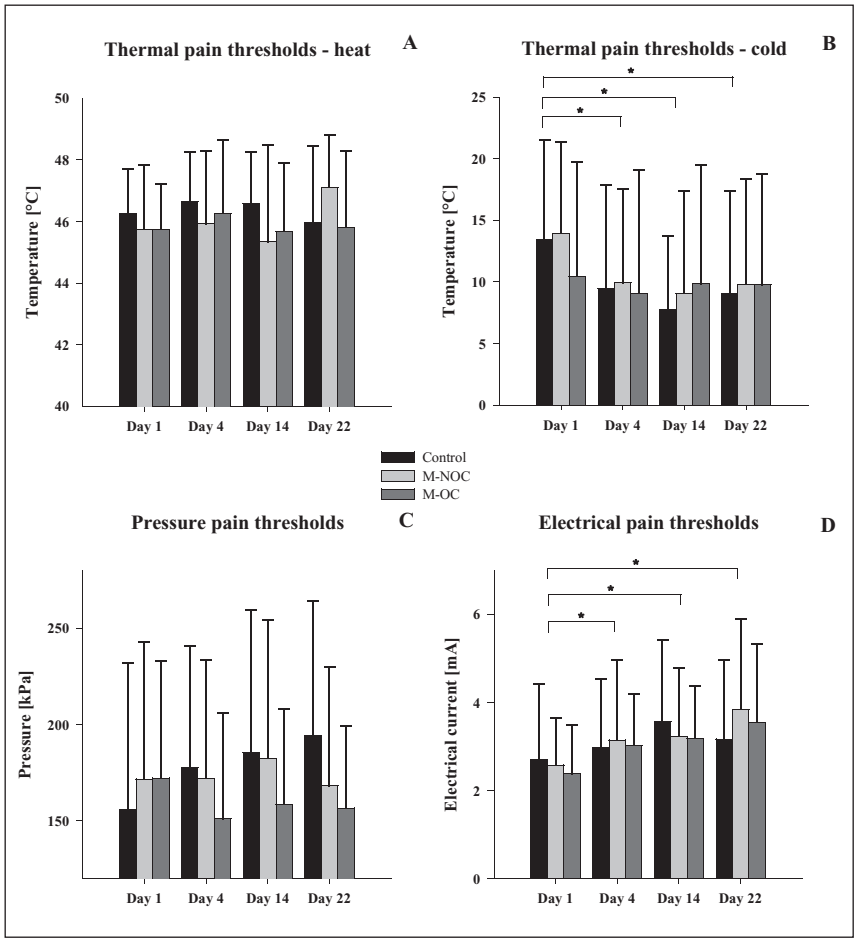

Fig 2.—(A-D) Pain thresholds (mean ± SD) during menstrual cycle from day 1 to day 22 in healthy controls, migraineurs using OC (M-OC) and not using OC (M-NOC): (A) heat, (B) cold and (C) pressure pain, and (D) electrical pain. Decreased heat pain thresholds are indicated by lowered temperature values whereas decreasing cold pain thresholds are indicated by elevated temperature values. Variations of pain thresholds during menstrual cycle, as determined by ANOVA (main effect "time"), were statistically specified by *post hoc t*-tests to detect differences between certain times of measurements. Statistical significance is denoted by "*" = $P < .05$.

Fig. 3). This suggests that healthy control subjects and patients suffering from migraine, irrespectively of the intake of OC, had similar detection thresholds.

Electrical detection thresholds (see Fig. 3C) displayed no significance regarding the factor "time" [F (2.488/89.585) = 1.952, $P = .137$] and the interaction of "time" and "group" [F (4.977/89.585) = 1.204, $P = .314$]. Although near to significant, the same was true for cold detection thresholds (see Fig. 3B) [factor "time": F (3/108) = 2.632, $P = .054$; interaction "time" and "group": F (6/108) = 0.591, $P = .737$]. The detection thresholds for warmth (see Fig. 3A) revealed a significant main effect of time [F (3/108) = 3.916, $P = .011$], with significant differences between day 1 and day 4 (*t*-test: T = –2.472, df = 38, $P = .018$) as well as day 1 and day 22 (*t*-test: T = –2.806, df = 38, $P = .008$) with the lowest levels on day 1. However, there were no significant results regarding the interaction of "time" and "group" [F (6/108) = 0.884, $P = .509$].

