Author’s response to reviews

Title: Specific motor cortex hypoexcitability and hypoactivation in COPD patients with peripheral muscle weakness

Authors:
Francois Alexandre (francois.alexandre@5-sante.fr)
Nelly Heraud (nelly.heraud@5-sante.fr)
Emilie Tremey (emilie.tremey@5-sante.fr)
Nicolas Oliver (nicolas.oliver@5-sante.fr)
Dominique Bourgouin (dominique.bourgouin@5-sante.fr)
Alain Varray (alain.varray@umontpellier.fr)

Version: 2 Date: 14 Nov 2019

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To the Editor:

We would like to thank you and the reviewers for the positive appreciation of our study and the valuable comments that have allowed us to improve our manuscript. All the questions raised by the reviewers were addressed in the new version of the manuscript and answered below. We provide a clean version of the manuscript and a version with track changes with the modifications highlighted in yellow. We hope that the modifications included in the new manuscript will be satisfactory.

Response to the reviewers:
Reviewer 1:
First, we thank you for the evaluation of our manuscript and your constructive remarks and suggestions, which undoubtedly have improved our paper. Please find our reply point-by-point below:

1) Were patients consecutively enrolled? In how many weeks/months? A consort diagram would be useful
We must recognize that this point was poorly developed in the first version of the manuscript. The participants of the study were enrolled over four different periods of time, each lasted between one to three months, between 2012 and 2014 (so not consecutively during the 2 years of recruitment, but consecutively within each period of recruitment). The COPD patients were recruited and tested at their entrance in two pulmonary rehabilitation center (Cliniques du Souffle La Vallonie, Lodève, and Les Clarines, Riom-ès-Montagne, France). The healthy controls were recruited through an ad in a local newspaper. We add this clarification on lines 51-54. We also add a flow diagram (figure 1) and consequently actualize each figure number in the manuscript.
2) Can you list all the drugs that can impact brain function for which you excluded the patients from the protocol? The drugs that can impact brain function for which we excluded the patients are the benzodiazepines (GABA agonist and Z-drugs), the antidepressants (tricyclic antidepressants, melatonergic antidepressants and selective serotonin/noradrenalin reuptake inhibitors) and the opioids (opioid receptor agonists). This clarification is added on lines 59-61.

3) Low PaO2 patients had also desaturation on effort? We did not record SpO2 during the neuromuscular tests, but oxygen desaturation during these tests was unlikely. In a previous study, we measured the SpO2 in 12 COPD patients during both submaximal and maximal isometric quadriceps contractions and we never observed any significant changes, as attested by the mean ΔSpO2 during contractions which was only 0.01 (Alexandre et al., Plos One, 2014). In addition, the 3 patients of the sample who had the lowest baseline SpO2 (i.e. 92%, which approximately equals to a PaO2 of 65 mmHg) neither exhibited any changes. Therefore, the occurrence of desaturation during the effort of the current protocol is unlikely, even in the patients with low PaO2 at rest. We add further discussion in relation to this point on lines 359-361.

4) What about smoking history of all subjects? Any difference related to MW? The mean pack-year was 45.5 ± 24 in the patients with muscle weakness, 38.9 ± 26.9 in the patients without muscle weakness and 7.3 ± 12.5 in healthy controls. There was a significant effect of group (F = 14.7, p < 0.001), but without any difference between the two groups of COPD patients (post-hoc p = 0.39). We add these results on lines 250 and in the table 2 of the manuscript, and discuss it briefly on lines 398-399.

5) What about years of disease? comorbidities (e.g. cardiaca)? • We did not have any information of the number of years of disease in the current sample. Although we should recognize that this information can be relevant to better describe the study population, this information is particularly difficult to obtain due to the specificity of this chronic disease, which is often diagnosed later after the first symptoms have occurred. As a direct consequence, the time spent after the diagnosis would not be able to give relevant information on how ancient the disease is. Furthermore, while it could be logical to think that the number of years of disease could partly explain the heterogeneity of muscle weakness in COPD (and thus the differences between the two groups of patients in our study), this idea is not supported by the literature data. For instance, Kharbanda et al. (Int. Journal of COPD, 2015) found that the onset of muscle weakness in COPD may precede the onset of symptoms, and both Kharbanda et al. (Int. Journal of COPD, 2015) and Seymour et al. (Eur Resp J, 2010) found a high proportion of patients with muscle weakness in the first stage of the disease (GOLD 1).
• Regarding comorbidities, the most prevalent were cardiac comorbidities (n= 10, 25% of the study sample), followed by obstructive sleep apnea (OSA n=9, 23% of the study sample) and thyroid dysfunction (n=6, 15% of the study sample). The distribution of comorbidities was quite similar in both groups of patients regardless of muscle weakness: 5 patients with cardiac comorbidities in each group, 4 patients with OSA in the COPDMW group vs 5 in the COPDNoMW group, and 3 patients with thyroid dysfunction in each group. Furthermore, there was no significant difference in the number of comorbidities between the two groups of patients (2.2 ± 1.1 in the COPDMW group vs 2 ± 1.2 in the COPDNoMW, p = 0.63). We have added data on comorbidities in the results section on lines 251 and in the table 2.
5) Are the data obtained in COPD patients with MW similar somehow to any other condition previously described?
The data obtained in COPD patients with MW are quite similar to data reported in the age-related muscle weakness in healthy elderly (Clark et al. Journal of Gerontology: Medical Sciences, 2015). Classically, the age-related muscle weakness is studied across comparisons of healthy elderly vs healthy young people, but not all elderly people have reduced muscle strength. Using a similar approach as ours, Clark et al. found no differences in voluntary activation between young adults and seniors when all the seniors were pooled as in several studies. However, when they dichotomized the seniors in two groups as a function of their maximal strength, the voluntary activation was reduced in the weakest seniors compared to the strongest ones. We have added the reference of Clark et al. in the introduction section of the manuscript on line 7.

