



Trait- and Frequency-Dependent Dysfunctional Habituation to Trigeminal Nociceptive Stimulation in Trigeminal Autonomic Cephalalgias

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Abstract: We investigated whether the stimulation frequency (SF), the pain phases, and different diagnoses of trigeminal autonomic cephalalgias (TACs) may influence the habituation to pain. We studied the habituation of the nociceptive blink reflex R2 responses at different SFs (.05, .1, .2, .3, .5, and 1 Hz), in 28 episodic cluster headache (ECH) patients, 16 during and 12 outside the bout; they were compared with 16 episodic paroxysmal hemicrania (EPH) during the bout and 21 healthy subjects. We delivered 26 electrical stimuli and subdivided stimuli 2 to 26 in 5 blocks of 5 responses for each SF. Habituation values for each SF were expressed as the percentages of the mean area value of second through fifth blocks with respect to the first one. A significant lower mean percentage decrease of the R2 area across all blocks was found at .2 to 1 Hz SF during ECH, outside of the ECH, and EPH compared with healthy subjects. We showed a common frequency-dependent deficit of habituation of trigeminal nociceptive responses at higher SFs in ECH and EPH patients, independently from the disease phase. This abnormal temporal pattern of pain processing may suggest a trait-dependent dysfunction of some underlying pain-related subcortical structures, rather than a state-dependent functional abnormality due to the recurrence of the headache attacks during the active period.

Perspective: TACs showed a frequency-related defective habituation of nociceptive trigeminal responses at the higher SFs, irrespectively of the diagnosis and/or the disease phase. We showed that the clinical similarities in the different subtypes of TACs are in parallel with a trait-dependent dysfunction in pain processing.

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Key words: Cluster headache, paroxysmal hemicrania, nociceptive blink reflex, habituation.

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Cluster headache (CH) and paroxysmal hemicrania (PH) are rare and highly disabling primary headaches, characterized by remitting attacks of strictly unilateral severe headache pain occurring in association with ipsilateral cranial parasympathetic autonomic signs or symptoms, including conjunctival injection, lacrimation, nasal congestion, and rhinorrhea, among others. CH and PH are both grouped as trigeminal autonomic

cephalalgias (TACs).¹³ They are clinically differentiated from each other in view of the duration (lasting 30–180 minutes in CH and 2–30 minutes in PH) and frequency of the pain attacks (occurring from once every other day to 8 times a day in CH; occurring several or many times a day in PH),¹³ of the response to preventive treatment, and of the epidemiological features. Although the precise brain structures anatomically and functionally responsible for these primary headache syndromes are still debated, neuroimaging, neurochemical, and neurophysiological data suggest a major role for the posterior hypothalamus and other brain areas anchored to the so-called “pain neuromatrix,” and for the central descending pain control systems in predisposition and recurrence of attacks of TACs.^{16,19,26} However, whatever the pathogenetic mechanisms of TACs, these should encompass the involvement of the trigeminal system. Indeed, abnormal functional activity of the trigeminal nociceptive system was several times disclosed using blink reflex (BR), a surrogate marker of the trigeminal nucleus caudalis functional integrity (see Coppola et al⁶ for a review). In the episodic form of CH (ECH), a deficit of BR habituation has been described during the active phase for conventional²⁷—stimulating tactile as well as nociceptive fibers—and nociceptive⁵—preferentially stimulating facial cutaneous nociceptive A δ fibers—trigeminal responses, with 1 notable exception.¹⁴ The clinical features and the hypothesized pathogenetic mechanisms of CH and PH partially overlap, thus it could be relevant to analyze and compare the activity of the nociceptive trigeminal system, by means of nociceptive BR (nBR) recording, in ECH subjects during and outside the bouts with that of episodic PH (EPH) subjects. Moreover, considering that habituation to nociceptive stimuli involves short- and long-lasting modification of synaptic transmission^{17,35} as well as segmental and suprasegmental pain control pathways,³ and that the frequency of a sequence of individual stimulation may deeply influence habituation, it would be of major interest to investigate the short- and long-term trigeminal mechanisms of pain processing by evaluating habituation of the nBR at varying stimulation frequencies (SFs) in ECH during the active as well as remission periods, and EPH during the active phase (outside the attacks), compared with healthy subjects (HS). The aims of this study were thus to investigate: 1) whether any differences of trigeminal nociceptive responses may be found in 2 different subtypes of TACs (ECH and EPH) during the active phase of the disease, 2) whether trigeminal nociceptive pathways may react differently to nociceptive stimulation in CH during and out of the bout, 3) whether different frequencies of nociceptive stimulation may elicit differences in the habituation to pain in TACs, and 4) whether any correlation may be found between habituation to nociceptive stimuli in the trigeminal pathways and clinical features in TACs patients. Considering the similarities in the clinical presentation of TACs, we reasoned that, compared with HS, CH and PH patients would show a common trait of trigeminal short- as well as long-term abnormal pain processing that would further depend on the clinical recurrence of the attacks.

