JOURNAL OF Neuroscience Research

Comparison of the effects of two therapeutic strategies based on olfactory ensheathing cells transplantation and repetitive magnetic stimulation after spinal cord injury in female mice

Guérout, Nicolas; Delarue, Quentin; Robac, Amandine; Massardier, Romane; Marie, Jean-Paul

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Review timeline: Submission date: 5 August 2020
Editorial Decision: Major Modification (23 December 2020)
Revision Received: 4 March 2021
Editorial Decision: Accept with Minor Edits (3 April 2021)
Revision Received: 12 April 2021
Accepted: 15 April 2021

Editor 1: Jeremy Hogeveen
Editor 2: Cristina Ghiani
Reviewer 1: Maria Rodriguez-Ayllon
Reviewer 2: Christopher Watson
Reviewer 3: Alena Svatkova

1st Editorial Decision

Decision letter
Dear Miss Meijer:

Thank you for submitting your work to the Journal of Neuroscience Research. The manuscript has now been reviewed by myself and three external expert reviewer. I sincerely apologize for the delay, finding reviewers during the pandemic has been a challenge. While the reviewers and I agree that the manuscript is on a critical topic given physical inactivity and obesity are major public health challenges and an understanding of the basic neurobiological impact of physical fitness interventions is essential, our enthusiasm was tempered by some major concerns with aspects of the manuscript. As a result, I cannot accept the manuscript in its current form, but would be willing to receive a revised version of the manuscript that addresses the reviewers' significant concerns.

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 and submit a point by point response to the editors' and reviewers' comments. If you require longer than 90 days to make the revisions, please contact Dr Cristina Ghiani (cghiani@mednet.ucla.edu). To submit your revised manuscript: Log in by clicking on the link below

(If the above link space is blank, it is because you submitted your original manuscript through our old submission site. Therefore, to return your revision, please go to our new submission site here (submission.wiley.com/jnr) and submit your revision as a new manuscript; answer yes to the question "Are you returning a revision for a manuscript originally submitted to our former submission site (ScholarOne Manuscripts)? If you indicate yes, please enter your original manuscript's Manuscript ID number in the space below" and including your original submission's Manuscript ID number (jnr-2020-Aug-8980) where indicated. This will help us to link your revision to your original submission.)

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

Best Wishes,

Dr Jeremy Hogeveen
Associate Editor, Journal of Neuroscience Research

JOURNAL OF Neuroscience Research

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

Associate Editor: Hogeveen, Jeremy
Comments to the Author:
Dear Ms. Meijer,

Thank you for submitting your work to the Journal of Neuroscience Research. The manuscript has now been reviewed by myself and three external expert reviewer. I sincerely apologize for the delay, finding reviewers during the pandemic has been a challenge. While the reviewers and I agree that the manuscript is on a critical topic given physical inactivity and obesity are major public health challenges and an understanding of the basic neurobiological impact of physical fitness interventions is essential, our enthusiasm was tempered by some major concerns with aspects of the manuscript. As a result, I cannot accept the manuscript in its current form, but would be willing to receive a revised version of the manuscript that addresses the reviewers' significant concerns.

Best Wishes,

Dr Jeremy Hogeveen
Associate Editor, Journal of Neuroscience Research

Additional editorial Comments:

Please format the abstract following the JNR guidelines.
Please carefully review the files attached to this email as they may contain the reviewer's comments.

Please add to your paper (after the Discussion and Acknowledgments, immediately before the References) a statement of authors' contributions. The statement must follow the CRediT Taxonomy. You can find examples of such statements in the author guidelines on-line at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1097-4547/homepage/ForAuthors.html.

GRAPHICAL ABSTRACT
Please upload a graphical abstract, which we are asking of all authors submitting original research articles. This is intended to provide readers with a visual representation of the conclusions and an additional way to access the contents and appreciate the main message of the work. What we require is a .tif image file and a .doc text file containing an abbreviated abstract. For the image, labels, although useful, must be kept to a minimum and the image should be 400 x 300, 300 x 400, or 400 x 400 pixels square and at a resolution of 72 dpi. This can be one of the figures from your article, or something slightly different, as long as it represents your study. Instructions for this can be found in our author guidelines online at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1097-4547/homepage/ForAuthors.html

Reviewer: 1

Comments to the Author
Reviewer report

Summary

Thank you very much for providing me the opportunity to review the manuscript entitled “The Relationship between White Matter, Cardiovascular Fitness, Gross Motor skills and Neurocognitive Functioning in Children”. This manuscript aims to investigate the role of white matter in the relationship between cardiovascular fitness or gross motor skills and neurocognitive functioning in healthy children. The research topic is relevant and has the potential to aid in the field of exercise screening questionaries.

Minor issues

Tittle

1. I would include the word “microstructure” in the title since there are also other white matter features

such as a white matter hyperintensity or white matter volume. "The Relationship between White Matter Microstructure, Cardiovascular Fitness, Gross Motor skills and Neurocognitive Functioning in Children". This comment is extensible to the whole manuscript.

Abstract

1. I would include the sample characteristics (N = 92, mean age 9.1 years, range 8.0 – 10.7) in the methods section of the Abstract.
2. I would change the first sentence of the abstract. Physical activity (i.e., any bodily movement produced by skeletal muscles that result in energy expenditure) and cardiorespiratory fitness (i.e., the overall capacity of the cardiovascular and respiratory systems and the ability to carry out prolonged strenuous exercise) are different concepts.
3. The aim of the study is not clear to me. I expected to see the mediating role of white matter microstructure in the relationship between CRF/gross motor skills and neurocognitive functioning when I read the aim. However, in the results section, you reported, independently, the association between CRF/gross motor skills and white matter and the association of CRF/gross motor skills with cognitive function but not the mediating role. If you tested also the mediating role but you didn't find any significant association, please, include also this information in the abstract section since it is the most novel finding of this paper.

Significance statement

1. There are other studies that investigated the relationship between physical activity, white matter, and cognitive function. Additionally, the relationship between cardiorespiratory fitness, white matter, and cognitive function has been investigated. Please, have a look at these studies from the FITKids, the ActiveBrains project, or the Generation R. It is true, that most of them, did not test the mediating role of white matter in the relationship between cardiorespiratory fitness and neurocognitive functioning in healthy children. Therefore, since my point of view, this is the most important finding of the paper, and therefore might be highlighted in this section.