6) Methodological considerations at page 19-20 can be moved elsewhere
As we did not find any guidelines in the instructions for authors, we checked in recent papers published in BMC Pulmonary Medicine the way to manage this section and we saw that it was systematically set at the end of the discussion (e.g. Gebremariam et al. 2019, Zhongqi Li et al. 2019, Vieira Santana et al. 2019, Elfu Feleke et al. 2019). Consequently, we propose at the moment not to move the methodological considerations section, but we would be pleased to do it, by moving it for example at the beginning of the discussion, if you deem it necessary in a new revision.

Reviewer 2:
First, we would like to thank you for the positive appreciation of our manuscript and your valuable comments that have allowed us to make improvements to our manuscript. We were able to take into account all of your comments, except the first one, pending further clarifications (the reason why we have failed at the moment to address your first concern is detailed just below), as you can see in our point-by-point reply below:

1) The introduction provides good background information and the flow is logical. However, the paragraph structure needs to be revised.
We really appreciate that you highlighted the content of the introduction section. At the same time, we feel very embarrassed because we fail to understand with certainty what you precisely want us to revise in the introduction section. We cannot yet see clearly what you mean by the “paragraph structure”: do you refer to a precise paragraph of the introduction section, or each of the 5 paragraphs of the section? At the moment, we deem prudent to not modify the introduction, and would prefer to wait for obtaining further information details regarding your request. Then we will be pleased to make any revisions you deem necessary.

2) Line 85-87: The participants were systematically familiarized with maximal voluntary contractions, femoral nerve stimulation and transcranial magnetic stimulation the day before the protocol. Can the authors elaborate on what was meant by familiarization? Were they explained what the tasks would be? Were they given physical training doing MVCs, stimulation etc?
The familiarization session was a physical training to familiarize the patients with isometric quadriceps contractions and with superimposed transcranial magnetic stimulation and femoral nerve stimulation. It included first transcranial magnetic stimulation and femoral nerve stimulation recruitment curves. Then it was followed by 3 maximal voluntary contractions and several submaximal voluntary contractions at 30 and 50% of MVC lasting 5 seconds or until the targets were correctly reached, with superimposed transcranial magnetic and femoral nerve stimulations. These details are added in the method section on lines 94-99.
3) Line 110-112: One pulse was delivered on the femoral nerve every 10 s, with the intensity beginning at 50 mA and increasing from 10 mA until no further increase in twitch mechanical response and M-wave amplitude occurred. Should this read increasing by 10 mA? I.e. in 10 mA increments? We apologize for the grammatical error you have identified. Your understanding of the sentence is the good one. We modify the sentence accordingly in the manuscript (line 123).

4) Was the femoral nerve stimulation and TMS at rest performed with the same body positioning as the MVC testing position? This will influence excitability and the details should be included. We confirm that the femoral nerve stimulation and TMS at rest were all performed with the same body positioning as the MVC testing position. Indeed, the participants were set and securely attached on the ergometer before the beginning of any experimental procedures, and the position was maintained until the end of the protocol. We agree that this is an important point that merit further clarification in the manuscript. We have added these details on lines 92-93.

5) Line 222-223: Moreover, they also exhibited significantly higher Hmax latency (t37=2.94, p<0.01) and silent period duration (t37=3.33, p<0.001) than controls. This should state "longer silent period duration" instead of "higher." We made the requested modification on line 239. Furthermore, we checked for potential similar mistakes in the manuscript and found another one in the abstract. We thus also corrected it accordingly.