Methods

The study was approved by the Neurimed Institute Ethics Committee and was carried out following the guidelines for proper human research conduct in accordance with the Declaration of Helsinki of the World Medical Association and its revisions. All of the participants gave their written consent and were informed that they could withdraw from the experiments at any time.

Study Population

According to the International Classification of Headache Disorders (ICHD) Third Edition (Beta Version),¹³ 28 subjects diagnosed as suffering from ECH (3.1.1, ICHD) and 16 from EPH (3.2.1, ICHD) were recruited among those seeking treatment at the Headache Clinic of the IRCCS Neuromed Institute, Pozzilli, Isernia, Italy, between January 2014 and February 2016, and were enrolled in a case-control study. In its episodic forms, CH and PH are characterized by the occurrence of series of attacks lasting for weeks or months (bouts), separated by remission periods lasting at least 1 month.¹³ All subjects with ECH and EPH experienced strictly unilateral pain and no side shift of headache attacks were reported in the past clinical history. Multiple diagnoses were not allowed. Patients were compared with 21 HS, without personal or family (first- or second-degree relatives) history of primary headaches or aura-like symptoms, recruited between university and hospital employees not directly involved in our own department. The recruitment of the HS had been performed by individuals not actively involved in the study by using a preliminary questionnaire. After the acceptance (26 subjects) they were enrolled using the described inclusion/exclusion criteria and were studied in parallel with the ECH and EPH groups.

For all participants exclusion criteria included secondary headaches, neurological disorders or clinical history (including family history) of neurological disorders, any systemic or psychiatric disorder, Beck Depression Inventory scale score >9, current use of antidepressant or antiepileptic medications (in the previous 2 months) or analgesics (in the previous 24 hours); clinical or instrumental evidence of any central or peripheral disease potentially causing sensory impairment; fibromyalgia, neuropathic pain, complex regional pain syndrome, chronic low back pain, or other pain conditions, according to current guidelines. No patient had been taking prophylactic medication (including indomethacin) for headache in the previous 7 to 15 days, and all were self-medicated with triptans and/or oxygen therapy as needed.

The ECH subjects were recorded during active (outside the attack; ECH-in) and remission (ECH-out) phase. EPH subjects were recorded during the active phase (outside the attack). ECH and EPH were considered to be in an active period when severe typical attacks had been occurring daily or almost daily for at least 10 days. ECH subjects were considered to be in a remission phase when no attacks had occurred for at least 2 months after the prophylactic treatment had been interrupted or a spontaneous remission occurred. ECH-in and ECH-out were different subjects.

Table 1. Demographic and Clinical Characteristics of the Study Population Expressed as Mean Values \pm SD or Frequency (%)

	HS	ECH-IN	ECH-OUT	EPH-IN	ONE-WAY ANOVA
n	21	16	12	16	
Male sex	10	16	10	8	
Age, years	44.6 \pm 6.8	38.7 \pm 11.4*	40.9 \pm 10.1*	51.1 \pm 8.9	F _{3,61} = 5.387, P = .002
Mean age at onset, years		27.2 \pm 10.5	29.2 \pm 10.9	34.3 \pm 14.2	F _{2,47} = .705, P = .794
Duration of disease, years		11.4 \pm 9.2	11.8 \pm 7.7	16.5 \pm 13.4	F _{2,47} = .982, P = .509
Attack frequency, days		1.9 \pm 1.1	2.0 \pm .8	5.9 \pm 2.9	P = .631†
Attack duration, minutes		61.2 \pm 38.3	91.4 \pm 42.6	22.0 \pm 7.3	P = .722†
Cluster period duration, weeks		6.0 \pm 4.0	8.3 \pm 5.8		P = .185†
Cluster periods per year		1.6 \pm .9	1.6 \pm .7		P = .769†
Episodic phase duration, weeks				15.6 \pm 12.4	
Primary chronic PH, %				50 (8)	
Laterality, %		16 (100)	12 (100)	14 (87.5)	
Right-side attacks, %		12 (75)	8 (66.6)	12 (75)	
Left-side attacks, %		4 (25)	4 (33.3)	2 (12.5)	
Alternating side attacks, %		0	0	2 (12.5)	
Autonomic symptoms, %		16 (100.0)	12 (100.0)	15 (93.7)	
Tearing, %		13 (81.2)	10 (83.3)	15 (93.7)	
Conjunctival injection, %		11 (68.7)	7 (58.3)	8 (50.0)	
Nasal congestion, %		13 (81.2)	8 (66.6)	9 (56.2)	
Rhinorrhea, %		7 (43.7)	50 (6.0)	7 (43.7)	
Eyelid edema, %		1 (6.2)	3 (25.0)	0 (0)	
Ptosis, %		1 (6.2)	1 (8.3)	0 (0)	
Hyperhidrosis, %		3 (18.7)	1 (8.3)	0 (0)	
Smokers, %		13 (81.2)	6 (50.0)	0 (0)	

Abbreviation: EPH-in, EPHs during the active phase.