I enclose to you the link of some papers that tested independently the relationship between physical activity or cardiorespiratory fitness and white matter or cognitive functioning in children. I have seen that some of them were cited but you should have a look at the rest of the papers I suggested to you.

https://pubmed.ncbi.nlm.nih.gov/31605827/
https://pubmed.ncbi.nlm.nih.gov/30618578/
https://pubmed.ncbi.nlm.nih.gov/31058358/
https://pubmed.ncbi.nlm.nih.gov/30809168/
https://pubmed.ncbi.nlm.nih.gov/24797659/
https://pubmed.ncbi.nlm.nih.gov/25191243/
https://pubmed.ncbi.nlm.nih.gov/31876665/
https://pubmed.ncbi.nlm.nih.gov/32719329/
https://pubmed.ncbi.nlm.nih.gov/24457421/

Introduction

Page 3, Lines 28-32: Are those studies in children? If not, I would use references only in children.
Page 3, Lines 32-33: There are a few studies that tested the effect and the relationship between physical activity and white matter microstructure. Please, include some references here (see the references I suggested in the Significant Statement comment).
Page 3, Line 43: Why you did not include Mean diffusivity? It is another common DTI measure that normally is included together white FA, RD, and AD measures.
Page 4, Line 34: Please include this study between the references (https://pubmed.ncbi.nlm.nih.gov/32719329/).
Page 6, line 5: I wouldn´t assume that cardiorespiratory fitness is a pathway across physical activity improves white matter. For that purpose, you should test the mediating role of cardiorespiratory fitness in the relationship between physical activity and white matter, or at least, you should cite a paper confirming that assumption. I would focus the paper on cardiorespiratory fitness instead of including the physical activity concept since it could be confusing. I extend this comment to the all manuscript.
Page 6, lines 3-9: The aims of this study are not clear to me.
Page 6, line 22: I see now, that you tested the mediating role. Please, include this information in the Abstract Section.

Methodology

Page 10, line 5. Did you use two different MRI scans? I would include it as a limitation of the study. Did you run any sensitivity analysis to check if there are differences between MRI scans?
Page 11, lines 14-22. After reading the statistical analysis, I see two different aims. Aim 1. To test the independent association of cardiorespiratory fitness and motor skills with white matter and neurocognitive functioning. Aim 2. To test the mediating role of white matter in the association of cardiorespiratory fitness and motor skills with neurocognitive functioning in the observed (significant) relationships.
Page 12, line 28. Did you adjust for multiple comparisons in the regression analyses?

Results

Page 17, lines 6-25. Please include a summary of these findings in the abstract.

Discussion

Page 18, lines 3-13. Please see my previous comment focused on physical activity and cardiorespiratory fitness concepts.
Page 18, line 53. I would discuss why this study found different results (https://pubmed.ncbi.nlm.nih.gov/32719329/).
Page 19, line 39. The article by Ruotsalainen et al. is focused on adolescents and the one from Opel et al. is focused on young adults. Is that possible that in children other mediators would explain the association between CRF and cognitive functioning?
Page 20, line 57. I would be cautious with this statement according to your mediation findings: "These findings support the idea that physical activity may induce changes in white matter integrity that benefit neurocognitive functioning in childhood

Reviewer: 2

Comments to the Author
Please see the attached text file for my comments.

Reviewer: 3

Comments to the Author
The manuscript presents interesting findings in a large sample of 92 healthy children, which is quite challenging to acquire, particularly in a population with mean age of 9.2 years. The authors demonstrated positive relationships between cardiorespiratory fitness, gross motor performance, neurocognitive functioning, and white matter integrity, measured using diffusion tensor imaging. As the authors suggested, their findings may have significant consequences when the sedentary lifestyle rapidly increases. The article is nicely written, although I have some comments/suggestions:

1. The authors did not analyse mean diffusivity, which is one of the major DTI parameters. Why?
2. Diffusion scanning protocols are quite distinct between the two sites. This has to be appropriately addressed in the analysis. The scanning sites should be retained in the final model used for the analysis (it is not clear to me if it was as authors stated "Scanning Site, Sex, Age and SES were added to the models and only significant covariates were retained in the final model") or authors have to prove that distinct scanning protocols did not impact the final outcomes.
3. The same applies to age, as an important confounding variable, which critically affects diffusion data in adults and even more in rapidly ongoing brain development in kids.
4. Based on the method section, the authors end up with an anisotropic voxel size 1x1x2.5mm, which may have critical consequences for tensor estimation. The authors should elaborate on this issue.
5. Authors claim that they excluded subjects based on the head motion; however, the exact exclusion motion parameters are unclear. The head motion needs to be quantified.
6. Why did authors utilize one-sided permutation testing to examine the relationship between FA and neurocognitive components score, and then did the two-sided analysis only in the regions within predefined ROIs? The two-sided analyses should be performed from the beginning. Also, authors should avoid analyzing AD and RD only within regions with significant FA. Changes in AD and RD might precede changes in FA, and such analyzes have to be conducted independently.
7. The BMI pointed out that 16% of children included in the analyses are overweighed or obese it will be of

interest to include BMI as a covariate.
8. I am missing the information on how the authors performed the mediation analysis in the method section.
9. While the relationship between neurocognition, gross motor performance, cardiorespiratory fitness in children might be relatively novel, similar associations have been already described in adults. Authors should briefly elaborate on similarities and/or differences between children and adult populations regarding relationships with explored parameters.
10. As a minor point, authors should avoid reporting relevant information in the parentheses such as "…and higher white matter integrity (higher FA, presumably accompanied by a profile of higher AD and lower RD)."

Authors' Response

Dear Dr. Jeremy Hogeveen and Dr. Cristina Ghiani,

Please find enclosed the revised version of our manuscript entitled: 'The Relationship between White Matter Microstructure, Cardiovascular Fitness, Gross Motor skills and Neurocognitive Functioning in Children' (ID jnr-2020-Aug-8980). We appreciate the constructive and helpful comments from you and the reviewers, and we thank you for offering us the opportunity to rework our manuscript.

Guided by the editorial and reviewer's comments, we put forward revisions of the original text to clarify the issues raised by the reviewers. We feel that the comments were addressed satisfactorily and have resulted in a substantially improved manuscript. Furthermore, we adjusted our abstract to the guidelines of JNR and added a graphical abstract, an Acknowledgement section and a statement of authors contributions.

We hope that the revised manuscript now meets the standards of publication in Journal of Neuroscience Research. Our point-by-point responses to the Reviewers' commentary accompany this letter.

Yours faithfully,

Anna Meijer, MSc*,
Petra J.W. Pouwels, PhD,
Joanne Smith, PhD,
Prof. Chris Visscher, PhD,
Prof. Roel J. Bosker, PhD,
Dr. Esther Hartman,
Prof. Jaap Oosterlaan, PhD
Marsh Königs PhD.
*Corresponding Author (a.meijer@vu.nl)

Reviewer: 1

Summary

Thank you very much for providing me the opportunity to review the manuscript entitled "The Relationship between White Matter, Cardiovascular Fitness, Gross Motor skills and Neurocognitive Functioning in Children". This manuscript aims to investigate the role of white matter in the relationship between cardiovascular fitness or gross motor skills and neurocognitive functioning in healthy children. The research topic is relevant and has the potential to aid in the field of exercise screening questionaries.

>> We thank the reviewer for the kind words regarding our manuscript.

Minor issues

Tittle

1. I would include the word "microstructure" in the title since there are also other white matter features such as a white matter hyperintensity or white matter volume. "The Relationship between White Matter Microstructure, Cardiovascular Fitness, Gross

Motor skills and Neurocognitive Functioning in Children". This comment is extensible to the whole manuscript.

