Activation response and functional connectivity change in rat cortex after bilateral transcranial direct current stimulation – an exploratory study

Dijkhuizen, Rick; Boonzaier, Julia; Straathof, Milou; Ardesch, Dirk Jan; Toorn, Annette van der; Vliet, Gerard van; Heijningen, Caroline L. van; Otte, Willem

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1st Editorial Decision

Decision letter
12-Feb-2020

Dear Dr Dijkhuizen:

Thank you for submitting your manuscript to the Journal of Neuroscience Research. We’ve now received the reviewers' and editors' feedback and have appended those reviews below. While the editors and the reviewers were quite interested in the subject of your study, they do not find the manuscript suitable for publication in its current form and rejection was recommended based on some critical observations concerning the methodology. Most importantly, a major concern is the small sample size, which makes this study vastly underpowered, even if it is a repeated measures design. Another concern is that the study seems to be too preliminary in its current form. Unfortunately, in comparison with other currently submitted manuscripts, it does not show a sufficiently high priority for acceptance. We strongly encourage the authors to resubmit their findings after considering all the recommended reviews.

If you feel that you can adequately address the concerns of the reviewers, you may revise and resubmit your paper within 90 days. It will require further review. Please explain in your cover letter how you have changed the present version. If you require longer than 90 days to make the revisions, please contact Dr Cristina Ghiani (cghiani@mednet.ucla.edu). You can submit your revised manuscript directly by clicking on the following link: *** PLEASE NOTE: This is a two-step process. After clicking on the link, you will be directed to a webpage to confirm. ***

https://mc.manuscriptcentral.com/jnr?URL_MASK=b0689c39082249eea4c224c8adc4cc2b

Thank you again for your submission to the Journal of Neuroscience Research; we look forward to reading your revised manuscript.
Best Wishes,

Dr Sandra Chanraud  
Associate Editor, Journal of Neuroscience Research

Dr Cristina Ghiani  
Co-Editor-in-Chief, Journal of Neuroscience Research

Statistics Editor: McArthur, David
Comments to the Author:

The small sample size here is strongly working against your evaluation of statistical significance and the reproducibility and generalizability of these findings. While the use of mixed effects models is encouraged, those too may not behave particularly well if samples are tiny and distributions are far from normal.

Why some interaction effects in the Results narrative are accompanied with statistical results but other are not is unclear.

Figure 3 and Supplementary Figures 2 and 3 show graphics that are box-and-whisker plots, but with tiny sample sizes these can be unexpectedly deceptive. Use of dotplots when sample sizes are low is strongly preferred; boxplots do not necessarily reveal the scope of nonnormality within distributions.

It is not entirely clear how many datapoints make up each box shown in the figures.

Reviewer: 1

Comments to the Author
Activation response and functional connectivity change in rat cortex after bilateral transcranial direct current stimulation

This interesting study aimed to identify the effects of transcranial current direct stimulation (tDCS) over the sensorimotor cortex on resting brain activity and functional connectivity using in vivo functional and perfusion MRI in rats. The results of this study showed that tDCS was able to induce an increased hemodynamic activation response in different cortical areas of interest, notably the sensorimotor regions. This is in agreement with recent studies carried out in humans combining tDCS and cerebral oxygenation measurements (DOI: 10.1111/ner.12632; DOI: 10.1016/j.neuroimage.2010.11.085) that should be reported and discussed.

Interestingly, a second stimulation session (carried out in a subset of rats) showed "inverse" output at the neural level. Due to important technical limitations sample size at the end is becoming an issue. It does not allow provide numerous hypothetical statements and interpretations in the Discussion and Conclusion which should be reconsidered.

Introduction
Page 5. L5-48. Authors spent too much time at the beginning of the introduction to report (in)consistent findings in clinical population using bilateral montage for tDCS. First, patients were not assessed in the present study. Second, choice of a bilateral montage as compared to others should be motivated more in terms of putative mechanisms at two levels: brain activation for some brain regions and functional connectivity patterns across different brain regions of interest.

Page 5. L58-60. Authors forgot some important / relevant studies dealing with concurrent measures of brain activation during (and not only after) tDCS intervention. Not only MRI should be indicated here; functional near infrared spectroscopy studies offer also promising perspectives (location, parameters of stimulation, intervals etc..) and valuable findings to consider in the rationale of the study.

-Khan B, Hodics T, Hervey N, Kondraske G, Stowe AM, Alexandrakis G. Functional near- infrared spectroscopy maps cortical plasticity underlying altered motor performance induced by transcranial direct current stimulation. J Biomed Opt 2015;18:116003.
- Muthalib, M., Besson, P., Rothwell, J., & Perrey, S. (2018). Focal hemodynamic responses in the stimulated hemisphere during high-definition transcranial direct current stimulation. Neuromodulation, 21,348-354. https://doi:10.1111/ner.12632
- Merzagora AC, Foffani G, Panyavin I et al. Prefrontal hemodynamic changes produced by anodal direct current stimulation. Neuroimage 2011;49: 2304–2310.
Page 6. Lines 17-20. Authors have right. However, the study does not address really this issue (specific patterns) thereafter. Further, preclinical tDCS study was not performed.

Page 6. Lines 29-34. Hypotheses (immediate effects? after-effects?) and/ or expected results were not proposed on the tDCS-induced changes of brain activity and network connectivity. In addition the latter was not presented in the scientific background. This is lacking. It is likely relevant to move beyond identification of regional cortical activations toward the characterisation of interactions between brain areas. But why? Authors should motivate this. Connectivity analyses of the brain have been the object of a growing interest in neuroimaging studies in recent years. As authors know, brain is a complex system par excellence characterised by the co-existence of functional segregated parts of the brain, and functional integration among these parts. So what the role of (bilateral) tDCS here? Which “mechanism of action” (lines 31-32) is investigated? whereas a rat model was used. Overall, added value and novelty of the present should be clearly acknowledged. Introduction needs to be restructured accordingly.

Methods
Page 7, line 36. “Precise positioning”. Finally, what was the exact location of the epicranial cannulas in terms of brain region (neuroatlas coordinates to report as it was done for image registration in MRI; tDCS-targeted area was considered)? It is visible on figure 1B. This location was the same for all rats? Please confirm. Page 8, lines 9-14. This dosage of current density is important since it corresponds to one issue in the literature explaining inconsistent results among human studies. These stimulation parameters can be considered as “optimal” (page 5, lines 44-50)? Please confirm that anodal stimulation was applied over the right hemisphere while cathodal was done on the left.

Pages 9-10. Due to the small sample size (especially in the session 2 of stimulation, n=4) effect size can be added when available. Final group sizes are seriously critical.
Page 10 (lines 42-46). Please indicate whether changes in BOLD signal were significant.

Discussion-Conclusion
Due to important limitations, this study has to be considered as a pilot study. Bold signals were modulated even for the visual areas. Please explain why brain activation occurs for such control areas. Systemic interference is one possibility.