*P < .05 ECH groups versus EPH-in in Bonferroni post hoc comparisons.

†ECH-in versus ECH-out unpaired t-test.

Clinical data of the participants are summarized in Table 1.

Neurophysiological Measurements

BR Measurement

The subjects were comfortably settled in an armchair in a quiet, temperature controlled room and were asked to sit back and relax, keeping their eyes open.

The nBR was elicited by a planar concentric electrode (Bionen, Florence, Italy). The stimuli (monopolar square-wave pulse with a duration of .3 ms delivered by a constant current stimulator—electric stimulator DS7A, Digitimer, Welwyn Garden City, United Kingdom) were applied 10 mm above the emergence of the supraorbital nerve on the usual headache side in all subjects and always on the right side in HS.

Electromyographic signals were recorded from both orbicularis oculi muscles via a standard pair of Ag/AgCl surface electrodes placed on the midline of the lower eyelid. Because a clear-cut lack of habituation was observed from the affected side, but not from nonaffected side, in previous BR studies in ECH,⁵ in the subsequent offline analysis, only the R2 reflex responses obtained from the ipsilateral affected side has been considered. The position of the reference electrodes is lateral to the eye. The ground electrode was placed on the subject's forehead. The filter bandpass settings were between 3 Hz and 3 kHz, with a sampling rate of 2.5 kHz. The analy-

sis time was 200 ms (CED Power interface 1401, Cambridge Electronic Design, Cambridge, United Kingdom; electronic amplifier BM623, Biomedica Mangoni, Pisa, Italy; electric stimulator DS7A, Digitimer).

In each participant, the sensory threshold (ST) was determined on the basis of a sequence of stimuli of increasing intensity (increased in .1 mA steps) delivered at pseudorandom intervals (\pm 10 seconds). Subjects were asked to indicate verbally the stimulation levels at which they became aware of sensory sensations. The staircase method was used to evaluate the reflex threshold (RT) for the R2 component of the BR by raising the stimulus intensity (in .1-mA steps) until a stable reflex response. The subjective pain sensation elicited by supraorbital nerve stimulation at RT was graded on an 11-item numeric rating scale for pain (0 = no pain; 10 = severe pain). For the RT assessment, to avoid R2 response habituation, the stimuli were delivered at pseudorandom frequencies between .033 and .025 Hz.²⁷

The stimulation intensity was then fixed at 1.5 times the RT to ensure an affordable persistence/reproducibility of the reflex response. The latency (L), visually determined as the take-off point from the baseline, and area under the curve (AUC) of the R2 component were automatically measured and expressed in milliseconds and microvolts \times seconds, respectively. For each component, the time window to calculate the AUC was defined according to the measurable latencies of the best defined template, at the beginning as well as at the end of the component, and was then kept constant in each subject.

For the L and AUC basal assessment at least 3 to 5 successful responses were recorded and averaged in all participants.

Habituation

To evaluate the habituation phenomenon of the R2 component of the nBR, a series of electrical stimuli delivered at different SFs (.05, .1, .2, .3, .5, and 1 Hz) were used. The stimulus intensity was set at 1.5 times the R2 RT. A sequence of 26 consecutive rectified electromyographic responses was recorded for each randomly chosen SF. The duration of each stimulus sequence varied from 8.66 minutes (.05 Hz) to 26 seconds (1 Hz), to respectively assess long- and short-term mechanisms of neural plasticity in response to pain. The first sweep of each sequence of responses was excluded from further analysis to avoid contamination with a startle response. In offline analysis, the sequence of responses for each SF was subdivided into 5 blocks of 5 and the R2 AUC values were calculated and averaged for each block of responses. The mean AUC values of the second to the fifth block expressed as the percentage of the mean AUC value of the first block, were taken as an index of habituation for each SF.

Statistical Methods

The plan of the analysis was designed a priori and is described as follows.