>> We agree with the reviewer's comment and have changed "*white matter*" into "*white matter microstructure*" throughout the manuscript and also omitted the use of 'white matter integrity'.

Abstract

2. I would include the sample characteristics (N = 92, mean age 9.1 years, range 8.0 – 10.7) in the methods section of the Abstract.

>> In response to this point we now describe the sample characteristics in the Methods section of the Abstract. This section now reads as follows:

"Methods *In total 92 children (mean age 9.1 years, range 8.0 – 10.7) were included in this study. Cardiovascular fitness and gross motor skill performance were assessed using…."*

3. I would change the first sentence of the abstract. Physical activity (i.e., any bodily movement produced by skeletal muscles that result in energy expenditure) and cardiorespiratory fitness (i.e., the overall capacity of the cardiovascular and respiratory systems and the ability to carry out prolonged strenuous exercise) are different concepts.

>> We agree with the reviewer that physical activity and cardiovascular fitness are different concepts and we changed the pertinent sentence in the Abstract as follows:

"*Recent evidence indicates that physical activity is both cardiovascular fitness and gross motor skill performance are related to enhanced neurocognitive functioning in children by influencing brain structure and functioning."*

4. The aim of the study is not clear to me. I expected to see the mediating role of white matter microstructure in the relationship between CRF/gross motor skills and neurocognitive functioning when I read the aim. However, in the results section, you reported, independently, the association between CRF/gross motor skills and white matter and the association of CRF/gross motor skills with cognitive function but not the mediating role. If you tested also the mediating role but you didn't find any significant association, please, include also this information in the abstract section since it is the most novel finding of this paper.

>> In response to this comment we have added the following information to the Abstract:

"The results revealed positive associations of both cardiovascular fitness and gross motor skills with neurocognitive functioning. Information processing and motor response inhibition were associated with FA in a cluster located in the corpus callosum. Within this cluster, higher cardiovascular fitness and better gross motor skills were both associated with greater FA, greater AD and lower RD. No mediating role was found for FA in the relationship of both cardiovascular fitness and gross motor skills with neurocognitive functioning. The results indicate that cardiovascular fitness and gross motor skills are related to neurocognitive functioning as well as white matter microstructure in children. However, this study provides no evidence for a mediating role of white matter microstructure in these relationships."

Significance statement

5. There are other studies that investigated the relationship between physical activity, white matter, and cognitive function. Additionally, the relationship between cardiorespiratory fitness, white matter, and cognitive function has been investigated. Please, have a look at these studies from the FITKids, the ActiveBrains project, or the Generation R. It is true, that most of them, did not test the mediating role of white

matter in the relationship between cardiorespiratory fitness and neurocognitive functioning in healthy children. Therefore, since my point of view, this is the most important finding of the paper, and therefore might be highlighted in this section.
I enclose to you the link of some papers that tested independently the relationship between physical activity or cardiorespiratory fitness and white matter or cognitive functioning in children. I have seen that some of them were cited but you should have a look at the rest of the papers I suggested to you.
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https://pubmed.ncbi.nlm.nih.gov/31058358/
https://pubmed.ncbi.nlm.nih.gov/30809168/
https://pubmed.ncbi.nlm.nih.gov/24797659/
https://pubmed.ncbi.nlm.nih.gov/25191243/
https://pubmed.ncbi.nlm.nih.gov/31876665/
https://pubmed.ncbi.nlm.nih.gov/32719329/
https://pubmed.ncbi.nlm.nih.gov/24457421/
>> We thank the reviewer for the most helpful literature suggestions. We have now added the studies to our manuscript. Please find the studies of Rodriguez-Ayllon, Derks, et al. (2020), Chaddock-Heyman et al. (2018), Esteban-Cornejo, Derks et al. (2019), in our Introduction on page 4, first paragraph, the study of Schaeffer et al., (2014) in the Introduction on page 3, last paragraph and the study of Rodriguez-Ayllon, Esteban-Cornejo, et al. (2020) in the Introduction on page 5, first paragraph and Discussion on page 17, first paragraph. We agree with the reviewer that we could emphasize our mediation analysis more in the significance statement. Therefore, we adapted the Significance statement as follows:
"This is the first study to investigate the role of white matter microstructure in the relationship of both cardiovascular fitness and gross motor skills with neurocognitive functioning in children. This study shows that cardiovascular fitness and gross motor skills relate to white matter microstructure, while no evidence was found for a mediating role of white matter microstructure in the relationship of both cardiovascular fitness and gross motor skills with neurocognitive functioning. This study adds to the cumulating evidence that physical activity exposure in childhood contributes to brain development, potentially through changes in cardiovascular fitness and gross motor skill development."
Introduction
6. Page 3, Lines 28-32: Are those studies in children? If not, I would use references only in children.
>> In response to the reviewer's comment we removed the reference of Erickson et al. (2014). Unfortunately, we are not aware of studies in children concerning the effects of physical activity on grey or white matter volume, Hence, we adapted the paragraph as follows:
Page 3, second paragraph: *"A recent neuroimaging studies meta-analysis concerning the effects of physical activity on brain structure and neurophysiological functioning supports the idea that physical activity indeed has beneficial effects on neurophysiological functioning in children, while little is known about the effects of physical activity on brain structure in children (Meijer et al., 2020). such as the volume of subcortical structures and grey and white matter (Erickson, Leckie, & Weinstein, 2014; Hyde et al., 2009). Of the few studies into the effects of physical activity on brain structure in children, all focused on white matter microstructure (Chaddock-Heyman et al., 2018; Krafft et al., 2014; Schaeffer et al., 2014).*