Reviewer: 2

Comments to the Author
In this study, Boonzaier and coauthors aim to investigate the effects induced by bilateral tDCS on resting brain activity and functional connectivity using in vivo MRI in an animal model. Main findings were an increase, although not significant, in BOLD signal in different cortical areas, and a decrease of functional connectivity within the cortical sensorimotor network after a first stimulation session with a subsequent increase after a second session, for which the authors suggested the involvement of neural adaptation mechanisms. The authors report new results, although by a methodological point of view the study lacks of a clear hypothesis. Main concern regards the small sample size evaluated in this study. I have some suggestions that the authors may want to take into consideration.

Introduction: The authors should argue better the theoretical hypothesis regarding the possible effects induced by bilateral tDCS for modulating neuronal activity and connectivity. Additionally, they should take in consideration that any result observed in healthy animal model is not applicable in brain disorders (i.e., stroke), considering as several neural activity changes related to cellular and molecular processes are detected after stroke, not only in perilesional tissue but also in more remote brain regions. Additionally, they should evaluate how the neural changes induced by a stroke could interfere with tDCS-induced effects at cortical level.

Experimental design. The rationale about the application of once stimulation or twice stimulations should be clearly explained and justified, as well as the choice not to do a fMRI during the second tDCS session. Additionally, I think that from a methodological point of view this study suffers for the lack of an evaluation of neural changes induced on-line by sham tDCS on BOLD signal, that could allow a direct comparison between the immediate effects induced by a real bilateral tDCS vs sham tDCS.
For the analysis of resting-state fMRI and perfusion MRI, it is unclear the rationale underlying the choice to calculate difference values comparing for the first stimulation, post-stimulation-1 vs pre-stimulation and for second stimulation, post-stimulation-2 vs post-stimulation-1. Have the authors compared post-stimulation-2 value vs pre-stimulation value?

In my opinion, every possible conclusion about the results is seriously affected by the very small sample size, also considering the exclusion of animals due to MR artifacts.

In the figure 1 about the experimental design, the authors should report how many animals participated in each MR imaging evaluation and stimulation session.

Concluding, I think that the findings reported by the authors are not sufficient to support their conclusions.

Reviewer: 3

Comments to the Author

The study aims to understand the mechanism of tDCS using rodent fMRI. As similar study can be and have been done in humans, the novelty and insights demonstrated in this manuscript is limited. Also, because of the limited number of animal and experimental design, the results appear to be preliminary. Specific comments are:

1. Similar attempts had been done in rats (eg, Takano et al. 2011) but was not mentioned at all in this paper. There are also papers in humans need to critically review.
2. fMRI Method: was the rat given muscle relaxant during mechanical ventilation? Without muscle relaxant, the spontaneous breathing will counteract the ventilator and affect the respiration rate and EtCO2.
3. Resting fMRI preprocessing: motion parameters alone is not sufficient for removing nuisance. Additional consideration for the physiological noise is needed.
4. The physiological conditions should be reported for each period the fMRI was measured.
5. The sample size after quality control is insufficient, making the results unconvincing.
6. The activation induced by tDCS (Fig.2) appears to colocalize with large vessel. Please elaborate and discuss the possible mechanisms and how that would impact BOLD and CBF response in the cortex.
7. The BOLD signal during the switching off of tDCS was measured but not reported. As some regions appear to have a more delayed activation, it would be interesting to compare the off-response.
8. There were significant changes in functional connectivity the sham control group. This raised a concern about the stability of the signal and/or experimental condition like indicated in points 1 and 2.
9. Fig.4: please only show the significantly changed connections.
10. Despite using histology to show no lesion induced by the procedure, there is no measures (eg, electrophysiology) to help understanding the mechanism of tDCS.
11. Lots of disparity among human studies are related to the way tDCS was applied. Although the paper pointed out this issue to highlight the need for animal study, this study didn’t look into that at all.
12. Considering the number of animal, it’s not clear why the degree of freedom was 58 on p.10.

Authors’ Response

Statistics Editor: McArthur, David

Our response: We thank the Statistics Editor for his time and efforts to critically review our paper and for the recommendations to improve it. We have incorporated the editor’s suggestions in the revised manuscript, as explained below.

Comments to the Author:
The small sample size here is strongly working against your evaluation of statistical significance and the reproducibility and generalizability of these findings. While the use of mixed effects models is encouraged, those too may not behave particularly well if samples are tiny and distributions are far from normal.

Our response: We understand the editor’s point. In response to his comment, we have made textual adaptations in the manuscript to underline the exploratory character of the study. We have further reduced the focus on statistical significance in the Discussion and Conclusion. For example, “…the main purpose of this exploratory study was to develop and apply an experimental in vivo setting for MRI during tDCS (in rats) to measure instant and resultant effects of cortical stimulation on whole-brain network status.” (page 4, lines 46-52)
"In conclusion, our exploratory study shows successful application of an MRI-compatible bilateral tDCS setup in an animal model. Our results demonstrate that bilateral tDCS over the sensorimotor cortex affects signals and signaling within and across the sensorimotor cortical network in the healthy rat brain. This includes modulation of neuronal activity and connectivity as measured with functional MRI. Further studies are needed to assess these effects under pathophysiological conditions, such as after stroke, where (bilateral) tDCS may contribute to functional recovery." (page 11, lines 29-38)

Why some interaction effects in the Results narrative are accompanied with statistical results but other are not is unclear.

Our response: We apologize for the confusion. The narrative results have been removed, as it was only intended to give a general description of the results.

Figure 3 and Supplementary Figures 2 and 3 show graphics that are box-and-whisker plots, but with tiny sample sizes these can be unexpectedly deceptive. Use of dotplots when sample sizes are low is strongly preferred; boxplots do not necessarily reveal the scope of nonnormality within distributions. It is not entirely clear how many datapoints make up each box shown in the figures.

Our response: We have adapted all boxplot figures to also show the individual data points.

Reviewer #1: Our response: We thank the reviewer for his/her time and efforts to critically evaluate our paper and for pointing out several aspects of this manuscript that needed improvement. We very much appreciate the reviewer's constructive comments and suggestions, and we have dealt with all his/her points in the revised manuscript, which we address separately below.

Comments to the Author: Activiation response and functional connectivity change in rat cortex after bilateral transcranial direct current stimulation
This interesting study aimed to identify the effects of transcranial direct current stimulation (tDCS) over the sensorimotor cortex on resting brain activity and functional connectivity using in vivo functional and perfusion MRI in rats. The results of this study showed that tDCS was able to induce an increased hemodynamic activation response in different cortical areas of interest, notably the sensorimotor regions. This is in agreement with recent studies carried out in humans combining tDCS and cerebral oxygenation measurements (DOI: 10.1111/ner.12632; DOI: 10.1016/j.neuroimage.2010.11.085) that should be reported and discussed.