We based the sample size on our previous data derived from a similar study of the habituation of the conventional BR R2 response in migraine without aura.²⁷ A priori power analysis was conducted to determine the minimal sample size needed to obtain a statistical power of .80 at an α level of .05 by using the difference in nBR R2 response habituation rate at 1 Hz SF between migraine without aura and HS. The a priori power analysis estimated a minimum total sample size of 12 participants and a minimum sample size per group of 6 participants. We decided to increase the sample size of the groups to achieve a similar number to that of the previous series. Mean values of demographic and clinical features as well as of neurophysiological (ST, RT, R2 L, R2 AUC) and related psychophysical values (numeric rating scale) clustered for group of participants (ECH-in, ECH-out, EPH, and HS) were considered in statistical analysis. Distribution of variables was tested using Kolmogorov-Smirnov analysis and considered normal for P value $>$.05. Parametric tests were used as all variables considered passed the test.

One-way analysis of variance (ANOVA) was used to compare the mean values of the clinical characteristics as well as of the neurophysiological and psychophysical measurements detected at baseline between the different groups of participants (HS, ECH-in, ECH-out, EPH). Similarly, unpaired t-tests were performed to compare the mean values of clinical characteristics between ECH-in and ECH-out.

To verify the effect of the clinical condition (HS, ECH-in, ECH-out, EPH) on the habituation rate (the percentage

change of the mean nBR R2 AUC value of the second to fifth block with respect to the first) at each SF, a 3-way ANOVA for repeated measures was performed, with factors group (4 levels: ECH-in, ECH-out, EPH, HS), SF (6 levels: .05, .1, .2, .3, .5, and 1 Hz) and blocks (5 levels: first, second, third, fourth, and fifth) to evaluate the differences between groups at each SF and habituation block from the second to the fifth and to compare in each group the percentage changes of the mean nBR R2 AUC value regarding the blocks from the first to the fifth at each SF.

Student t-tests with Bonferroni correction for multiple comparisons were used for post hoc analysis. The level of significance was set at .05. All values were reported as mean \pm SD. Pearson correlation was used to search for correlations among electrophysiological parameters and clinical variables. Values of $P <$.05 were considered statistically significant.

The Statistical Package for the Social Sciences (SPSS) for Windows, version 19.0, was used for all analyses (IBM Corp, Armonk, NY).

Experimental Procedure

The experimental session was conducted between 9:00 and 11:00 AM to minimize any possible effect of diurnal variation. Participants were required to be nicotine-, caffeine-, and drug-free in the 8 hours (including sleep time) before the experiments.

To avoid the influence of the headache pain phase on the neurophysiological responses, subjects were studied during the headache-free period. ECH subjects were attack-free for not less than 4 hours and EPH for not less than 3 hours before and after the recording session (they were telephoned to verify this).

Each participant underwent 2 experimental consecutive sessions consisting of a baseline neurophysiological and psychophysical recording followed by an evaluation of the nBR habituation rate. To avoid any carryover effect from one SF to the next, participants rested between each SF for not less than 20 minutes. To guarantee the blinded condition, enrollment (A.P.), neurophysiological acquisitions (R.D.I.), and data analysis (M.G.A.) were made by different physicians.

Results

This is the primary analysis of these data, and there have been no previous publications using this data set.

No significant differences emerged in terms of mean age, mean age at onset, and duration of the disease in ECH-in, ECH-out, and EPH when individually compared with HS. On the contrary, a significant higher mean age was detected in the EPH group compared with ECH-in as well as ECH-out. No differences emerged between ECH-in and ECH-out in term of attack duration, attack frequency per day, cluster period duration, and number of cluster periods per year. All clinical data are reported in [Table 1](#).

Table 2. Mean Values \pm SD of the Neurophysiological (ST, RT, L) and Psychophysical Parameters (NRS) of the nBR R2 Responses Ipsilateral to the Stimulation Site

	<i>ECH-IN</i>	<i>ECH-OUT</i>	<i>EPH-IN</i>	<i>HS</i>	<i>ONE-WAY ANOVA</i>
ST, mA	.5 \pm .3	.6 \pm .4	.6 \pm .2	.7 \pm .2	$F_{3,61} = .95, P = .418$
RT, mA	2.4 \pm .8	3.3 \pm 2.5	2.6 \pm 1.5	2.1 \pm .8	$F_{3,61} = 2.62, P = .058$
L, ms	43.0 \pm 5.3	43.3 \pm 5.6	44.4 \pm 4.6	39.2 \pm 4.1	$F_{3,61} = 2.48, P = .078$
NRS RT	3.8 \pm 2.9	4.7 \pm .5	3.2 \pm .9	4.1 \pm .9	$F_{3,61} = 2.18, P = .100$

Abbreviations: NRS, numeric rating scale; EPH-in, EPHs during the active phase.