7. Page 3, Lines 32-33: There are a few studies that tested the effect and the relationship between physical activity and white matter microstructure. Please, include some references here (see the references I suggested in the Significant Statement comment).
>> We thank the reviewer for his/her valuable literature suggestions. All relevant studies into the effects of physical activity on white matter microstructure have now been added to the pertinent text:
"Of the few studies into the effects of physical activity on brain structure in children, all focused on white matter microstructure (Chaddock-Heyman et al., 2018; Krafft et al., 2014; Schaeffer et al., 2014)."
8. Page 3, Line 43: Why you did not include Mean diffusivity? It is another common DTI measure that normally is included together whit FA, RD, and AD measures.
>> The rationale to limit our analyses of white matter microstructure to FA, AD and RD was twofold. First, this choice followed the mainstream of available literature concerning cardiovascular fitness/gross motor skills, neurocognitive functioning and white matter microstructure. Most of the studies that focused on these variables focused on FA (Bengston et al., 2005; Opel, 2019) or FA in combination with RD and/or AD (Chaddock-Heyman et al., 2018; Schaeffer et al., 2014). The studies which took besides FA also MD, RD an AD into account only reported significant results for FA and RD (Ruotsalainen et al., 2020; Krafft et al., 2014; Rodriguez-Ayllon, Esteban-Cornejo, et al. 2020). Therefore, we chose for FA as our primarily outcome measurement. To further exploit the nature of the obtained effects we decided to include RD and AD parameters in additional analyses. Second, and most importantly, MD is mathematical combination of AD and RD (Bihan et al., 2001) and because we tried to limit the number of analyses, we decided to focus only at FA, AD and RD.
Le Bihan, D., Mangin, J. F., Poupon, C., Clark, C. A., Pappata, S., Molko, N., & Chabriat, H. (2001). Diffusion tensor imaging: concepts and applications. Journal of Magnetic Resonance Imaging: An Official Journal of the International Society for Magnetic Resonance in Medicine, 13(4), 534-546.
9. Page 4, Line 34: Please include this study between the references
https://pubmed.ncbi.nlm.nih.gov/32719329/
>> We thank the reviewer for the suggestion. We added the study of Rodriguez-Ayllon and collegues (2020) to our Introduction on page 5, first paragraph.
"In contrast, the study of by Rodriguez-Ayllon, Esteban-Cornejo, et al. (2020) did not find a significant relationship between cardiovascular fitness and FA in obese children."
10. Page 6, line 5: I wouldn´t assume that cardiorespiratory fitness is a pathway across physical activity improves white matter. For that purpose, you should test the mediating role of cardiorespiratory fitness in the relationship between physical activity and white matter, or at least, you should cite a paper confirming that assumption. I would focus the paper on cardiorespiratory fitness instead of including the physical activity concept since it could be confusing. I extend this comment to the all manuscript.
>> Physical activity and cardiovascular fitness are indeed different constructs, but, as pointed out by Caspersen et al. (1985), Aires et al. (2010) and Sallis et al. (1997), also strongly related. We believe that physical activity is the most important pathway to affect cardiovascular fitness. However, we agree with the Reviewer that cardiovascular fitness also has other determinants, such as genetic make-up or nutrition and therefore we should be careful not to suggest that physical activity and cardiovascular fitness represent one and the

same construct. In response to the reviewer's comment, we have therefore changed the following paragraph as follows (please see page 6, Introduction, second paragraph):
The present study aims to investigate the role of white matter in the relationship between physical activity and neurocognitive functioning in healthy children, targeting both cardiovascular fitness and gross motor skill development as potential pathways through which physical activity may promote white matter integrity and neurocognitive functioning.
into*:*
"The present study aims to investigate the relationships of both cardiovascular fitness and gross motor skills with (1) neurocognitive functioning and (2) white matter microstructure with relevance for neurocognitive functioning, and (3) whether white matter microstructure mediates the relationship of both cardiovascular fitness and gross motor skills with neurocognitive functioning."
We further clarified the concepts of physical activity and cardiovascular fitness and the relationship between the two. Please see page 4 of the Introduction (first paragraph):
"Developmental studies demonstrate age-related increases in FA in most white matter regions until the late adolescence (Lebel, Walker, Leemans, Phillips, & Beaulieu, 2008; Peters et al., 2012). Hence, white matter microstructure may be particularly sensitive for physical activity during the window of strong brain development in childhood and adolescence. This idea is supported by a recent study indicating that physical activity (sports participation and outdoor play time) is associated with higher FA in healthy preadolescent children (Rodriguez-Ayllon, Derks, et al., 2020). Moreover, cardiovascular fitness (Esteban-Cornejo et al., 2019) and motor skills (Langevin, MacMaster, Crawford, Lebel, & Dewey, 2014) seems to be also associated to white matter microstructure in children. Cardiovascular fitness and motor skills are strongly related to physical activity exposure (Aires et al., 2010; Stodden et al., 2008) and physical activity is considered as an important determinant of cardiovascular fitness levels and motor skill development during childhood and adolescence (Aires et al., 2010; Ortega, Ruiz, Hurtig-Wennlöf, & Sjöström, 2008; Sallis et al., 1997; Stodden et al., 2008). Taken together, this suggests that cardiovascular fitness and gross motor skills are related to white matter microstructure in children and that this relationship may be influenced by the exposure to physical activity.
Aires, L., Andersen, L. B., Mendonça, D., Martins, C., Silva, G., & Mota, J. (2010). A 3-year longitudinal analysis of changes in fitness, physical activity, fatness and screen time. *Acta paediatrica, 99*(1), 140-144.
Caspersen, C. J., Powell, K. E., & Christenson, G. M. (1985). Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. Public health reports (Washington, D.C. : 1974), 100(2), 126–131.
Ortega, F. B., Ruiz, J. R., Hurtig-Wennlöf, A., & Sjöström, M. (2008). [Physically active adolescents are more likely to have a healthier cardiovascular fitness level independently of their adiposity status. The European youth heart study]. *Rev Esp Cardiol, 61*(2), 123-129.
Sallis, J. F., McKenzie, T. L., Alcaraz, J. E., Kolody, B., Faucette, N., & Hovell, M. F. (1997). The effects of a 2-year physical education program (SPARK) on physical activity and fitness in elementary school students. Sports, Play and Active Recreation for Kids. *American journal of public health, 87*(8), 1328-1334.
11. Page 6, lines 3-9: The aims of this study are not clear to me.
>> In response to the reviewer's remark, we changed the following sentence:

"The present study aims to investigate the role of white matter in the relationship between physical activity and neurocognitive functioning in healthy children, targeting both cardiovascular fitness and gross motor skill development as potential pathways through which physical activity may promote white matter integrity and neurocognitive functioning."
To:
"*The present study aims to investigate the relationships of both cardiovascular fitness and gross motor skills with (1) neurocognitive functioning and (2) white matter microstructure with relevance for neurocognitive functioning, and (3) whether white matter microstructure mediates the relationship of both cardiovascular fitness and gross motor skills with neurocognitive functioning."*

12. Page 6, line 22: I see now, that you tested the mediating role. Please, include this information in the Abstract Section.

>> Please see our response to comment 4 of this reviewer.

Methodology

13. Page 10, line 5. Did you use two different MRI scans? I would include it as a limitation of the study. Did you run any sensitivity analysis to check if there are differences between MRI scans?

>> We obtained the same scans on two different scanning locations. However, we indeed used two different scanners (GE Discovery MR750 3T and a Phillips Intera 3T). We used a detailed scanning protocol to ensure the scans were comparable. To ensure that possible differences did not affect our results, we included Scanning Site as covariate in all our analyses (please see page 11, Methods, second paragraph). To emphasize our strategy, we included this as a limitation on page 18.

"Due to practical reasons, we scanned at two locations with scanners from different vendors. Accordingly, we have matched scanning protocols and included Scanning site as covariate in all analyses."

14. Page 11, lines 14-22. After reading the statistical analysis, I see two different aims. Aim 1. To test the independent association of cardiorespiratory fitness and motor skills with white matter and neurocognitive functioning. Aim 2. To test the mediating role of white matter in the association of cardiorespiratory fitness and motor skills with neurocognitive functioning in the observed (significant) relationships.