Correlation Between Pain Thresholds and Headache Intensity (Regression and Co-Variance Analyses).—Among the 2 migraine groups, linear regression analyses were performed to examine the relationship between headache intensity and the various pain thresholds. As shown in Table 2, the contribution of the headache intensity (as predictor variable) explaining variance in pain thresholds (as the

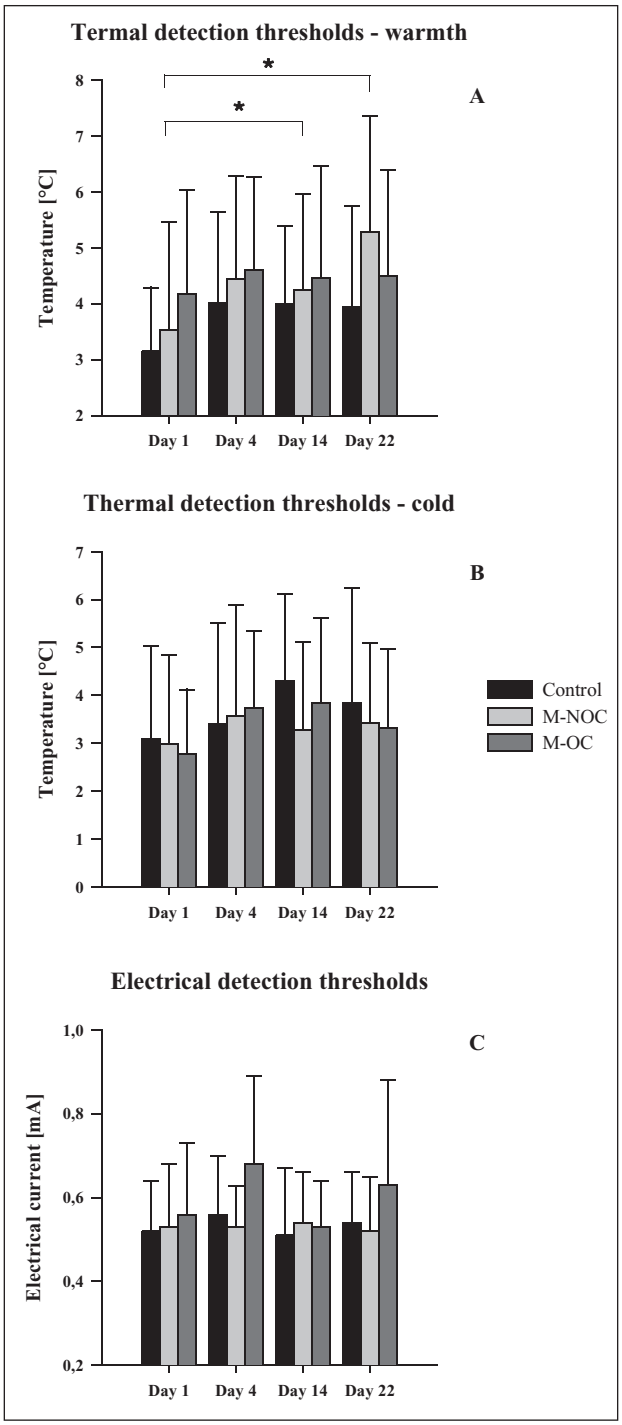

Fig 3.—(A-C) Detection thresholds (mean ± SD) during menstrual cycle from day 1 to day 22 in healthy controls, migraineurs using OC (M-OC) and not using OC (M-NOC): (A) warmth, (B) cold, and (C) electrical pain. Data in case of thermal thresholds (ie, warmth and cold) are expressed in relation to the baseline temperature of 32°C. Variations of detection thresholds during menstrual cycle, as determined by ANOVA (main effect "time"), were statistically specified by *post hoc t*-tests to detect differences between certain times of measurements. Statistical significance is denoted by "*" = $P < .05$.

criterion variables) was consistently small and insignificant, with the exception that headache intensity predicted significantly the pressure and electrical pain thresholds on day 22. Surprisingly as regards direction of relationship, high pain intensity predicted high pain thresholds. However, even for these 2 thresholds, the ANOVA with corrected residual values of regression analysis (ie, statistically freed from the influence of headache intensity), yielded a comparable result pattern as reported above for the raw values (chapter B). This suggests that the time courses of headache intensity and pain thresholds over the menstrual cycle were largely unrelated.