Nociceptive BR Baseline Parameters

The nBR R2 responses were elicited in all subjects. No statistically significant differences emerged at baseline in ST, RT, L, and AUC of the nBR R2 component between the groups of subjects (HS, ECH-in, ECH-out, EPH). Mean values \pm SD are reported in Table 2.

Nociceptive BR Habituation

As the main outcome, a clear difference emerged between the 4 groups of subjects in the habituation of the ipsilateral R2 component of the nBR.

No significant differences emerged in nBR R2 AUC mean values of the first block of responses at every SF between the groups of subjects (ECH-in, ECH-out, EPH, and HS; Table 3).

The 3-way ANOVA for repeated measures revealed a significant effect for factor Group \times SF \times Blocks interaction ($F_{60;1,220} = 1.947; P = .0001$).

Post hoc analysis revealed a significant deficit in habituation rate in all blocks from the second to fifth at 1, .5, .3, and .2 Hz in ECH-in, ECH-out, and EPH compared with HS (Figs 1A–D), with the exception of the ECH-out group, which showed a not significant habituation deficit in the third and fifth block at 1 and in the third block .3 Hz SF compared with HS (Figs 1A and C). No significant differences emerged in habituation rate at .1 and .05 Hz SF between groups (Figs 1E and F), as well as no differences emerged between ECH-in, ECH-out, and EPH in all SFs considered.

Post hoc analysis revealed a significant habituation rate of the mean nBR R2 AUC across the 5 blocks of responses (from second to fifth compared with the first one) at any SF from 1 to .05 Hz (Fig 1) in all groups of participants, with the exception of the second block compared with the first one at .1 Hz in ECH-out and EPH

(Fig 1E), of the second block compared with the first one at .05 Hz in all groups, of the third block compared with the first one at .05 Hz in ECH-in and ECH-out, and of the fourth block compared with the first one at .05 Hz in ECH-out (Fig 1F).

The sequence of the 25 nBR R2 consecutive responses at .2 Hz SF, grouped into 5 blocks of 5 averaged and rectified responses each in a representative ECH subject and HS are shown in Figs 2A and B, respectively.

Pearson test disclosed a significant correlation between nBR parameters and clinical variables in ECH, but not in EPH patients. In fact, in the ECH-in subjects there was a positive correlation between the habituation rate of nBR R2 responses at .1 Hz SF and the number of cluster periods per year ($r = .541, P = .030$).

Discussion

The main findings of this study were that: 1) ECH as well as EPH patients showed less habituation of the nBR R2 responses compared with HS, 2) ECH subjects showed less nBR R2 response habituation irrespectively of being in- or outside of the bout, 3) in ECH as well as EPH subjects, repetitive supraorbital nociceptive stimulation induced less nBR R2 response habituation at faster (1, .5, .3, and .2 Hz), but not at slower (.1 and .05 Hz), SF compared with HS, and 4) at SF of .1 Hz in ECH-in subjects, the rate of nBR R2 response habituation was positively related to the number of cluster periods per year.

In previous reports, less habituation of the R2 polysynaptic component of the BR has been detected using conventional^{12,27} and nociception-specific⁵ electrodes of stimulation over the supraorbital branch of the trigeminal nerve in ECH patients during the bouts. In this report we document, to our knowledge, for the first time, that the same trigeminal electrofunctional abnormality found

Table 3. Mean AUC Values \pm SD of the First Block of the nBR R2 Responses at Different SFs on the Symptomatic Side in Patients and on the Right Side in HS

<i>FREQUENCY</i>	<i>ECH-IN</i>	<i>ECH-OUT</i>	<i>EPH-IN</i>	<i>HS</i>	<i>ONE-WAY ANOVA</i>
1 Hz	.4 \pm .2	.6 \pm .5	.8 \pm 1.0	.7 \pm .5	$F_{3,61} = 1.39, P = .253$
.5 Hz	.4 \pm .2	.8 \pm 1.0	.8 \pm .9	.6 \pm .3	$F_{3,61} = 1.86, P = .146$
.3 Hz	.6 \pm .3	.9 \pm .7	.9 \pm .6	.8 \pm .5	$F_{3,61} = 1.06, P = .372$
.2 Hz	.6 \pm .2	.8 \pm .6	.9 \pm .8	.6 \pm .2	$F_{3,61} = 1.82, P = .153$
.1 Hz	.9 \pm .7	1.1 \pm .8	1.1 \pm 1.2	.8 \pm .4	$F_{3,61} = .92, P = .435$
.05 Hz	1.1 \pm .8	1.1 \pm .8	1.3 \pm .8	.9 \pm .5	$F_{3,61} = 1.04, P = .380$

Abbreviation: EPH-in, EPHs during the active phase.