>> Please see our response to comment 4 of this reviewer.

15. Page 12, line 28. Did you adjust for multiple comparisons in the regression analyses?

>> To account for multiple testing, we used threshold-free cluster enhancement in all MRI analyses (Winkler, Ridgway, Webster, Smith, & Nichols, 2014).

Winkler, A. M., Ridgway, G. R., Webster, M. A., Smith, S. M., & Nichols, T. E. (2014). Permutation inference for the general linear model. Neuroimage, 92, 381-397.

Results

16. Page 17, lines 6-25. Please include a summary of these findings in the abstract.

>> Please see our response to comment 4 of this Reviewer.

Discussion

17. Page 18, lines 3-13. Please see my previous comment focused on physical activity and cardiorespiratory fitness concepts.

>> We thank the Reviewer in drawing our attention to these inconsistencies and we adapted the paragraph as follows (Page 15, first paragrapg:

"This study is the first study that investigated the role of white matter microstructure in the

relationship of both physical activity and neurocognitive functioning in healthy children, targeting both cardiovascular fitness and gross motor skills with neurocognitive functioning as potential pathways through which physical activity might act on the brain. The results show that both cardiovascular fitness and gross motor skills are related to neurocognitive functioning and white matter microstructure with relevance to neurocognitive functioning. which in turn was shown to be related to neurocognitive functioning. However, no evidence was found for a mediating role of white matter microstructure in the relationship between both cardiovascular fitness and gross motor skills with neurocognitive functioning. These findings support the idea that physical activity may induce changes in white matter integrity that benefit neurocognitive functioning in childhood."

18. Page 18, line 53. I would discuss why this study found different results (https://pubmed.ncbi.nlm.nih.gov/32719329/).

>> We thank the reviewer for this suggestion and have now added the most interesting study of Rodriguez-Ayllon et al. (2020) to our Discussion on Page 17, first paragraph:

"However, our results concerning cardiovascular fitness are in contrast with the results of a recent cross-sectional study which indicated no significant relationship between physical fitness and FA (Rodriguez-Ayllon et al., 2020). An important difference between their and our study is that Rodriguez-Ayllon and colleagues included only obese children, which may indicate that the association between cardiovascular fitness and white matter integrity may differ between healthy and clinical populations. However, it is currently unknown whether the proposed underlying mechanisms linking cardiovascular fitness to white matter microstructure may act similarly in obese children (or other clinical groups) and healthy children (Meijer et al., 2020). Future research may further clarify the possible differences between healthy and clinical populations."

19. Page 19, line 39. The article by Ruotsalainen et al. is focused on adolescents and the one from Opel et al. is focused on young adults. Is that possible that in children other mediators would explain the association between CRF and cognitive functioning?

>> We agree with the reviewer that during childhood other mediators may explain the association between cardiovascular fitness and neurocognitive functioning. Age may play a crucial role in this relationship. To point this out in our manuscript we have put forward the following adaptions on page 17, second paragraph:

"These findings are in contrast with earlier studies of Opel et al. (2019) and Ruotsalainen et al. (2020) in adults and adolescents which both indicated a mediating role of FA in the relationship between cardiovascular fitness and executive functioning. These contradictory results could be explained by the idea that white matter plasticity is age-dependent and that during adolescence or young adulthood white matter microstructure is more sensitive for physical activity-induced effects than during preadolescence."

20. Page 20, line 57. I would be cautious with this statement according to your mediation findings: "These findings support the idea that physical activity may induce changes in white matter integrity that benefit neurocognitive functioning in childhood

>> In response to this comment we have put forward the following adaptions in the Discussion on page 18-19:

"Although white matter microstructure could not be indicated as a mediator in the relationship in both cardiovascular fitness and gross motor skills with neurocognitive functioning, our findings do support the idea that cardiovascular fitness and gross motor skill are related to white matter microstructure which in turn was shown to be related to

neurocognitive functioning in healthy children. These findings emphasize the relevance of physical activity for brain development."

Reviewer: 2

Summary: This study used DTI to assess the mediating effect of WM metrics on the association between cardiovascular fitness and neurocognitive functioning. As the authors note, this is the first such study of healthy children. This is an important area of research to uncover the effects of fitness since physical inactivity and obesity (and related health issues) are on the rise.

>> We thank the reviewer for the kind words and his/her helpful feedback on our manuscript.

Major comments:

1. The Abstract makes no mention of mediation even though it seems to me to be an important part of the manuscript. If the authors are not constrained by word limit, I think they should add the mediation-related details and results to the Abstract.

>>Please see our response to comment 4 of Reviewer 1.

2. While "white matter integrity" is a commonly-used phrase in the literature, it is not descriptive in and of itself. I am sure the authors are familiar with the commentary in DK Jones et al. (2013) in NeuroImage (volume 73) which argues against using this term. While am not suggesting the authors rewrite the entire manuscript, I think at the very least they should define what they consider "integrity" to mean. I understand that the 2nd paragraph of the Introduction (last sentence) attempts this, but perhaps the authors can include some more evidence from the literature supporting their view. For example, since in the Abstract they focus on the corpus callosum, perhaps the usage of the term is more justifiable in large WM bundles without (many) crossing fibers. On the other hand, the later paragraphs of the Introduction seem to treat the (potential) underlying factors in greater depth (in addition to similar text in the Discussion).

Note this key quote in Jones et al. on p. 250 of their paper:

"Any further interpretations [RE differences in FA] ... must be backed by strong theoretical foundations or additional data from other sources".

I won't penalize the authors for what has continued to be standard terminology, and what may be just a semantic pet-peeve, but I think it would be beneficial for the field as a whole to abandon such a term. I will leave it up to the authors to determine whether they agree that such changes could improve the manuscript. I do think, though, that when white matter integrity refers solely to FA (e.g., in the Results and in Figure 3 caption and the text boxes) it should just say FA, as that is what was measured and used in the analyses.

>> We thank the Reviewer in drawing our attention to the paper of Jones et al. (2013). After reading this valuable article, we agree that our terminology should be adjusted. Therefore, we decided to abandon the term "white matter integrity" and use "white matter microstructure" and FA instead. We adapted this throughout the manuscript and more clearly explained the measurement potential of DTI in the Introduction (please see Page 3, last paragraph):

"Diffusion tensor imaging (DTI) allows the assessment white matter microstructure by quantification of water molecule diffusion characteristics (Le Bihan et al., 2001). The most commonly used DTI measure is fractional anisotropy (FA), which is considered to be higher in tightly bundled, structurally compact fibers with high integrity (Beaulieu, 2002). Other DTI parameters are axial diffusivity (AD) and radial diffusivity (RD), possible markers for axonal density (Song et al., 2003) and myelination (Song et al., 2002) respectively. Although DTI

parameters are measures of water diffusion and thus indirect measures of structural connectivity (Jones, Knösche, & Turner, 2013), a profile of higher FA and AD and lower RD is thought to be compatible with higher white matter integrity."