Time Course of 17-β-Estradiol.—ANOVA revealed a significant main effect of "group" [$F(2/34) = 30.157$; $P < .001$]. *Post hoc t*-tests indicated that the control group showed higher estrogen levels than migraineurs ($T = 4.622$, $df = 23$, $P < .001$) and – among the migraine group – subjects without intake of OC (M-NOC) had higher concentrations than those using OC (M-OC) ($T = 3.155$, $df = 22$, $P = .050$) (see Fig. 4).

Salivary estrogen displayed cyclic variations over the time points tested as suggested by a significant main effect of the factor "time" [$F(1.744/59.306) = 3.296$, $P = .050$]. In particular, there were statistical significant differences in salivary concentrations of estrogen on day 1 vs day 14 (*t*-test: $T = 2.166$, $df = 36$, $P = .037$ with higher concentrations on day 1), day 4 vs day 22 (*t*-test: $T = –2.473$, $df = 36$, $P = .018$ presenting higher levels on day 22), and day 14 vs day 22 (*t*-test: $T = –2.418$, $df = 36$, $P = .021$ indicating higher levels on day 22).

No interactions between "time" and "group" could be observed [$F(3.489/59.306) = 1.552$, $P = .205$].

DISCUSSION

This is the first approach to investigate the influence of OC on migraine headache and various somatosensory detection and pain thresholds (warmth, cold as well as heat, cold, pressure, and electrical

Table 2.—Results of Linear Regressions Analyses (R^2, *P* value) With Pain Thresholds (as the Criterion Variable) and Headache Intensity (as the Predictor Variable)

	Thermal Pain Thresholds – Heat		Thermal Pain Thresholds – Cold		Pressure Pain Thresholds		Electrical Pain Thresholds	
Time Point	R^2	*P*	R^2	*P*	R^2	*P*	R^2	*P*
Day 1	0.033	.371	0.001	.876	0.000	.945	0.024	.448
Day 4	0.067	.203	0.000	.935	0.004	.758	0.020	.494
Day 14	0.019	.505	0.012	.588	0.004	.754	0.078	.167
Day 22	0.050	.275	0.001	.855	0.293	.004	0.257	.008

stimuli) during different phases of menstrual cycle. Healthy volunteers not using OC were included into the control group. There was a significant difference on day 4 with regard to pain intensity of migraine, indicating that M-OC had more severe attacks. There were no significant differences between the migraine groups with and without use of OC as well as between migraineurs and healthy controls in detection and pain thresholds. Accordingly, OC use seems to affect migraine pain without general effects on pain processing.

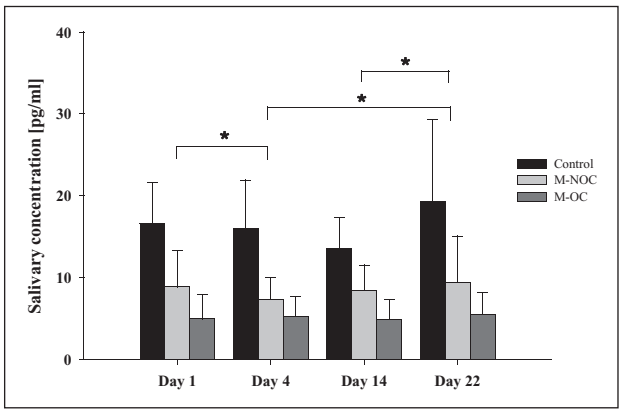

Fig 4.—Course of salivary estrogen (mean ± SD) on days 1, 4, 14, and 22 of menstrual cycle in the 3 groups tested (healthy controls, migraineurs not using OC [M-NOC], and migraineurs using OC [M-OC]). Variations of salivary estrogen during menstrual cycle, as determined by ANOVA (main effect "time"), were statistically specified by *post hoc t*-tests to detect differences between certain times of measurements for all 3 groups combined. Statistical significance is denoted by "*" = $P < .05$. Further statistical evaluation regarding the between-subject factor "group" revealed higher estrogen concentrations of the control group and – among the migraine patients – higher levels for M-NOC (results of corresponding *post hoc t*-tests for between-group differences are not marked in the diagram).