NOTE. First block nBR R2 AUC (microvolts \times seconds).

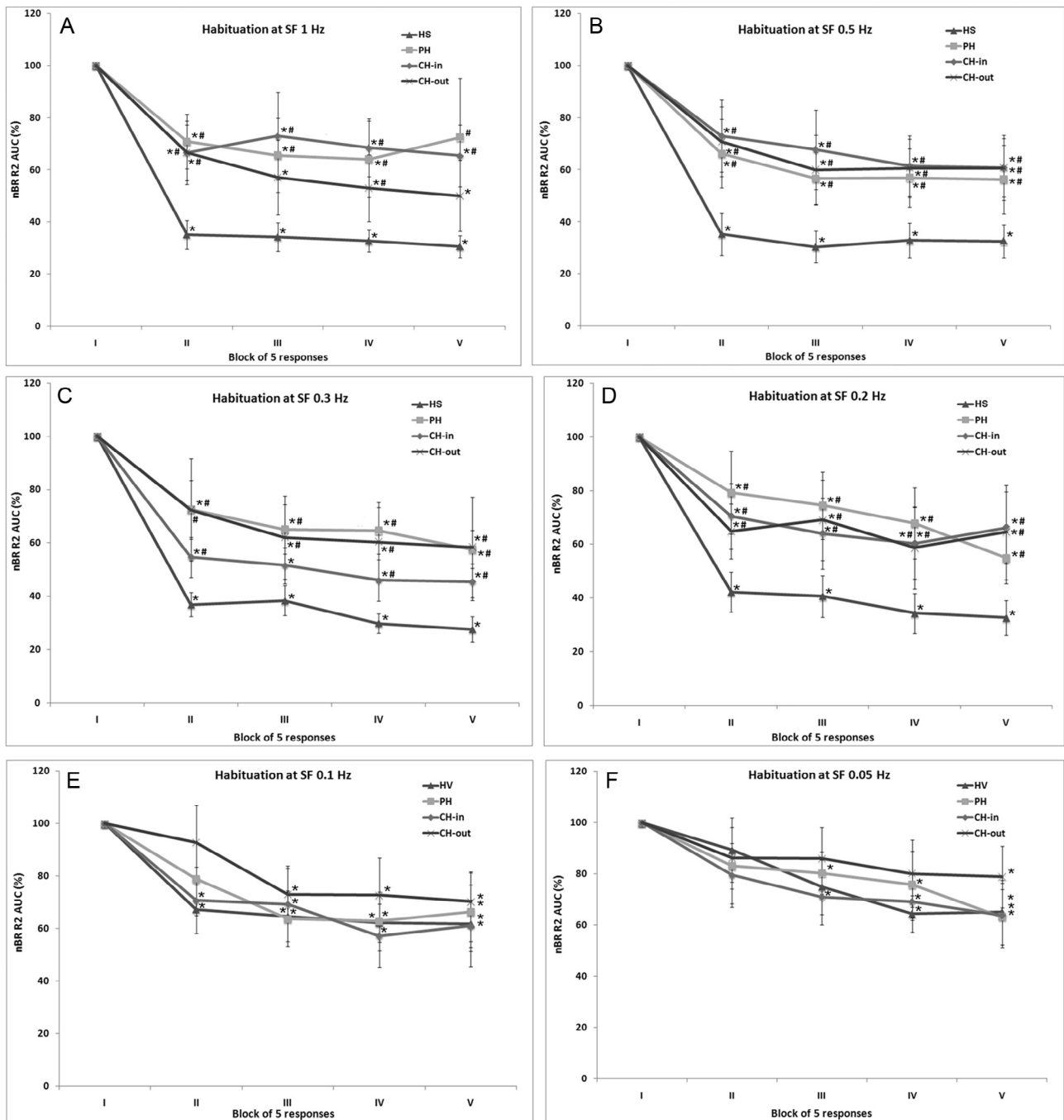


Figure 1. Habituation of the ipsilateral nBR R2 AUC in 5 blocks of 5 averaging at increasing SFs (A) 1 Hz, (B) .5 Hz, (C) .3 Hz, (D) .2 Hz, (E) .1, and (F) .05 Hz, expressed as a percentage of the first block. Data are shown as mean values and SDs of the mean. CH-in, CH during the active phase; CH-out, CH during the remission phase. Bonferroni test: * $P < .05$ versus baseline; # $P < .05$ versus HS.

in ECH is also present in subjects with EPH, another TAC. Our data strongly support that in parallel with the similarities in clinical features these 2 TACs share a common pathogenetic abnormal pain processing.