3. Is there a reason both age and grade are included in the regression models? These would seem to me to be redundant (possibly collinear). I see that you use stepwise regression but there have been many criticisms of this technique that can be found easily in the literature. It would be better to use predictors based on theory (e.g., if there are significant developmental differences between grades 3 and 4 that are important enough to be used as a separate predictor).

>>We agree with the reviewer that it is important to select predictors based on theory and therefore our selection of covariates (Age, Grade, BMI and SES) was based on theory. Both the variables Age and Grade may have impact on neurocognitive functioning and brain functioning during childhood (Brod et al., 2017; Diamond, 2013). Furthermore, age could also vary within grade. To reduce the number of measures in order to increase the statistical power, we decided to select only the significant covariates in our models. To take the possibility of collinearity into account we decided to use a backward selection model instead of a forward selection model (Heinze et al., 2018). Our approach has resulted in models in which Age and Grade were never included in the same model. Therefore, multi-collinearity could not confound our findings in in the final models.

Brod, G., Bunge, S. A., & Shing, Y. L. (2017). Does one year of schooling improve children's cognitive control and alter associated brain activation?. Psychological science, 28(7), 967-978.

Diamond, A. (2013). Executive functions. Annual review of psychology, 64, 135-168.

Heinze, G., Wallisch, C., & Dunkler, D. (2018). Variable selection–a review and recommendations for the practicing statistician. Biometrical journal, 60(3), 431-449.

4. There is not nearly enough information in the Methods regarding mediation analyses. What software/libraries were used? What analyses, specifically, are referred to in the final sentence of the Methods? Was multiple testing correction applied?

>> In response to the reviewer's comment, we have now added the following information to the Methods section on page 11, last paragraph:

"Third, the role of white matter integrity in the relation between cardiovascular fitness and neurocognitive functioning, and between gross motor skills a neurocognitive functioning was tested using mediation analysis. The mediation analysis was performed using the PROCESS SPSS macro developed by Hayes (2017). All indirect effects were tested using 5000 bootstrap samples and bias-corrected bootstrap confidence intervals"

Minor comments:

1. There are some minor edits to grammar throughout that are required (e.g., comma placement, adding/removing words). For example, on manuscript p. 3 in the middle of the second paragraph, it should read "...allows the assessment *of* white matter microstructure...".

>> In response to this point, the manuscript was carefully checked for language errors.

Reviewer: 3

Comments to the Author

The manuscript presents interesting findings in a large sample of 92 healthy children, which is quite challenging to acquire, particularly in a population with mean age of 9.2 years. The authors demonstrated positive relationships between cardiorespiratory

fitness, gross motor performance, neurocognitive functioning, and white matter integrity, measured using diffusion tensor imaging. As the authors suggested, their findings may have significant consequences when the sedentary lifestyle rapidly increases. The article is nicely written, although I have some comments/suggestions.

>>We thank the reviewer for the kind words and his/her helpful feedback on our manuscript.

1.The authors did not analyse mean diffusivity, which is one of the major DTI parameters. Why?

>>Please see our response to comment 8 of Reviewer 1.

2. Diffusion scanning protocols are quite distinct between the two sites. This has to be appropriately addressed in the analysis. The scanning sites should be retained in the final model used for the analysis (it is not clear to me if it was as authors stated "Scanning Site, Sex, Age and SES were added to the models and only significant covariates were retained in the final model") or authors have to prove that distinct scanning protocols did not impact the final outcomes.

>>We used the same scanning protocol to ensure the scans were comparable. However, to ensure that possible differences between scanning sites would affect our results, we indeed included Scanning Site as covariate in all our analyses. We agree with the Reviewer that this should be pointed out more clearly. The following changes have been made to enhance clarity of the approach taken (please see Page 11, second paragraph):

"To control for possible effects of scanning site and demographic variables, the covariates: Scanning Site, Sex, BMI, Age and SES were added to the models. Only significant covariates were retained in the final model. To account for possible differences between scanning sites, we added Scanning Site in all final models."

3. The same applies to age, as an important confounding variable, which critically affects diffusion data in adults and even more in rapidly ongoing brain development in kids.

>> We agree with the reviewer that age is an important possible confounding variable in this population. Therefore, we checked in all analyses whether age significantly contributed to the statistical model. However, in none of our analyses age was found to significantly contribute to the dependent variables tested. This finding be explained by the small age range in our sample (8.0 – 10.7 years old).

4. Based on the method section, the authors end up with an anisotropic voxel size 1x1x2.5mm, which may have critical consequences for tensor estimation. The authors should elaborate on this issue.

>> We acquired the original DTI images with isotropic resolution, which were interpolated during reconstruction only in-plane. This strategy led to anisotropic voxels as input for tensor estimation and we cannot exclude small effects on the results. However according to Dyrby et al. (2014) the effect of interpolation is limited. To point this out we included this as a limitation in the Discussion on page 18 (second paragraph):

"All DTI images were acquired with isotropic resolution, which were interpolated during reconstruction only in-plane. This led to anisotropic voxels as input for tensor estimation. According to Dyrby et al. (2014) the effect of interpolation is unlikely to impact the results.

Dyrby, T. B., Lundell, H., Burke, M. W., Reislev, N. L., Paulson, O. B., Ptito, M., & Siebner, H. R. (2014). Interpolation of diffusion weighted imaging datasets. NeuroImage, 103, 202-213.

5. Authors claim that they excluded subjects based on the head motion; however, the

exact exclusion motion parameters are unclear. The head motion needs to be quantified.
>> We did not exclude any participant based on head motion. Scans of poor quality due to head motion were directly repeated. To correct for the minor distortions due to head motion we used the pre-processing pipeline of FSL eddy. Hence, all our DTI scans were of reasonable quality. Please refer to the descriptions of this strategy in the following sections:
Methods section Page 9: "*Pre-processing of DTI included estimation and correction of susceptibility induced distortions using topup, and correction of eddy currents and head motion using FSL eddy, including detection and imputation of outlier slices (average number of imputed slices: 21 [range 0-71] per subject; out of the total number of ~2000 slices (Andersson, Skare, & Ashburner, 2003; Smith et al., 2004).*"
Methods section Page 10: *"Scans of poor quality due to head motion during scanning were directly repeated."*
6. Why did authors utilize one-sided permutation testing to examine the relationship between FA and neurocognitive components score, and then did the two-sided analysis only in the regions within predefined ROIs? The two-sided analyses should be performed from the beginning. Also, authors should avoid analyzing AD and RD only within regions with significant FA. Changes in AD and RD might precede changes in FA, and such analyzes have to be conducted independently.
>> We agree with the Reviewer that two-sided testing is preferred. However, in order to reduce the number of comparisons, we decided to make one exception in the preprocessing procedure. We used only one-sided permutation testing for the first step in our analysis approach: creating FA masks with relevance to neurocognitive functioning. For all hypothesis testing analyses two sides permutation testing was performed.
We indeed analyzed only AD and RD within in the regions in which we found significant associations for FA. FA was taken as the primary measure of white matter microstructure and we used AD and RD to further investigate the nature of the obtained effects on FA. Furthermore, we tried to limit the number of analyses conducted in order to avoid type 1 errors. Hence, we believe this approach was the best possible strategy.
7. The BMI pointed out that 16% of children included in the analyses are overweighed or obese it will be of interest to include BMI as a covariate.
>> We agree with the Reviewer that BMI is a covariate of interest. Therefore, we added BMI as covariate in all our analyses. This has slightly changed our results concerning the association of both cardiovascular fitness and gross motor skills with neurocognitive functioning. BMI is now included as significant covariate in our model of both cardiovascular fitness and gross motor skills with Attention Accuracy. For the results we refer to Table 3 and Table 4 on Page 14 in the Results section. In all other analyses BMI did not significantly contribute. We adapted the following sections:
Methods section Page 11 (first paragraph): "*Demographic variables (Sex, Grade [three or four], Age, BMI and SES) were selected in each model as covariates using a stepwise backward selection approach, providing a data-driven selection of relevant covariates for each dependent variable."*
Methods section Page 11 (last paragraph): *"To control for possible effects of demographic variables, the covariates: Sex, Age, BMI and SES were added to the models. Only significant covariates were retained in the final model."*
Results section page 14: *"Of the covariates: Scanning Site, Sex, Age, BMI and SES, only Scanning site showed a significant association with FA, hence all models included Scanning*