Only detection thresholds for warmth as well as pain thresholds for cold and electrical current showed cyclic variations in general, with an increased sensitivity during menstruation in all three cases. Detection thresholds for cold and electrical current as well as pain thresholds for heat and pressure did not present with similar variations. Furthermore, migraine headache was significantly linked with the pain thresholds for pressure and electrical current on day 22, suggesting that more severe migraine bouts were associated with higher thresholds at this time.

Salivary estrogen levels were higher in the control than in the migraine group. Additionally, migraineurs without intake of OC had higher estrogen levels than those using OC. The general course of the estrogen levels with decreasing levels from menstruation to mid cycle and further increases to the luteal phase could reproduced in the present study. Our findings will now be discussed in detail.

Migraineurs using OC showed more severe headache attacks on day 4 of menstrual cycle. These data accord with the findings of LeResche et al[3] for TMD: Investigating the influence of OC in TMD patients, they found highest TMD ratings during menstruation. Interestingly, OC did not affect detection or pain thresholds in our study on day 4. At this time, headache intensity was not related to pain thresholds because the linear regression analysis revealed a positive correlation between headache intensity and

mechanical as well as electrical thresholds only on day 22. Thus, OC seem to increase intensity of headache attacks in migraine during the menstrual phase via pathways that do not affect pain thresholds.

Our findings in terms of missing OC effects on detection or pain thresholds agree with previous works on healthy subjects using cold pressor test[13] or pressure pain stimuli,[14] respectively. In contrast to this, lower pressure pain and tactile thresholds of the temporalis and masseter muscle were reported by Drobek et al[12] for healthy women under OC medication. This was explained by a release of NO by exogenous estrogen in OC. Dao and colleagues[8] studied the effects of OC under a more clinical point of view in patients with myofascial pain, a related condition to TMD. They could demonstrate more stable daily pain ratings of OC users and that – although not significant – the intake of OC tended to be associated with elevated pain ratings. The authors hypothesized NO-dependent mechanisms responsible for these findings. This putative positive correlation between OC intake and increase in pain via NO-dependent factors would concur with our results showing more intense headache attacks in OC users on day 4 of menstrual cycle. NO plays a key role in migraine pathophysiology, too, as it can be released from blood vessels, perivascular nerve endings or brain tissue and trigger migraine attacks.[21,22] Estrogens are able to induce a release of NO.[19,20] Our finding that exogenous estrogens in terms of OC altered headache pain intensity but not detection and pain thresholds of migraineurs might suggest that NO affects in migraineurs nociceptive pathways for headache generation but not for pain processing in general.

Summarizing the results of earlier studies and our present experiment, investigating the effects of OC on both clinical and experimental pain in healthy subjects and patients suffering from chronic pain, one has to conclude that they are contradictory and seem to depend on further parameters, such as type of subjects tested (eg healthy volunteers or patients suffering from TMD) or type of pain inducing techniques.[8,9,11-14,29] By intake of OC, female hormones such as estrogens and progesterone are applied. Both hormones are able to influence nociception in animal studies[30] but little is known about their interactive effects. Therefore, the influence of OC on nociceptive processes may depend not only on estrogen but also on progesterone, which may result into complex and non-linear relationships.

Cephalic and extra-cephalic changes of pain thresholds (ie, hyperalgesia or allodynia) are evident in various headache disorders and extra-cephalic ones were reported particularly for severe headaches forms such as migraine or tension-type headache (for review see[31]). Sensitization of meningeal or trigeminal neurons was hypothesized to be present in migraine. As possible mechanisms for extra-cephalic hyperalgesia, hyperexcitability developing along trigeminal pathways or a temporal deficiency of pain-inhibiting systems was discussed by Burstein et al.[26] They described normal thresholds in the absence of migraine headache and lower ones during migraine attacks accompanied by allodynia in both cephalic and extra-cephalic sites.[32] Lower pain thresholds were also found in patients suffering from chronic migraine by quantitative sensory testing and Semmes-Weinstein monofilaments.[33] In this study, we have investigated detection and pain thresholds in an extra-cephalic location on the forearm and our results displayed no differences between migraineurs and the control group of healthy volunteers. In line with this, Domingues et al reported no differences between controls and migraineurs as well as between migraine with or without aura using the cold pressure test.[34] We found no differences within the migraine group between M-OC and M-NOC although M-OC showed significant higher clinical headache intensity scores on day 4 than M-NOC. Our data might denote that the proposed central sensitization of trigeminal neurons is not evident outside migraine attacks and is further influenced neither menstrually nor by exogenous estrogens. As extra-cephalic hypersensitivity occurs predominantly in severe headache forms,[31] it might be that central sensitization was too weak in our migraine population.