Interestingly, in ECH the habituation deficit was observed ictally¹² as well as interictally,^{5,27} but always during the bouts. In this study, we expand on this by documenting that, in ECH, this electrophysiological phenomenon persists even after the complete resolution of the cluster period. Indeed, and in contrast to the recordings per-

formed in HS in whom higher frequencies of stimulation result in more pronounced response decrement—in agreement with the behavioral characteristics of habituation²⁸—the habituation deficit appears to be very stable in all groups of patients with TACs, independently from the diagnosis (CH or PH) or the clinical condition (CH in or CH out of the active period). Therefore, it is unlikely that this abnormal trigeminal pain processing is only due to the recurrence of CH attacks that characterize the bouts, such as a state-dependent

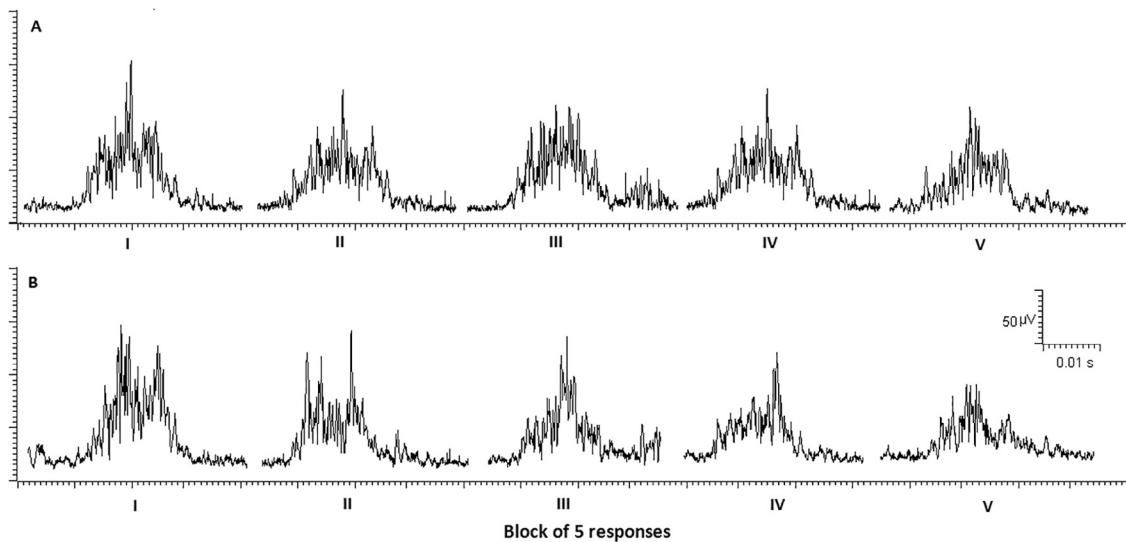


Figure 2. The sequence of the 25 nBR R2 consecutive responses at .3 Hz SF, grouped into 5 blocks of 5 averaged and rectified responses each in a representative CH (A) and healthy (B) subject.

condition, but may be inherent to the CH pathology itself, probably representing a trait-dependent aspect of the CH and maybe of other TACs.

Overall, our previous studies and the present consistently showed that in CH the reduced habituation to trigeminal stimulation represents an endophenotypic abnormality, because it is clearly detectable in and out of bouts as well as during and outside the attacks. These findings mark a clear pathophysiological difference with migraine, in which BR reduced habituation is clearly detectable during the interictal phase,^{7,18,25} but disappears with increasing attack frequency⁷ and during the ictal period.¹⁸

Results from the present study are in line with previous neuroimaging studies of pain-modulating areas, including hypothalamus, showing persistent functional and structural abnormalities in subjects with CH during the active as well as remission phase,^{4,20,23,32} which could be considered as permanent underlined abnormalities that prevent the habituation of the brainstem nBR to occur. Therefore, considering the central role of the hypothalamus in CH through its modulatory effect on the trigeminal nociceptive system^{2,22} and the previous evidence pointing to an even more pronounced BR deficit of habituation in ECH than that frequently found in migraine interictally, we have hypothesized that the hypothalamus could be one of the major determinants of the habituation deficit observed in subjects with CH.²⁷ Our speculation is further supported by the evidence that in animal models genetically modified to overexpress corticotrophin-releasing hormone resulting in chronic hypothalamus-pituitary-adrenal axis hyperactivity, reduced response habituation of the startle reflex, originating from the brainstem, was observed,⁹ which is comparable with our findings obtained with reflex blinking in subjects with ECH.

