Dear Editors, dear Reviewers,

thank you for reviewing our manuscript, the general positive reply and all the valuable comments, which we want to address in the following point by point.

Reviewer 1 reports:

Gianluca Coppola, MD, PhD (Reviewer 1):

Inclusion/exclusion criteria should be clarified: medication-free for at least 14 days is ambiguous. Did you include patients with preventive medication in the last 3 months? Did you ask patients to stop medications for at least 14 days before performing the study? And, for medication-free do you mean preventive or acute?

Thank you for this important point. All the included migraine patients had a low to moderate mean attack frequency. All of them except one patient never took a prophylactic treatment; the patient with prophylactic medical treatment stopped prophylaxis more than three months before
the study. Furthermore, all migraineurs were without acute attack therapy in the last two weeks before the study. We added this description in the manuscript.

Statistics is not clear. If Authors have performed repeated measure ANOVA, why they performed unpaired t test as post-hoc? Statistics should be performed using post-hoc included in the rm-ANOVA test.

Thank you for this important comment.

Since, we only compared two different groups (migraineurs vs. Ctr), as far as I know, we do not necessarily need to perform posthoc analysis such as Bonferroni or Sidak. But, as it was your wish, we applied posthoc Bonferroni-correction for multiple testing. We ran the ANOVA for repeated measures within both groups and found a significant difference. Then we performed an unpaired t-test to find out which time point(s) were statistically different between the both groups. To the best of our knowledge, this is statistically a correct way to analyse our data. For sake of clarity we described this more carefully in the manuscript.

Results:

Inclusion/exclusion criteria for healthy controls should be included in the Material section, not in the results. Moreover, having included "healthy subjects" with an occasional primary headache different from migraine is at least a contradiction. This imply that you won't or cannot found subjects without a headache, that, of course, it is hard to found, but possible. Could you comment on this? To be added as study's limitation at the end of the Discussion.

Furthermore, if "healthy" subject is "healthy" why he/she has such a MIDAS score? This is the first time I saw a MIDAS (Migraine Disability Assessment) value of healthy controls. I mean, your controls are not completely healthy, and even not matched for gender (I personally calculated a Chi-square= 3.956, with a p= 0.043 see Table 1). Groups must be matched for age and gender.

Thank you for these comments. First of all, regarding the gender-matching we want to apologize for the confusion, since the numbers in Table 1 were not correct: as you can undoubtedly see in
the uploaded original statistics files and also in line 150 in the results part of the original manuscript version, we had 14 females and 6 males in the migraine group and not 16 females and 4 males, as written in Table 1 of the original manuscript version. Accordingly, our calculated Chi-square value of 1.667 with a corresponding p-value of 0.20 is correct, which means that both groups are not different for gender and age. We, of course, corrected the numbers in Table 1 in the revised version of the manuscript (line 170).

Secondly, none of the subjects in the control group fulfilled current diagnostic criteria for migraine or any other primary headache. Nevertheless, it is very difficult to find healthy subjects in the required number without any headaches at all in their past history. To detect such occasional headaches such as related to a flu etc. we also applied the MIDAS score to the control group. And as expected, we indeed had some subjects within the control group with such occasional headaches, but not fulfilling current diagnostic criteria for a specific primary headache, which resulted in this MIDAS score. For sake of clarity we added more detailed descriptions in the manuscript in the method section.

RM-ANOVA F values are missing and must be added. The same for post-hoc (within the RM-ANOVA model) p values.

Please, remove the phrase in bold "Error! Reference source not found" in the Cardiovascular response paragraph.

We added all the RM-ANOVA F values in the manuscript, as suggested. We also added the p-values for the posthoc Bonferroni-correction. The corresponding phrase “Error! Reference source not found” was not in our version; thus the phrase presumably appeared after the upload. We will doublecheck our revised version of the manuscript for it after having finished the upload.

Discussion:

There are some evidences in the literature that brainstem mechanisms subserving cold pressor test (or heterotopic pain stimulation or DNICs) are impaired in migraine. These data should be discussed in depth.
Thank you for this valuable stimulus for the discussion of our data. We incorporated the suggested data in our discussion.

Catello Vollono (Reviewer 2): Comments:

Dear Authors, Dear Editor,

this is an interesting study evaluating the cranial ANS in migraineurs, however, I suggest that the Authors perform a revision of paper.

In the study, the Authors addressed 'whether there is distinct modulation of the cranial and cardiovascular ANS in migraine patients by applying the cold pressor test to a cohort of migraine patients compared to healthy controls and measured pupillary response, heart rate and blood pressure.'

This is an important topic since the assessment of ANS in previous studies in migraine showed conflicting results. The authors reported their findings in a relatively clear presentation.

Certain major revisions may help the strength of their efforts. Subsequently, I have several concerns regarding the methods as well as the results and the interpretation of their findings.

1. The Authors should change the objectives of the study in 'In the present study, we aimed to assess the cranial ANS in migraine patients by applying the cold pressor test to a cohort of migraine patients compared to healthy controls and measured pupillary response.', as they do not have any 'sensitive' tools to investigate the cardiovascular autonomic nervous system.

Thank you for this important advice. You are completely right with your comment that our dataset mainly addresses and investigates in detail the cranial ANS in migraine patients, while the cardiovascular ANS only been roughly investigated. Thus, we completely followed your advice and rearranged the topic of the manuscript in all sections more towards the cranial ANS; the cardiovascular ANS parts instead were significantly toned down in all sections.
2. Single measurement of blood pressure and heart frequency, in fact, are unable to define exactly the ANS balance. So, the findings regarding the cardiovascular ANS, assessed without HRV study or other not gold standard tools (i.e. continuous recording of blood pressure), are to be considered only 'collateral'.

We completely agree and are very thankful for this important and essential comment, which we tried to follow as far as possible throughout the whole manuscript.

3. Consensually, I suggest that the Authors change in this way, the title, the abstract, the discussion, and the conclusion of the study.

We completely agree and consequently we changed the title, the abstract, the introduction, the method section, the discussion and the conclusion accordingly

4. Another method to assess cranial ANS response is the evaluation of degree of pupillary diameter, a measure of ANS balance modification. The Authors could consider also these data in order to increase the power of their findings.

Yes, this is indeed a good measure of modification of ANS balance. But, unfortunately, we did not perform it appropriately and only concentrated on the parameters described in the study.

5. The Authors could take in account that other Authors report that variations of PLR and HRV are not associated also in healthy subjects. (Daluwatte C, Miles JH, Yao G. Simultaneously measured pupillary light reflex and heart rate variability in healthy children. Physiol Meas. 2012 Jun;33(6):1043-52.)
Thank you for the literature source, which we cited and discussed with regard to our dataset (line 298).


Thank you very much for the intellectual input. We included the suggested relevant literature for the discussion of our dataset.

7. I suggest that the Authors discuss the results without different paragraphs, considering the limitation of the cardiovascular ANS findings.

The paragraphs were merged as suggested.