In our study, detection thresholds for warmth as well as pain thresholds for cold and electrical stimuli showed cyclic fluctuations with increasing thresholds. This accords with the meta-analysis by Sherman and LeResche,[29] indicating that cyclic changes depend on the respective pain-inducing stimulus. The fact that

cyclic fluctuations of thresholds were evident in both controls and migraine subject might indicate that cyclic variation depends more on the respective pain-inducing technique than on the intake of OC or the existence of migraine.[35]

Collecting saliva is less unpleasant and stressful than venipuncture. This approach is therefore less likely to produce stress analgesia and to interact, by that, with the results of experimental pain tests. Studies have demonstrated saliva techniques to be reliable for assessment of steroid hormones.[36] In our study, salivary estrogen levels displayed the lowest concentrations for M-OC and this group reported significant more intense headache attacks on day 4 of menstrual cycle. Because OC can inhibit ovulation it is possible that significantly low concentrations of salivary estrogen might occur in M-OC. Our results agree with the work of McGregor et al,[24] showing that low plasma levels of estrogens in the menstrual phase may lead to migraine attacks whereas higher concentrations provide protection against migraine headache. Interestingly, salivary concentrations of estrogen were higher in the control group than in migraineurs. This is in contrast to the findings of Epstein et al[37] describing elevated levels of estrogen and progesterone in migraineurs, especially in the late luteal phase. However, they determined hormonal concentrations in plasma whereas we provide the first data on salivary concentrations of estrogen in migraineurs. Further tests are necessary to confirm our results.

There are some limitations to our study. The experimental protocol and the recruitment of participants were very time- and effort-consuming, making it impossible to investigate more cycles and more subjects. However, other studies are even smaller, eg, the study by Dao et al, who included 12 female myofascial pain patients.[8] Menstrual cycle can vary inter-individually although it occurs individually regularly. By using self report or diaries of menstrual cycle without further validation (eg, by hormonal assessment), it might be that women differed regarding the exact phase of menstrual cycle or that some were anovulatory. We did not include healthy volunteers using OC as a further control group, but in their diary study LeResche and coworkers did not either.[3] There are miscellaneous OC products with a different composition of female hormones. As some women do not tolerate all of these OC, we were not able to standardize the OC medication. Both cephalic and extra-cephalic changes of pain thresholds are evident in migraine – as discussed above – which may reflect regional and generalized alterations of the nociceptive system. Thus, assessing pain thresholds at the extremities in this study reflected the generalized alterations and may have missed the regional ones.

Taken together, we could show that OC users had more intense migraine headache at the end of the menstrual phase, which was not associated with altered detection and pain thresholds. We failed to demonstrate a general influence of OC on various detection and pain thresholds. There were menstrual variations in some of the detection and pain thresholds with no differences between groups. Migraine attacks were associated with higher thresholds for pressure as well as electrical pain on day 22 of menstrual cycle.

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STATEMENT OF AUTHORSHIP

Category 1

(a) Conception and Design
Michael Teepker, Mira Peters, Bernd Kundermann, Helmut Vedder, Karsten Schepelmann, Stefan Lautenbacher

(b) Acquisition of Data
Michael Teepker, Mira Peters, Bernd Kundermann, Helmut Vedder, Karsten Schepelmann, Stefan Lautenbacher

(c) Analysis and Interpretation of Data
Michael Teepker, Mira Peters, Bernd Kundermann, Helmut Vedder, Karsten Schepelmann, Stefan Lautenbacher

Category 2

(a) Drafting the Manuscript
Michael Teepker, Mira Peters, Bernd Kundermann, Helmut Vedder, Karsten Schepelmann, Stefan Lautenbacher

(b) Revising It for Intellectual Content
Michael Teepker, Mira Peters, Bernd Kundermann, Helmut Vedder, Karsten Schepelmann, Stefan Lautenbacher

Category 3

(a) Final Approval of the Completed Manuscript
Michael Teepker, Mira Peters, Bernd Kundermann, Helmut Vedder, Karsten Schepelmann, Stefan Lautenbacher

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