6) Lines 236-237: The data are presented in Figures 2a and 2b. Both QMVC and QPt were significantly lower in COPDMW compared with the COPDNoMW and control groups (F2,57=10.73, p<0.001 and F2,57=4.46, p<0.01, respectively). As an ANOVA was performed, the authors should first state whether there was an overall significant effect of group and then discuss the comparisons to improve transparency of results. This should be done for all group comparisons. (This was demonstrated in the Table however so perhaps it is okay). Although it was clearly presented in the tables, as you noted, we agree that it could be somewhat confusing in the text. As requested, before each post-hoc comparison, a statement is thus added to state whether there was an overall significant effect of group (lines: 255-256, 258-259, 282, 285 and 288).

7) Regarding comparisons, the authors need to explicitly state when there was an overall difference between the three groups or when some comparisons were a pooled two group comparison (lines 209-211). These two types of comparisons were described in the Statistical analyses section. However, there is a lack of clarify for some statements in the Results - "Hmax latency was significantly higher in the two COPD groups compared with controls (F2,36=4.26, p<0.05), but the central motor conduction time was not different between the three groups (p=0.33)." These data are also pooled for the COPD patients in Table 1. Was an ANOVA performed followed by post hoc analyses to determine differences?

Once again, we should recognize after a careful reading that the results section could lead to confusion in some aspects. We believe that the fact that we were constrained to use different statistical tests for comparisons between groups have probably complicated the reading. As we explained in the statistical analyses section, the pooled COPD patients and the controls were only compared by the mean of unpaired t-tests for parametric data (or non-parametric Mann-Whitney U test). For the 3 group comparisons, ANOVAs have systematically been used, followed by post-hoc test when the ANOVAs F interaction were significant. In 4 cases where no data were available for healthy controls (PaO2, PaCO2, SpO2 during 6MWT and number of comorbidities), the two groups of COPD patients were compared using a student t-test.
We propose the following modifications to address your concern:

- In the statistical analyses section:
  - On lines 215-217, we have switched the part describing the tests used for the comparison of controls with the pooled patients, with the part describing the ANOVA procedure for the 3 group comparisons (in order to improve clarity, because the results were presented in this order in the result section).
  - On lines 218-220, we have specified the two cases when the COPDMW and COPDNoMW were compared using t-tests.

- In the results section:
  - On lines 280, 282, 285 and 300, we have added some F values and degree of freedom that were missing, because in the first version, they were only provided when the interaction was significant (although not necessary, this can help to minimize potential confusion on which test was used).
  - On lines 257, 259, 260, 264, 284, 287, 289 and 298, we now systematically specify when the p-values are those of post-hoc tests (once again, to minimize potential confusion on which test was used).

8) Some of the statements in the Discussion should be qualified. The following statement is too all encompassing. "In sum, because higher corticospinal inhibition and impaired neuromuscular transmission are unlikely to be involved in the reduced quadriceps strength of the patients with muscle weakness, the most likely mechanism responsible for muscle weakness, and thus for reduced voluntary activation, is decreased excitation from the brain, which is supported by the observation of lower gray matter density in the motor cortex (precentral gyrus) in COPD". Suggest to break the sentence up into two parts and have the first sentence end after "…reduced quadriceps strength of the patients with muscle weakness,". The second part of this sentence negates the contribution of peripheral muscle factors i.e. atrophy, myopathic changes, contributing to muscle weakness. Thus, the second part of the sentence should be qualified accordingly to include some reference to the contribution of peripheral muscle factors contributing to weakness.

We agree that this statement could have delivered the awkward message (that we especially wanted to avoid in our manuscript), that neural factors are more prominent than peripheral factors in peripheral muscle weakness. Inversely, our results show that both peripheral and neural factors are involved in the COPD peripheral muscle weakness, as we stated in the conclusion of the manuscript on line. We thus modify this statement accordingly, by specifying the context of the statement, which only concerned the neural component of peripheral muscle weakness, and not the overall peripheral muscle weakness factors (line 390).

9) One of the next statements should be placed in a greater context as well: "In the current study, the weak patients had lower resting PaO2 levels. However, the implication of this result in the reduced cortical activation is unlikely, since hypoxemia is not a sufficient condition to induce cerebral hypoxia, due to the cerebrovascular reactivity that compensates any reduction in PaO2 by an increase in cerebral blood flow [51]." Cerebrovascular reactivity may compensate for a reduction in PaO2 by an increase in cerebral blood flow in some individuals but to make this blanket statement for your sample of COPD patients is unfounded. Cerebral vascular disease and hypoxemia are considered to be major factors that can contribute to cognitive impairment and regional brain changes in people with COPD.

We agree with you that our position might have been too strong regarding this specific point of the manuscript. This was in link with a hypothesis developed in our team (See Alexandre et al. 2015, medical hypotheses). However, we agree that our data cannot directly support this hypothesis. Thus, we
propose a rewriting of this paragraph on lines 394-402, taking into account your concern, with a weaker positioning and also including some new elements of discussion in connection with the first reviewer’s suggestion (about smoking history)