However, because habituation is a learning process that belongs to well known synaptic plasticity phenomena thought to underlie processes like learning and memory formation,²⁹ we argue that this pattern of BR habitua-

tion deficit reflects a more general malfunction in synaptic plasticity mechanisms in response to pain, which characterize TACs. This interpretation found support in previous observation in CH and PH subjects of dysfunction of another form of synaptic plasticity, the sensitization process in response to pain, detected at the spinal level using nociceptive flexion reflex.^{1,26,30} Moreover, it must be knowledge that this abnormal processing of pain at the brainstem and spinal levels seen in CH as well as PH subjects, was not evident at the cortical level using cognitive event-related potentials,^{10,11} which contrasts with the opposite finding in migraine between attacks.⁶

Another striking finding of the present study is that, by varying the interstimulus interval, we observed that the reduced nBR R2 habituation pattern is detectable only with a short duration of stimulus sequence. This peculiar frequency dependence of behavioral response was reproducible in ECH (in- and outside of the bout) as well as EPH patients. The short- (STD) and long-term habituation/depression are main experimental models of activity-dependent synaptic transmission plasticity, which are mechanistically distinct. Compared with long-term habituation/depression, which is hypothesized as the neural substrate for experience-dependent plastic modification of neural circuit,³⁶ STD has a shorter time scale, usually inducing temporary modifications of synaptic efficacy. The induction of STD mainly depends on Ca^{2+} accumulation and dynamic depletion of excitatory as well as inhibitory neurotransmitters consumed during the synaptic signaling process at the axon terminal of a presynaptic neuron, which finally mediate neuronal response properties such as frequency adaptation.^{15,37} Therefore, because in our subjects there was no change in baseline electromyography activity of orbicularis oculi muscles, a good hint of integrity of facial motoneuronal activity, we argue that this abnormal temporal pattern of nociceptive stimuli processing might originate from multiple short-term synaptic mechanism interaction at the level of brainstem trigeminal sensory neurons, with a

principal involvement of neurons, which mediate tuning and responses to high-frequency stimuli. We can speculate that these trigeminal nucleus caudalis abnormalities in synaptic plasticity mechanisms might be either primary dysfunctions or secondary consequences of deficiencies in functionally connected areas such as posterior hypothalamus, and descending opiate and aminergic pain control systems.^{3,21,26,31,33} Indirect evidence favoring deficiency in descending aminergic control in CH come from the clinical improvement of CH patients seen after dopamine agonists administration,^{8,24} which, in a single case, was accompanied by normalization of the habituation deficit of the nBR R2 component.⁸ However, whatever the culprits of the nBR habituation deficit, these elusive mechanisms may not only set the functional properties of the TAC patients' brain, but also might contribute to set the severity of clinical features. This is supported by the present observation that, at SF of .1 Hz, the rate of nBR R2 response habituation was positively related to the number of cluster periods per year in ECH-in subjects. These findings, together with our previous results in another group of ECH-in subjects of positive correlations between nBR R2 habituation and the number of days elapsed from the beginning of the bout and the daily attack frequency,⁵ indicate that the overall performance of the trigeminal system is strongly related to the evolution of clinical features during the active periods rather than the consequences of single attacks. Interestingly, in a resting-state magnetic resonance imaging study, some researchers observed that the lower functional connectivity between the hypothalamus and the cerebellum—another area involved in nociceptive modulation—the fewer number of cluster periods per year,³⁴ highlighting once more the major role of the hypothalamus in initiating and maintaining the recurrence of the disease.

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Some limitations of the present study must be considered and include that CH subjects explored during the active phase are not the same studied during the remission phase, that in CH groups the gender distribution was not homogeneous with that of the PH and HS groups, and that we did not collect data on PH during the remission phase. Altogether these limitations are related to the very low prevalence of CH as well as PH, but also to the effort to avoid as potential source of bias the inclusion of subjects using preventive medications.

Conclusions

By evaluating the nBR R2 habituation rate, we found evidence for an abnormal temporal pattern of nociceptive trigeminal stimuli processing in ECH during the active as well as remission periods, and EPH during the active phase (outside the attacks), compared with HS. Corticosubcortical defective control of pain, persistent hypothalamic dysfunction, and intrinsic abnormal short-term synaptic plasticity of the trigeminal system could be involved as pathogenetic factors. Supplementary studies of nBR are needed and of interest. Future work will repeat this analysis in other primary headache types belonging to the group of TACs, such as short-lasting unilateral neuralgiform headache attacks with conjunctival injection and tearing (SUNCT)/short-lasting unilateral neuralgiform headache attacks with cranial autonomic symptoms (SUNA), hemicrania continua, and, more intriguing, probable TAC. Moreover, it would be of interest to verify whether targeted therapies that are known to be effective in preventive treatment of TACs, such as for instance the calcium antagonist verapamil, could potentially improve the disease and, at the same time, normalize abnormal short time scale depressive plastic mechanisms at the trigeminal level.

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