Our response: Thank you, we have included these references into the Discussion; see the section 'Hemodynamic changes in response to tDCS' (page 9, lines 35-40): "Previous studies in humans [13,32] and rats [15] have reported a BOLD activation response to tDCS, which was particularly noticeable in regions underneath the stimulation sites. Our results are in line with those findings in this study."

Interestingly, a second stimulation session (carried out in a subset of rats) showed "inverse" output at the neural level. Due to important technical limitations sample size at the end is becoming an issue. It does not allow provide numerous hypothetical statements and interpretations in the Discussion and Conclusion which should be reconsidered.

Our response: We have adjusted and reduced the hypothetical statements in the Discussion (pages 10, lines 26-60) and Conclusion (pages 11, lines 29-38) to fit with the exploratory nature of this study.

Introduction
Page 5. L5-48. Authors spent too much time at the beginning of the introduction to report (in)consistent findings in clinical population using bilateral montage for tDCS. First, patients were not assessed in the present study. Second, choice of a bilateral montage as compared to others should be motivated more in terms of putative mechanisms at two levels: brain activation for some brain regions and functional connectivity patterns across different brain regions of interest.

Our response: In response to the reviewer's suggestion, we have toned down the Introduction in terms of the reporting of (in)consistent findings in the clinical population and we have provided more explanation on our motivation to investigate bilateral tDCS, for example:
"The extent of these modulations largely depends on current intensity and electrode montage (unilateral vs bilateral), which can alter cortical excitability in a polarity-specific manner [5]. Anodal tDCS has been assumed to augment neuronal excitability while cathodal tDCS would diminish it [4,6]. With regard to motor function, it has been suggested that bilateral tDCS, with the anode over the non-dominant motor cortex and the cathode over the dominant motor cortex, may yield stronger effects on motor learning as compared to unilateral stimulation [7,8]." (see Introduction, page 4, lines 9-17).

Page 5. L58-60. Authors forgot some important / relevant studies dealing with concurrent measures of brain activation during (and not only after) tDCS intervention. Not only MRI should be indicated here; functional near infrared spectroscopy studies offer also promising perspectives (location, parameters of stimulation, intervals etc.) and valuable findings to consider in the rationale of the study. -Khan B, Hodics T, Hervey N, Kondraske G, Stowe AM, Alexandrakis G. Functional near-infrared spectroscopy maps cortical plasticity underlying altered motor performance induced by transcranial direct current stimulation. J Biomed Opt 2015;18:116003.
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- Merzagora AC, Foffani G, Panyavin I et al. Prefrontal hemodynamic changes produced by anodal direct current stimulation. Neuroimage 2011;49: 2304-2310.

Our response: We thank the reviewer for these suggestions. These references have been incorporated into the Introduction (page 4, line 17-19):
"Direct and delayed effects of tDCS at a whole-brain network level may be effectively measured with in vivo neuroimaging tools, such as optical spectroscopy/imaging [12-14] and magnetic resonance imaging (MRI) [15]."

Page 6. Lines 17-20. Authors have right. However, the study does not address really this issue (specific patterns) thereafter. Further, preclinical tDCS study was not performed.

Our response: Thank you. This reference has been removed.

Page 6. Lines 29-34. Hypotheses (immediate effects? after-effects?) and/or expected results were not proposed on the tDCS-induced changes of brain activity and network connectivity. In addition the latter was not presented in the scientific background. This is lacking. It is likely relevant to move beyond identification of regional cortical activations toward the characterisation of interactions between brain areas. But why? Authors should motivate this. Connectivity analyses of the brain have been the object of a growing interest in neuroimaging studies in recent years. As authors know, brain is a complex system par excellence characterised by the co-existence of functional segregated parts of the brain, and functional integration among these parts. So what the role of (bilateral) tDCS here? Which “mechanism of action” (lines 31-32) is investigated? whereas a rat model was used. Overall, added value and novelty of the present should be clearly acknowledged. Introduction needs to be restructured accordingly.

Our response: We thank the reviewer for these comments. We have now restructured the majority of the Introduction to provide a better explanation of the background, objectives and hypothesis of our study. For example, we now state in the Introduction that: "...the main purpose of this exploratory study was to develop and apply an experimental in vivo setting for MRI during tDCS (in rats) to measure instant and resultant effects of cortical stimulation on whole-brain network status. This could aid in the elucidation of neuromodulatory actions of tDCS. We hypothesized that bilateral tDCS of the sensorimotor cortex induces a polarity-specific direct cortical activation response and, as an after-effect, polarity-specific modulation of functional connectivity within the sensorimotor cortical network." (page 4, lines 46-52).

Methods
Page 7, line 36. "Precise positioning". Finally, what was the exact location of the epicranial canulas in terms of brain region (neurolatlas coordinates to report as it was done for image registration in MRI; tDCS-targeted area was considered)? It is visible on figure 1B. This location was the same for all rats? Please confirm.

Our response: We have added the following clarification in the Materials and Methods, section tDCS - Surgical placement of bilateral, epicranial canulas’ (page 5, line 38-41): “The canulas were positioned bilaterally over the sensorimotor cortex, which included the primary (M1) and secondary (M2) motor cortices and the fore-limb region of the somatosensory cortex (S1FL) (1.0
mm anterior to bregma and 3.0 mm lateral from the midline).” This positioning was the same for all rats.

Page 8, lines 9-14. This dosage of current density is important since it corresponds to one issue in the literature explaining inconsistent results among human studies. These stimulation parameters can be considered as “optimal” (page 5, lines 44-50)?

Our response: We agree that this is an interesting point of discussion and have commented on this in an additional section, ‘Histological effects of bilateral tDCS’, of the revised Discussion (page 11, lines 8-17):

"The stimulation parameters (current intensity of 200 µA, current density of 57.1 A/m2 and charge density of 51.4×103 C/m2) used in this study did not lead to histological damage. This suggests that these stimulation parameters, in line with proposed safety settings reported by Liebetanz and colleagues [24,25], are safe. Stimulation dosage is a critical aspect in the translation between animal and human tDCS studies. However, comparison of stimulation parameters may not be straightforward [45] and requires further computational and physiological validation."

Please confirm that anodal stimulation was applied over the right hemisphere while cathodal was done on the left.

Our response: We apologize that this may not have been clear. We now mention this more specifically in the Materials and Methods, in the ‘MRI-compatible tDCS’ section (page 6, lines 3-6), as well as in the legend of Figure 1B.

"A tDCS session consisted of 15-minute stimulation inside the MRI scanner, at a current intensity of 200 µA (anodal over the right sensorimotor cortex and cathodal over the left sensorimotor cortex)."

Pages 9-10. Due to the small sample size (especially in the session 2 of stimulation, n=4) effect size can be added when available. Final group sizes are seriously critical.