Site as covariate."

8. I am missing the information on how the authors performed the mediation analysis in the method section.

>> Please see our comment on question 4 of Reviewer 2.

9. While the relationship between neurocognition, gross motor performance, cardiorespiratory fitness in children might be relatively novel, similar associations have been already described in adults. Authors should briefly elaborate on similarities and/or differences between children and adult populations regarding relationships with explored parameters.

>> We thank the reviewer for this suggestion. We believe that the similarities between pediatric and adult populations are valuable and therefore added the studies of Opel et al. (2019) and Ruotsalainen et al. (2020) to the Discussion section (Page 17):

"These findings contrast with earlier studies of Opel et al. (2019) and Ruotsalainen et al. (2020) in adults and adolescents, which both indicated a mediating role of FA in the relation between cardiovascular fitness and executive functioning. These contradictory results could be explained by the idea that white matter plasticity is age-dependent and that during adolescence and young adulthood white matter microstructure is more sensitive for physical activity induced effects than during preadolescence."

10. As a minor point, authors should avoid reporting relevant information in the parentheses such as "…and higher white matter integrity (higher FA, presumably accompanied by a profile of higher AD and lower RD)."

>> We thank the reviewer for this suggestion and adapted this throughout the manuscript.

2nd Editorial Decision

Decision Letter

Dear Miss Meijer:

I'm pleased to inform you that your manuscript has been accepted pending few minor changes, that 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 15 days. Please explain in your cover letter how you have changed the present version and submit a point-by-point response to the editors' and reviewers' comments. The journal has adopted the "Expects Data" data sharing policy, which states that all original articles and reviews must include a Data Availability Statement (DAS). Please see https://authorservices.wiley.com/author-resources/Journal-Authors/open-access/data-sharing-citation/data-sharing-policy.html#standardtemplates for examples of an appropriate DAS. Please include the DAS in the manuscript as well.

If you require longer than 15 days to make the revisions, please contact Dr Cristina Ghiani (cghiani@mednet.ucla.edu). To submit your revised manuscript: Log in by clicking on the link below https://wiley.atyponrex.com/submissionBoard/1/c7c1ff6f-811a-469e-97cf-f29be0b53d09/current

(If the above link space is blank, it is because you submitted your original manuscript through our old submission site. Therefore, to return your revision, please go to our new submission site here (submission.wiley.com/jnr) and submit your revision as a new manuscript; answer yes to the question "Are you returning a revision for a manuscript originally submitted to our former submission site (ScholarOne Manuscripts)? If you indicate yes, please enter your original manuscript's Manuscript ID number in the space below" and including your original submission's Manuscript ID number (jnr-2020-Aug-8980.R1) where indicated. This will help us to link your revision to your original submission.)

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

JOURNAL OF Neuroscience Research

Best Wishes,

Dr Jeremy Hogeveen
Associate Editor, Journal of Neuroscience Research

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

Reviewer: 1
Comments to the Author

Summary

Thank you very much for providing me the opportunity to review the manuscript entitled "The Relationship between White Matter Microstructure, Cardiovascular Fitness, Gross Motor skills and Neurocognitive Functioning in Children". This manuscript aims to investigate the role of white matter microstructure in the relationship between cardiovascular fitness or gross motor skills and neurocognitive functioning in healthy children. The research topic is relevant and has the potential to aid in the field of exercise screening questionaries. Additionally, the authors have addressed most of the issues presented in the first submitted draft.

Minor issues

Introduction

Page 3, Lines 28: I would explain a bit the term "neurocognitive functioning". It is not clear to me if you mean fMRI data or cognitive function. After reading the methods, results and discussion I understand that you included cognitive function indicators. Please, clarify it. Clarify also which cognitive indicators you will include into the "neurocognitive functioning" term.
Page 3, Lines 30: Please have a look at this systematic review: https://pubmed.ncbi.nlm.nih.gov/31554668/
Page 6, Line 34: "The present study aims to investigate the relationships of both cardiovascular fitness and gross motor skills with (1) neurocognitive functioning and (2) white matter microstructure with relevance for neurocognitive functioning". I would delete "with relevance for neurocognitive functioning".

Results

Page 12, lines 26. Please standardize the term neurocognitive functioning. In the method, these variables are called "Neurocognitive functioning tasks" in the results "neurocognitive measures" and "neurocognitive components". It makes confusing the reading and understanding of the article.

Discussion

Page 15, lines 41-42. "These findings support the idea that physical activity may induce changes in white matter microstructure that benefit neurocognitive functioning in childhood". This sentence is confusing.

Conclusion

Page 19, line 14. "These findings emphasize the relevance of physical activity for brain development". Why these findings support the idea physical activity may induce changes in WM? If you would like to include this statement in the discussion, I would explain it a bit more in this section. However, due this article does not include physical activity variables, I would delete this part from the conclusion. I understand what the authors mean (probably physical activity might have an impact on white matter microstructure, not necessarily through physical fitness improvement). However, it is not clear to me in the manuscript.

Reviewer: 2

Comments to the Author
The authors have addressed my previous comments in this revision, and aside from perhaps some minor grammar editing I do not think this requires another revision.

Reviewer: 3

Comments to the Author
The authors satisfactory addressed all my comments and concerns.