Our response: As a measure of effect size, we included the 95% confidence interval for all the beta estimates of the performed mixed linear models, which provides a measure of the magnitude of the effect. See Results, ‘Immediate effects of bilateral tDCS on BOLD signal’ (page 8, lines 18-23) for the newly added p-values:

"Within one minute after onset of tDCS, the BOLD signal increased with 0.68 ± 0.84 % (mean difference ± SD from baseline; p = 0.13, FDR-corrected) in the sensorimotor cortex underneath the cathode (left) (Figure 2b). BOLD signal changes in the sensorimotor cortex underneath the anode (right) were smaller (0.27 ± 0.30 % from baseline: p = 0.13, FDR-corrected)."

Also, see Results, ‘After-effects of bilateral tDCS on resting-state functional connectivity’ for the p-values that were originally shown (page 8, lines 49-52, 57-58):

"...In the left sensorimotor cortex, underneath the cathode, we found a significant interaction effect between group and time (beta = 0.169, 95% confidence interval = 0.0596-0.277, t(degrees-of-freedom: 58) = 3.10, p = 0.0030).... For the right sensorimotor cortex, underneath the anode, a significant time effect (beta = 0.134, 95% confidence interval = 0.0499-0.217, t(58) = 3.19, p = 0.0023) was found."
In this study, Boonzaier and coauthors aim to investigate the effects induced by bilateral tDCS on resting brain activity and functional connectivity using in vivo MRI in an animal model. Main findings were an increase, although not significant, in BOLD signal in different cortical areas, and a decrease of functional connectivity within the cortical sensorimotor network after a first stimulation session with a subsequent increase after a second session, for which the authors suggested the involvement of neural adaptation mechanisms.

Our response: In response to the reviewer’s suggestion, we have restructured the introduction to better explain the background, objectives and hypothesis of our study (see also our response to Reviewer #1). For example, we now state in the Introduction that (page 4, lines 46-52):

"...the main purpose of this exploratory study was to develop and apply an experimental in vivo setting for MRI during tDCS (in rats) to measure instant and resultant effects of cortical stimulation on whole-brain network status. This could aid in the elucidation of neuromodulatory actions of tDCS. We hypothesized that bilateral tDCS of the sensorimotor cortex induces a polarity-specific direct cortical activation response and, as an after-effect, polarity-specific modulation of functional connectivity within the sensorimotor network.”

In conclusion we emphasized the exploratory nature of our study and that further studies are needed to assess the effects under pathophysiological conditions, such as stroke (page 11, lines 31-38).

Experimental design. The rationale about the application of once stimulation or twice stimulations should be clearly explained and justified, as well as the choice not to do a fMRI during the second tDCS session.

Our response: We now explain in the Materials and Methods, in the ‘In vivo MRI, Imaging protocol and timeline’ section (page 6, lines 22-32) why we included a second stimulation session, and that simultaneous fMRI data was not acquired during the second tDCS session due to technical reasons:

“The rats then underwent a first period of tDCS (n = 7) or sham stimulation (n = 6), followed by post-stimulation-1 measurements with resting-state fMRI and ASL to assess the acute after-effects of tDCS. In addition, two short (5 minutes) fMRI acquisitions were performed at the start and end of the first
tDCS period, respectively, to assess the activation response during and directly after stimulation (tDCS group (n=7)). In a subset of rats (tDCS group: n = 4; sham stimulation group: n = 4), a second 15-minute tDCS session was performed (without simultaneous fMRI, due to technical reasons), followed by a post-stimulation-2 measurement with resting-state fMRI and ASL, to evaluate whether a second stimulation would have similar or dissimilar effects.

Additionally, I think that from a methodological point of view this study suffers for the lack of an evaluation of neural changes induced on-line by sham tDCS on BOLD signal, that could allow a direct comparison between the immediate effects induced by a real bilateral tDCS vs sham tDCS.

Our response: We agree; this has been added to the Discussion as a limitation, see 'Study limitations' (page 11, lines 20–26): "Also, we did not include electrophysiological measurements that could provide additional insights into to the mechanism of action of bilateral tDCS."

For the analysis of resting-state fMRI and perfusion MRI, it is unclear the rationale underlying the choice to calculate difference values comparing for the first stimulation, post-stimulation-1 vs pre-stimulation and for second stimulation, post-stimulation-2 vs post-stimulation-1.

Our response: We agree that the rationale for the difference value comparisons for stimulation-1 (post1-pre value) and stimulation-2 (post2-post1 value) may have been unclear. To clarify, these comparisons were performed to investigate the independent effects of the first and second stimulations on resting-state fMRI and perfusion MRI. We have added this explanation to the manuscript; see the Materials and Methods, 'Statistical analyses' section, (page 7, lines 34–35): "These comparisons were performed to investigate the independent effects of the first and second stimulations on functional connectivity and CBF."

Have the authors compared post-stimulation-2 value vs pre-stimulation value?

Our response: This comparison would provide an overall effect of both stimulations. In response to the reviewer's suggestion, we ran an additional analysis, also including the post2-pre delta value in the linear mixed model. The results from this analysis were the same as from the initial analysis reported in the manuscript:

- A significant time*group interaction effect in the left sensorimotor network
- A significant time effect in the right sensorimotor network
- No significant effects in the left or right sensorimotor network

The linear mixed model compared post1-pre to post2-pre (1 stimulation session compared to 2 stimulation sessions).

Below is an overview of the results of the additional analysis. We have however, not incorporated this into the main manuscript, since it does not add any further clarification to the effects already seen when comparing the independent effects of stimulation-1 (post1-pre value) and stimulation-2 (post2-post1 value), and assessment of the combined effect was not a study goal.

Figure 1: Changes in the inter- and intrahemispheric functional connectivity following bilateral tDCS. These graphs illustrate the independent effects of tDCS on functional connectivity after stimulation 1, stimulation 2 as well as the overall effects of both stimulations (stimulation 1 and 2). (A) Change in intrahemispheric (left and right) and (B) interhemispheric functional connectivity (Δ(Fisher’s z’)) between regions of interest within the sensorimotor and visual cortices, following stimulation (tDCS or sham stimulation) session 1 and 2. L=left, R=right, M1=primary motor cortex, M2=secondary motor cortex, S1HL=hind-limb region of the somatosensory cortex, S1FL=fore-limb region of the primary somatosensory cortex, S2=secondary somatosensory cortex, V1=primary visual cortex, V2=secondary visual cortex.

| Resting-state | fMRI | Statistical analyses: |
|---------------|------|-----------------------|
| LEFT HEMISPHERE |
| Time * group interaction: F(116) = 6.278, p = 0.0026. |
| Post1-pre post2-post1: b=0.165; 95% CI: 0.064 – 0.266. |
| Post1-pre post2-pre: b=0.146; 95% CI: 0.045 – 0.247. (Additional result) |
| RIGHT HEMISPHERE |
| Time effect: F(116) = 23.589, p< 0.0001. |
| Post1-pre post2-post1: b=0.135; 95% CI: 0.051 – 0.220. |

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Figure 2: CBF change after bilateral tDCS. These graphs illustrate the independent effects of tDCS on CBF after stimulation 1, stimulation 2 as well as the overall effects of both stimulations (stimulation 1 and 2). Black dots represent individual data points. L=left, R=right.

Perfusion MRI Statistical analyses

LEFT HEMISPHERE
Time * group interaction: F(46) = 0.269, p = 0.7353.
Post1-pre post2-pre: b= 0.048; 95% CI: 0.036 – 0.133. (Additional result)
Post1-pre post2-pre: b= 5.012; 95% CI: 22.947 – 32.972. (Additional result)

RIGHT HEMISPHERE
Time effect: F(46) = 0.009; p=0.9905.
Post1-pre post2-pre: b=0.358; 95% CI: 26.976 – 27.693.
Post1-pre post2-pre: b=1.779; 95% CI: 25.555 – 29.115. (Additional result)

In my opinion, every possible conclusion about the results is seriously affected by the very small sample size, also considering the exclusion of animals due to MR artifacts.

Our response: We agree and have added this as a limitation of the study in the Discussion, see Study limitations (page 11, lines 20-26): "Our study, which is exploratory in nature, is limited by the small sample size, which was further affected by a number of technical issues that led to the drop-out of some animals. Also, we did not include electrophysiological measurements that could provide additional insights into to the mechanism of action of bilateral tDCS." The 'Conclusion' section has been adjusted accordingly (page 11, lines 29-38).

In the figure 1 about the experimental design, the authors should report how many animals participated in each MR imaging evaluation and stimulation session.

Our response: In response to the reviewer’s suggestion we have adapted Figure 1 to show how many animals contributed to the study and how many animals were used in each of the MR imaging evaluation and stimulation sessions. All boxplot figures have also been adjusted to illustrate individual animals/data-points in each graph.

Concluding, I think that the findings reported by the authors are not sufficient to support their conclusions.

Our response: In response to the reviewer’s criticism, we have adjusted our conclusions to fit that of an exploratory study (page 11, lines 29-38): "In conclusion, our exploratory study shows successful application of an MRI-compatible bilateral tDCS setup in an animal model. Our results demonstrate that bilateral tDCS over the sensorimotor cortex affects signals and signaling within and across the sensorimotor cortical network in the healthy rat brain. This includes modulation of neuronal activity and connectivity as measured with functional MRI. Further studies are needed to assess these effects under pathophysiological conditions, such as after stroke, where (bilateral) tDCS may contribute to functional recovery."

Reviewer #3:

Our response: We thank the reviewer for his/her time and efforts to critically evaluate our paper and for pointing out several aspects of this manuscript that needed improvement. We have tried to address all his/her specific comments in the revised manuscript, which we address separately below.

Comments to the Author

The study aims to understand the mechanism of tDCS using rodent fMRI. As similar study can be and have been done in humans, the novelty and insights demonstrated in this manuscript is limited. Also, because of the limited number of animal and experimental design, the results appear to be preliminary. Specific comments are:

1. Similar attempts had been done in rats (eg, Takano et al. 2011) but was not mentioned at all in this paper. There are also papers in humans need to critically review.
Our response: Takano et al. (2011) is now referenced in the Introduction (page 4, lines 28-30) and Discussion (page 9, lines 37-38). See Introduction: "Direct and delayed effects of tDCS at a whole-brain network level may be effectively measured with in vivo neuroimaging tools, such as optical spectroscopy/imaging [12–14] and magnetic resonance imaging (MRI) [15]." See Discussion, ‘Hemodynamic changes in response to tDCS’ section: “Previous studies in humans [13,32] and rats [15] have reported a BOLD activation response to tDCS, which was particularly noticeable in regions underneath the stimulation sites.”

2. fMRI Method: was the rat given muscle relaxant during mechanical ventilation? Without muscle relaxant, the spontaneous breathing will counteract the ventilator and affect the respiration rate and EtCO2.

Our response: In response to the reviewer’s comment: The scanning and anesthesia protocols in our lab have been developed and optimized to prevent spontaneous breathing against the ventilator without the use of muscle relaxants. Physiological stability during all MRI acquisitions was maintained through continuous monitoring of expired CO2 levels, and adjustments of ventilation rate or volume when necessary.

3. Resting fMRI preprocessing: motion parameters alone is not sufficient for removing nuisance. Additional consideration for the physiological noise is needed.

Our response: We are aware of the potential effects of physiological noise. Regression of motion parameters have previously been demonstrated to remove most of respiratory noise from resting-state fMRI data in rodents (Kalthoff et al., 2011. Neuroimage 54, 2828-2839). Inspecting the motion correction parameters showed that the translational motion in our study was in the order of maximum 20% of the voxel size, so we believe additional corrections for motion were not necessary. We did not perform any direct corrections for physiological noise because the physiological recordings were not complete for all rats. The relatively short resting-state fMRI repetition time (TR) resulted in the breathing frequency to occur outside the frequency band we used for the analysis. Hence the data was less prone to aliasing of physiological noise (Pais-Roldán et al., 2018. Front. Neurosci. 12, 788). We mechanically ventilated the animals to have a constant breathing rate during resting-state fMRI. The band-pass filtering removed physiological noise-related frequencies outside of our frequency range of interest (i.e. below 0.01 and above 0.1 Hz was removed).

Kalthoff, D., Seehafer, J. U., Po, C., Wiedermann, D. & Hoehn, M. Functional connectivity in the rat at 11.7T: Impact of physiological noise in resting state fMRI. Neuroimage 54, 2828–2839 (2011).

Pais-Roldán, P., Biswal, B., Scheffler, K. & Yu, X. Identifying respiration-related aliasing artifacts in the rodent resting-state fMRI. Front. Neurosci. 12, 788 (2018).

4. The physiological conditions should be reported for each period the fMRI was measured.

Our response: In response to the reviewer’s suggestion we have added a section about the physiological conditions during the two fMRI periods in the Results; see section ‘Immediate effects of bilateral tDCS on BOLD signal’ (page 8, lines 35-37): “Physiological conditions during simultaneous tDCS and fMRI were stable (see Supplementary Material, Figure S3).”

In the revised supplementary material, we have listed the following physiological parameters, measured during fMRI and during tDCS: expired CO2, SpO2, breathing rate, heart rate and temperature. The physiological parameters are shown in Supplementary Figure S3 (page 6), in which the parameters are given for fMRI during tDCS ON (start of the first tDCS-session) and for fMRI during tDCS OFF (end of the first tDCS-session).