Authors' Response

Dear Dr. Jeremy Hogeveen and Dr. Cristina Ghiani,

JOURNAL OF Neuroscience Research

Please find enclosed the revised version of our manuscript entitled: 'The Relationship between White Matter Microstructure, Cardiovascular Fitness, Gross Motor skills and Neurocognitive Functioning in Children' (ID jnr-2020-Aug-8980.R1).
We appreciate the second round of constructive and helpful comments and we thank you for offering us the opportunity to further polish our manuscript. We feel that all the comments are now addressed satisfactorily.
We hope that the revised manuscript now meets the standards of publication in Journal of Neuroscience Research. Our point-by-point responses to the Reviewers' commentary accompany this letter.
Yours faithfully,
Anna Meijer, MSc*,
Petra J.W. Pouwels, PhD,
Joanne Smith, PhD,
Prof. Chris Visscher, PhD,
Prof. Roel J. Bosker, PhD,
Dr. Esther Hartman,
Prof. Jaap Oosterlaan, PhD
Marsh Königs PhD.
*Corresponding Author (a.meijer@vu.nl)

Reviewer: 1

Comments to the Author

Summary

Thank you very much for providing me the opportunity to review the manuscript entitled "The Relationship between White Matter Microstructure, Cardiovascular Fitness, Gross Motor skills and Neurocognitive Functioning in Children". This manuscript aims to investigate the role of white matter microstructure in the relationship between cardiovascular fitness or gross motor skills and neurocognitive functioning in healthy children. The research topic is relevant and has the potential to aid in the field of exercise screening questionaries. Additionally, the authors have addressed most of the issues presented in the first submitted draft.

>> We would like to thank this reviewer for reading our revised manuscript.

Minor issues

Introduction

Page 3, Lines 28: I would explain a bit the term "neurocognitive functioning". It is not clear to me if you mean fMRI data or cognitive function. After reading the methods, results and discussion I understand that you included cognitive function indicators. Please, clarify it. Clarify also which cognitive indicators you will include into the "neurocognitive functioning" term.

>> In response to this comment, we changed "*neurocognitive functioning*" to "*behavioral measures of neurocognitive functioning*".

Page 3, Lines 30: Please have a look at this systematic review: https://pubmed.ncbi.nlm.nih.gov/31554668/

>> We thank the reviewer for this literature suggestion. We have now added this systematic review to our manuscript.

Page 3, second paragraph: "*A recent **systematic review and** meta-analysis concerning the effects of physical activity on brain structure and neurophysiological functioning supports the idea that physical activity indeed has beneficial effects on neurophysiological functioning in children, while little is known about the effects of physical activity on brain structure in children (Meijer et al., 2020; **Valkenborghs et al., 2019**).*"

Page 6, Line 34: "The present study aims to investigate the relationships of both cardiovascular fitness and gross motor skills with (1) neurocognitive functioning and (2) white matter

microstructure with relevance for neurocognitive functioning". I would delete "with relevance for neurocognitive functioning".

>> In response to this comment we deleted "with relevance for neurocognitive functioning"

Page 6, last paragraph: The present study aims to investigate the relationships of both cardiovascular fitness and gross motor skills with (1) neurocognitive functioning and (2) white matter microstructure **with relevance for neurocognitive functioning**, and (3) whether white matter microstructure mediates the relationship of both cardiovascular fitness and gross motor skills with neurocognitive functioning.

Results

Page 12, lines 26. Please standardize the term neurocognitive functioning. In the method, these variables are called "Neurocognitive functioning tasks" in the results "neurocognitive measures" and "neurocognitive components". It makes confusing the reading and understanding of the article.

>> We agree with the reviewer that differences in terminology are confusing. Therefore, we now clearly distinguish the terms neurocognitive functioning measures which refer to all outcome measures of the neurocognitive tasks (see Table 1) and neurocognitive functioning components which refers to the six components derived from the principal component analysis. We amended the following terms in the Methods and Results section:

- "*neurocognitive functioning tasks*" to "*neurocognitive functioning measures*"
- "*neurocognitive components*" to "*neurocognitive functioning components*"
- "*executive function components*" to "*neurocognitive functioning components*"

Discussion

Page 15, lines 41-42. "These findings support the idea that physical activity may induce changes in white matter microstructure that benefit neurocognitive functioning in childhood". This sentence is confusing.

>> In response to this comment we deleted the pertinent sentence.

"This study is the first study that investigated the role of white matter microstructure in the relationship of both cardiovascular fitness and gross motor skills with neurocognitive functioning. The results show that both cardiovascular fitness and gross motor skills are related to neurocognitive functioning and white matter microstructure which in turn was shown to be related to neurocognitive functioning. However, no evidence was found for a mediating role of white matter microstructure in the relationship between both cardiovascular fitness and gross motor skills with neurocognitive functioning. These findings support the idea that physical activity may induce changes in white matter microstructure that benefit neurocognitive functioning in childhood."

Conclusion

Page 19, line 14. "These findings emphasize the relevance of physical activity for brain development". Why these findings support the idea physical activity may induce changes in WM? If you would like to include this statement in the discussion, I would explain it a bit more in this section. However, due this article does not include physical activity variables, I would delete this part from the conclusion. I understand what the authors mean (probably physical activity might have an impact on white matter microstructure, not necessarily through physical fitness improvement). However, it is not clear to me in the manuscript.

>> In response to this comment we changed this sentence to:

"In conclusion, the present study shows that cardiovascular fitness and gross motor skills are associated with enhanced performance in a specific set of neurocognitive functions (i.e. relating to the speed and variability of information processing and motor response inhibition) as well as enhanced FA in a cluster of white matter tracts with overlapping relevance for neurocognitive functions (i.e. predominantly in the corpus callosum and corona radiata). Although white matter microstructure could not be indicated as a mediator in the relationship in both cardiovascular fitness and gross motor skills with neurocognitive functioning, our findings do support the idea that cardiovascular

fitness and gross motor skill are related to white matter microstructure which in turn was shown to be related to neurocognitive functioning in healthy children. These findings emphasize the relevance of physical activity for brain development. ***Although more research is needed to substantiate these results, they might indicate that physical activity exposure contributes to brain development and children's neurocognitive functioning through cardiovascular fitness and gross motor skills.***

Comments to the Author

The authors have addressed my previous comments in this revision, and aside from perhaps some minor grammar editing I do not think this requires another revision.

>> In response to this comment, the manuscript was carefully checked for grammar errors. We would like to thank this reviewer for reading our revised manuscript.

Reviewer: 3

Comments to the Author

The authors satisfactory addressed all my comments and concerns.

>>We would like to thank this reviewer for reading our revised manuscript and are pleased that the reviewer agrees with the changes that have been made.

3rd Editorial Decision

Decision Letter

Dear Miss Meijer:

Thank you for submitting your manuscript "The Relationship between White Matter Microstructure, Cardiovascular Fitness, Gross Motor Skills and Neurocognitive Functioning in Children" by Meijer, Anna; Pouwels, Petra J.W.; Smith, Joanne; Visscher, Chris; Bosker, Roel J.; Hartman, Esther; Oosterlaan, Jaap; Königs, Marsh.

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 Jeremy Hogeveen
Associate Editor, Journal of Neuroscience Research

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

Authors' Response

4th editorial decision

Decision Letter

JOURNAL OF Neuroscience Research

Author response