5. The sample size after quality control is insufficient, making the results unconclusive.

Our response: We agree that our sample size is small and have acknowledged this in the Discussion as a limitation of our study (see ‘Study limitations’, page 11, lines 20-26): “Our study, which is exploratory in nature, is limited by the small sample size, which was further affected by a number of technical issues that led to the drop-out of some animals.” Additionally, we have emphasized the exploratory nature of this study in both the Introduction (page 4, lines 46-49) and Conclusion (page 11, lines 29-32), for example we stated the following in the Introduction:
... the main purpose of this exploratory study was to develop and apply an experimental in vivo setting for MRI during tDCS (in rats) to measure instant and resultant effects of cortical stimulation on whole-brain network status."

6. The activation induced by tDCS (Fig.2) appears to colocalize with large vessel. Please elaborate and discuss the possible mechanisms and how that would impact BOLD and CBF response in the cortex.

Our response: In response to the reviewer’s suggestion, we have explained that tDCS may stimulate smooth muscle cells in vessel walls and consequently cause a BOLD response around large vessels (see Discussion, page 9, lines 40-44): "The observed increases in BOLD signal during bilateral tDCS may reflect direct effects of tDCS on cortical vessels, or a hemodynamic response to neuronal activation through neurovascular coupling [33]. A direct effect of tDCS on smooth muscle cells in the vessel walls could explain co-localization of BOLD signal increase with large vessels [34]."

7. The BOLD signal during the switching off of tDCS was measured but not reported. As some regions appear to have a more delayed activation, it would be interesting to compare the off-response.

Our response: In response to the reviewer’s comment, we have now included analysis of the BOLD signal immediately after tDCS as well; see Figure 2c and the Results, section 'Immediate effects of bilateral tDCS on BOLD signal’ (page 8, line 31-34). “For all ROIs, when tDCS was switched off, the BOLD signal remained at the same level within the first two minutes. Accordingly, no significant differences were measured between the BOLD signals during and after stimulation in the second fMRI session (Figure 2c; Supplementary Material, Figures S1 and S2).”

8. There were significant changes in functional connectivity the sham control group. This raised a concern about the stability of the signal and/or experimental condition like indicated in points 1 and 2.

Our response: To look into this more carefully, we ran a linear model in which we determined whether delta values for stimulation 1 and stimulation 2 differed between control and sham animals. We found a significant interaction effect; which means that the effect of both stimulations was different between control and sham animals. In the figure below (Supplementary Figure S5, page 9) functional connectivity changes are apparent in the sham group, but they are quite random (some are positive, some are negative). The levels of change are smaller and more variable as compared to the tDCS group. There is no clear explanation for the observed changes in the sham group. We speculate that it might be due to prolonged anesthesia exposure. Nevertheless, changes were different between the sham and tDCS groups, which confirm the indication of a tDCS effect.

9. Fig.4: please only show the significantly changed connections.

Our response: Although we appreciate the suggestion of the reviewer, it does not fit with our statistical modeling approach. We used a linear mixed model that included all connections within the sensorimotor network in a single model, and therefore the output of the model provides statistical significance of the whole model. It does not provide information about the significance of change at the level of single connections. This has been clarified in the Materials and Methods, ‘Statistical analyses’ section (page 7, line 40-45): "The model was hierarchically structured with random intercepts and network connections nested within animals. Consequently, the linear mixed model includes all network connections into a single model. The output informs on statistical significance for the whole model, but does not provide statistical information for single connections.” Another option would be to perform further analyses on each connection separately, but this will lead to multiple comparisons requiring correction for multiple testing and ultimately limited chances for detecting significant results.

10. Despite using histology to show no lesion induced by the procedure, there is no measures (eg, electrophysiology) to help understanding the mechanism of tDCS.

Our response: We agree, this has been added to the Discussion as a limitation of this study, see 'Study limitations’ (page 11, lines 20-26): “...we did not include electrophysiological measurements that could provide additional insights into to
the mechanism of action of bilateral tDCS."

11. Lots of disparity among human studies are related to the way tDCS was applied. Although the paper pointed out this issue to highlight the need for animal study, this study didn’t look into that at all.

Our response: We have added a section about this in the Discussion, see ‘Histological effects of bilateral tDCS’ (page 11, lines 14-17): “Stimulation dosage is a critical aspect in the translation between animal and human tDCS studies. However, comparison of stimulation parameters may not be straightforward [45] and requires further computational and physiological validation.”

12. Considering the number of animal, it’s not clear why the degree of freedom was 58 on p.10.

Our response: We agree that this was unclear. In order to clarify, we have added a reference to the following text in the Supplementary Material under ‘Statistical analyses’ (page 4, lines 16-21): “The degrees of freedom were determined in the mixed linear model. We used ten connections in one model for the left and right sensorimotor network. The linear mixed linear model estimates the degrees of freedom for the complete dataset, which included two rats from the sham group and four rats from the tDCS group. The degrees of freedom were then calculated as (2x10) – 1 + (4x10) – 1 = 58.”

2nd Editorial Decision

Decision Letter

Dear Dr Dijkhuizen:

Thank you for submitting your manuscript to the Journal of Neuroscience Research. We've now received the reviewer feedback and have appended those reviews below. I'm glad to say that the reviewers are very enthusiastic. Still there are some few changes to be made before your manuscript is fully accepted for publication. I expect that these points should be relatively straightforward to address. If there are any questions or points that are problematic, please feel free to contact me. I am glad to discuss.

We ask that you return your manuscript within 30 days. Please explain in your cover letter how you have changed the present version. If you require longer than 30 days to make the revisions, please contact Dr Cristina Ghiani (cghiani@mednet.ucla.edu). You can submit your revised manuscript directly by clicking on the following link: *** PLEASE NOTE: This is a two-step process. After clicking on the link, you will be directed to a webpage to confirm. ***

https://mc.manuscriptcentral.com/jnr?URL_MASK=abb46aade6de419a8bdb4f6d3e7a85b5

Thank you again for your submission to the Journal of Neuroscience Research; we look forward to reading your revised manuscript.

Best Wishes,

Dr Sandra Chanraud
Associate Editor, Journal of Neuroscience Research

Dr Cristina Ghiani
Co-Editor-in-Chief, Journal of Neuroscience Research

Associate Editor: Chanraud, Sandra

Comments to the Author:

It is a pleasure to accept your manuscript entitled “Activation response and functional connectivity change in rat cortex after bilateral transcranial direct current stimulation” in its current form for publication in Journal of Neuroscience Research. However, you should modify as much as possible the paper based upon the comments made by one reviewer.

Please note that although the manuscript is accepted, the files will now be checked to ensure that everything is ready for publication, and you may be contacted if final versions of files for publication are required.

Thank you for your fine contribution. On behalf of the Editors of Journal of Neuroscience Research.

Sincerely,

Dr Sandra Chanraud.
Reviewer: 3

Comments to the Author
1. The anaesthesia and dosage used and physiological conditions (temperature, heart rate, respiration, SpO2 or pO2/pCO2) during fMRI scan are essential and should be briefly summarized in the method section.
2. The sample size is quite small and reduced a lot in the post-stimulation measurements. This may be the reason why most results are insignificant or comparable between the tDCS and sham groups. The authors should consider adding more animals or tone down the difference they observed.
3. The BOLD activation shown in Fig.2a colocalized with large vessels, indicating a strong vascular effect, which is explained in the discussion. Such vascular effect can strongly confound the BOLD signal observed in cortical areas. One way to evaluate the confound could be to use the large vessel signal as a covariate in GLM. Please also discuss this issue and possible ways to minimize that.
4. Lack of task fMRI (e.g., forepaw stimulation) to evaluate the modulatory effects on cortical excitability.
5. There is no correction of baseline signal drift in fMRI. This is particularly critical for long scan with very low task frequency in the tDCS-fMRI study. Besides, as the data appears to be quite noisy, the authors could consider applying low-pass filter to their data to reduce the high-frequency noise.
6. 1.5% isoflurane is a bit high for rat fMRI. This may partly explain the weak BOLD signal and weak or negative bilateral functional connectivity between M1, S1FL, V2 in the sham control group. Besides, bursting-suppression activity may dominate the resting fMRI at this isoflurane level.
7. Why only the first few minutes were studied in tDCS-fMRI?
8. Please show example EPI so that readers can evaluate the image quality with the tDCS implants.
9. As the electrodes are quite big, the broad tDCS effects on functional connectivity is likely due to the very distributed current field.
10. P.13 “Hyperpolarizations reduce neural activity, lead to an increase in physiological signal-to-noise ratio, and consequently increase synchronization within the stimulated area.” Does that mean the increased synchronization is due to physiological synchronization but not neural synchronization? This statement seems to suggest that functional connectivity findings are just physiological artifacts.
11. P.14 “these differences emphasize the need for systematic and thorough studies on the physiological effects of tDCS.” Please be specific what kind of physiology should be examined.

Reviewer: 1

Comments to the Author
I would like to thank the authors for their consideration of the comments and subsequent amendments to the manuscript. With the additional information I hope the manuscript will allow a wider audience to interpret their first findings. I have no further major comments.

This exploratory study dealing with challenges in combining state-of-the-art techniques should be highlighted in the title, as for instance - Activation response and functional connectivity change in rat cortex after bilateral transcranial direct current stimulation - Experimental approaches or model?

Authors’ Response

Authors’ Response
24/07/2020

*Please note, referenced manuscript page numbers (by the author) refer to the page number at the bottom of the page.

Reviewers’ comments:

Reviewer #3:
We thank the reviewer for his/her time and efforts to critically evaluate our paper and for the recommendations to improve it. We have dealt with all his/her points in the revised manuscript, which we address separately below.

Comments to the Author:
1. The anaesthesia and dosage used and physiological conditions (temperature, heart rate, respiration, SpO2 or pO2/pCO2) during fMRI scan are essential and should be briefly summarized in the method section.

We agree with the reviewer and we have moved some text about the anesthesia dosage and physiological monitoring from the Supplementary material to the main Methods section (see page 7 and 8, line 60 and lines 3-16, respectively): "Anesthesia and physiological monitoring
Shortly before in vivo MRI, animals were endotracheally intubated for mechanical ventilation with 2%
isoflurane in O2/air (4:1). During MRI acquisition the respiration rate and end-tidal CO2 were closely monitored with a capnograph (Multinex 4200, Dataspore Corporation, Paramus, NJ, USA). Heart rate and blood oxygen saturation were monitored with a pulse oximeter (Nonin 8600V, Nonin Medical Inc., Plymouth, MN, USA), and body temperature was maintained at 37.0 ± 1.0 °C using a feed-back mechanism with a warm water circuit. Fifteen minutes prior to functional fMRI acquisition, the isoflurane percentage was lowered to 1.5% to reduce anesthetic effects on subsequent functional MRI scans.

2. The sample size is quite small and reduced a lot in the post-stimulation measurements. This may be the reason why most results are insignificant or comparable between the tDCS and sham groups. The authors should consider adding more animals or tone down the difference they observed.

We agree with the reviewer and have added the following to the Discussion (page 14, lines 33-36):
“However, our sample size was small and further experiments are needed to confirm these specific findings.”

3. The BOLD activation shown in Fig.2a colocalized with large vessels, indicating a strong vascular effect, which is explained in the discussion. Such vascular effect can strongly confound the BOLD signal observed in cortical areas. One way to evaluate the confound could be to use the large vessel signal as a covariate in GLM. Please also discuss this issue and possible ways to minimize that.

We have mentioned this as an option for follow-up studies in the Discussion; see the Study Limitations section (page 15, lines 5-9):
“Prospective follow-up studies should also consider strategies that minimize possible confounding effects, such as ... inclusion of signal from large vessels as a covariate in analyses.”

4. Lack of task fMRI (eg, forepaw stimulation) to evaluate the modulatory effects on cortical excitability.

We agree and now mention in the Discussion section (page 14 and 15, lines 60 and 3, respectively):
“Also, we did not measure the modulatory tDCS effects on cortical excitability, ....”

5. There is no correction of baseline signal drift in fMRI. This is particularly critical for long scan with very low task frequency in the tDCS-fMRI study. Besides, as the data appears to be quite noisy, the authors could consider applying low-pass filter to their data to reduce the high-frequency noise.

We have mentioned this as a suggestion for follow-up studies in the Discussion; see the Study Limitations section (page 15, lines 5-9):
“Prospective follow-up studies should also consider strategies that minimize possible confounding effects on the BOLD fMRI signal, such as .... correction for signal drift....”

6. 1.5% isoflurane is a bit high for rat fMRI. This may partly explain the weak BOLD signal and weak or negative bilateral functional connectivity between M1, S1FL, V2 in the sham control group. Besides, bursting-suppression activity may dominate the resting fMRI at this isoflurane level.

We are aware of the effects of (isoflurane) anesthesia on the BOLD signal, and have now mentioned the following in the Discussion (page 15, lines 5-9):
“Prospective follow-up studies should also consider strategies that minimize possible confounding effects on the BOLD fMRI signal, such as lower anesthesia levels....”

7. Why only the first few minutes were studied in tDCS-fMRI?

The largest changes in fMRI signal are to be expected during switching on and off of the stimulator. We measured for 5 minutes to allow stabilization of the signal, which we indeed observed pre- and post-stimulation. We refrained from continuous fMRI for the 10 minutes of tDCS inbetween, to limit the fMRI data file size (page 8, lines 34-35).
“To limit the fMRI data file size, we discontinued scanning during the ca. 10 minutes of tDCS in-between.”

8. Please show example EPI so that readers can evaluate the image quality with the tDCS implants.

We would like to refer the reviewer to Figure 4A, which shows a representative EPI.

9. As the electrodes are quite big, the broad tDCS effects on functional connectivity is likely due to the very distributed current field.
We have added the following to the Discussion (see page 12 and 13, lines 60 and 3, respectively):
“In addition, the electrodes were relatively large compared to the rat brain, which probably contributed to the widespread response.”

10. P.13 “Hyperpolarizations reduce neural activity, lead to an increase in physiological signal-to-noise ratio, and consequently increase synchronization within the stimulated area.” Does that mean the increased synchronization is due to physiological synchronization but not neural synchronization? This statement seems to suggest that functional connectivity findings are just physiological artifacts.

We apologize for the confusion; it is hypothesized that the increased synchronization is indeed due to increase neuronal synchronization. The text in the Discussion has been adapted accordingly (page 14, lines 3-13):
“It was hypothesized that this increase in functional connectivity following cathodal tDCS is due to neuronal hyperpolarization. Hyperpolarizations would effectively reduce local neuronal noise, lead to an increase in signal-to-noise, and increase neuronal synchronization within the stimulated area.”

11. p.14 “these differences emphasize the need for systematic and thorough studies on the physiological effects of tDCS.” Please be specific what kind of physiology should be examined.

We have specified this by referring to neurophysiology in the revised Discussion (page 14, line 21):
“These differences emphasize the need for systematic and thorough studies on the neurophysiological effects of tDCS.”

Reviewer #1:
Comments to the Author
I would like to thank the authors for their consideration of the comments and subsequent amendments to the manuscript. With the additional information I hope the manuscript will allow a wider audience to interpret their first findings. I have no further major comments.

This exploratory study dealing with challenges in combining state-of-the-art techniques should be highlighted in the title, as for instance - Activation response and functional connectivity change in rat cortex after bilateral transcranial direct current stimulation - Experimental approaches or model?

We thank the reviewer for his/her time and efforts to critically evaluate our paper and for pointing out several aspects of this manuscript that needed improvement. We have now indicated in the title that our paper concerns an exploratory study (see title page):
"Activation response and functional connectivity change in rat cortex after bilateral transcranial direct current stimulation – an exploratory study”

3rd Editorial Decision
Decision Letter
Dear Dr Dijkhuizen:

Thank you for resubmitting your manuscript to the Journal of Neuroscience Research. We've now received the reviewer feedback and have appended those reviews below. The reviewers and editors are overall very enthusiastic and supportive of the study. However, your manuscript is not yet suitable for publications and few more changes must be made before it is accepted. These points should be relatively straightforward to address. If there are any questions or points that are problematic, please feel free to contact me. I am glad to discuss.

We ask that you return your manuscript within 30 days. Please explain in your cover letter how you have changed the present version. If you require longer than 30 days to make the revisions, please contact Dr Cristina Ghiani (cghiani@mednet.ucla.edu). You can submit your revised manuscript directly by clicking on the following link: *** PLEASE NOTE: This is a two-step process. After clicking on the link, you will be directed to a webpage to confirm. ***

https://mc.manuscriptcentral.com/jnr?URL_MASK=1c517335df7049beba92b3f2b31bf55b

Thank you again for your submission to the Journal of Neuroscience Research; we look forward to reading your revised manuscript.
Best Wishes,

Dr Sandra Chanraud
Associate Editor, Journal of Neuroscience Research

Dr Cristina Ghiani
Editor-in-Chief, Journal of Neuroscience Research

Associate Editor: Chanraud, Sandra
Comments to the Author:
Please consider modifying your MS accordingly to the last remarks from both one reviewer and our Statistical Editor before publication.
Thank you for your fine contribution. On behalf of the Editors of Journal of Neuroscience Research.
Sincerely,
Dr Sandra Chanraud.

Statistics Editor: McArthur, David
Comments to the Author:
The continued use of box and whisker plotting when sample sizes are tiny is not useful. A simple reason is that when the number of cases is as small as 2, the resulting boxplot cannot be anything but fully symmetrical, yet the confidence in that symmetry is necessarily close to nil. Thus such boxplots are only minimally useful at best. The situation improves as one adds cases but not but much. Kindly delete the boxplots but keep the dotplots wherever they are displayed.

Reviewer: 3

Comments to the Author
1. Despite being mechanically ventilated, the spO2 level fluctuated a lot (Fig.S3). From the graph it is unclear whether the large variation is due to large individual difference or large fluctuation over time. If there is large individual variation, then the physiological control was sub-optimal and may contribute to more variable results. If there is large fluctuation over time, it may confound the fMRI readout as fMRI signal is highly depends on oxygenation level.
2. Correcting baseline drift would improve the data quality and results. It is strange that this is not done.

Authors’ Response
Dear Drs. Chanraud and Ghiani,
Thank you for reviewing our work and for giving us the opportunity to revise our paper.

We have revised the manuscript according to the suggestions of Reviewer #3 and the Statistical Editor. First, we have replaced boxplots by dotplots. Second, we have explained that intraindividual spO2 recordings could be affected by measurement drift, but that levels were always within the normal range. Third, we have mentioned that no baseline drift correction was applied; our tDSC-fMRI measurements were normalized to the mean baseline signal, and our resting-state fMRI analyses excluded signals below 0.01 Hz, which should largely correct for baseline drift.

We hope that our revised manuscript now meets the standard of your journal and we are looking forward to hearing from you.

Yours sincerely,
On behalf of all authors,

Julia Boonzaier, MSc
Rick M. Dijkhuizen, PhD

Center for Image Sciences
University Medical Center Utrecht
Decision Letter

Dear Dr Dijkhuizen:

Thank you for submitting your manuscript "Activation response and functional connectivity change in rat cortex after bilateral transcranial direct current stimulation – an exploratory study" by Boonzaier, Julia; Straathof, Milou; Ardesch, Dirk Jan; Toorn, Annette van der; Vliet, Gerard van; Heijningen, Caroline L. van; Otte, Willem; Dijkhuizen, Rick.

You will be pleased to know that your manuscript has been accepted for publication. Thank you for submitting this excellent work to our journal.

In the coming weeks, the Production Department will contact you regarding a copyright transfer agreement and they will then send an electronic proof file of your article to you for your review and approval.

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Would you be interested in publishing your proven experimental method as a detailed step-by-step protocol? Current Protocols in Neuroscience welcomes proposals from prospective authors to disseminate their experimental methodology in the rapidly evolving field of neuroscience. Please submit your proposal here: https://currentprotocols.onlinelibrary.wiley.com/hub/submitaproposal

Congratulations on your results, and thank you for choosing the Journal of Neuroscience Research for publishing your work. I hope you will consider us for the publication of your future manuscripts.

Sincerely,

Dr Sandra Chanraud
Associate Editor, Journal of Neuroscience Research

Dr Cristina Ghiani
Editor-in-Chief, Journal of Neuroscience Research

Reviewer: 3

Comments to the Author

The authors have addressed my concerns.