## PEER REVIEW HISTORY



## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | Effect of regulating gut microbiota using probiotics on functional changes in the brain: protocol for a systematic review |
| **AUTHORS** | Liu, Lu; Ni, Xixiu; Tian, Tian; Li, Xiao; Li, Fengmei; Sun, Mingsheng; Chen, Jiao; Zhou, SiYuan; Zhao, Ling |

## VERSION 1 – REVIEW

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| **REVIEWER** | Prof. Bagher Larijani<br>Endocrinology and Metabolism Research Institute, Tehran, Iran. |
| **REVIEW RETURNED** | 04-Mar-2020 |

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| **GENERAL COMMENTS** | Dear Authors,<br>The topic of this systematic review is interesting and novel and results would be practical. There are some comments to improve the study:<br>- I think it is better to include Scopus database in search strategy. The results will be more comprehensive.<br>- Regarding to search terms, Acidophilus and prebiotic have not been selected correctly, please omit these words and add important terms like microbiome, intestinal bacteria and other related words to gut microbiota.<br>- Since probiotics and prebiotics have different underlying mechanisms, results of articles in these fields should be reported separately or prebiotic related studies should be eliminated.<br>- Regarding to selection criteria, explain more about the randomization in trial. What is your decision about non-randomized clinical trial?<br>- What is your plan for meta-analysis of the results?<br><br>Best Regards |

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| **REVIEWER** | Andrea Ticinesi<br>Unniversity Hospital of Parma, Italy |
| **REVIEW RETURNED** | 09-Mar-2020 |

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| **GENERAL COMMENTS** | This manuscript reports the protocol for a systematic review exploring the effects on brain activity of the administration of probiotics. The topic is original and worth of investigation. The results of the systematic review will be very useful for designing future studies to test the effects of probiotics on brain function in different neuro-psychiatric disorders.<br>I have the following comments:<br>1) The authors focus almost exclusively on probiotics. However, growing evidence suggests that the best strategy for modulating gut microbiota may instead be the administration of prebiotics (functional foods), that can stimulate the growth of bacterial |

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| | populations not limited only to one or few species. A focus also on prebiotics could represent an important point of strength of the study.<br>2) The clinical context of the studies that will be selected for the systematic review is unclear. I think that it is useful to specify in the criteria of selection whether the studies were performed in healthy subjects or in patients with neuro-psychiatric diseases. The type of diseases should be also specified.<br>3) More in general, the authors should focus more on the clinical context and clinical implications of the studies they will consider for the systematic review.<br>4) Outcome measures are someway unclear. What do the authors mean by "changes in clinical symptoms"? This point should be better detailed. Again, a better focus on the clinical context of studies is needed.<br>5) Outcomes are mentioned twice in the text. Repetitions should be avoided. |

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| **REVIEWER** | Helen Macpherson and Nathan Nuzum<br>Deakin University, Australia |
| **REVIEW RETURNED** | 13-Mar-2020 |

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| **GENERAL COMMENTS** | Review<br>“Effect of regulating gut microbiota using probiotics on brain activity: protocol for a systematic review”<br>This paper provides the protocol for a systematic review examining the effects of probiotics on functional magnetic resonance imaging outcomes. This is the first planned systematic review to cover this topic.<br>Major Concerns<br>Throughout the manuscript it should be made clearer what conditions will be included in your systematic review. Are you only looking at studies that investigated anxiety disorders, depression or are you also including studies that looked at cognitive changes (related to specific cognitive tasks) in healthy individuals? The inclusion criteria should also be updated to specifically reflect the conditions you are interested in. It needs to be clearer what brain activity changes will be investiaged as ‘brain activity’ by itself is not a very specific term. In addition, authors state they will evaluate clinical symptoms, but clinical symptoms of what disease(s)? And what rating(s) of clinical symptoms will be used? Or, if a range of methods to determine clinical symptoms of the same condition are used how will authors collate and compare this information?<br>In relation to the above points, the title could be changed to be clear on what ‘brain activity’ is exactly being investigated. There is no clarity regarding the fMRI outcomes or why fMRI was selected as the imaging modality. Does this review include task based fMRI and resting state functional connectivity? fMRI outcomes need to be clearly defined. “Functional alteration area of the brain” is a vague outcome, does not map to specific fMRI techniques, and will be influenced by the fMRI study design.<br>The introduction could be restructured to better show why the topic of gut bacteria and how it may relate to brain activity is important. Perhaps authors could first mention the meta-analysis showing the levels of Lactobacillus, Bifidobacterium and Faecalibacterium prausnitzii in irritable bowel syndrome (IBS) patients compared with healthy controls, then mention the clinical trials showing neurological disorders being linked to gastrointestinal dysfunction and then move on to introducing how probiotics were shown to alleviate anxiety symptoms in healthy individuals, and how they |

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| | improved depression severity in individuals with major depressive disorder. Collectively this information shows that gut bacteria is related to various diseases, and that modulating gut bacteria through probiotics/prebiotics/synbiotics may offer a potential treatment option. Then the introduction can cover more specifically how prebiotics have been shown to relate to brain activity.<br>On page 5 line 39: The authors state “The above results show that probiotics affect central nervous system diseases and this effect may be exerted through the microbiota-gut-brain axis.” This is followed by “A study has systematically reviewed the effects of probiotics on central nervous system function in animals and humans suggesting that more research using both behavioral and neuroimaging measures on healthy volunteers and patients is needed in the future.17” These two sentences are somewhat contradictory as it is stated that probiotics do affect central nervous system diseases (as indicated by studies 14,15 and 16), but then state how a systematic review determined more research is needed. Please clarify.<br><br>Page 3, line 43 and on page 7, line 17: The authors indicate that the search has already been conducted. Why have databases already been searched if this is a systematic review protocol?<br><br>Minor Concerns<br>Grammatical/Spelling errors change to the suggestions as below<br>The abstract requires further English review.<br>Page 3, line 32: ‘…aim to summarize the literature…”<br>Page 4, line 29: ‘meta-analyses’<br>Other Minor concerns<br>Page 5, line 50: Specifically, what studies do the authors mean by ‘…clinical trials…’, if this is study 14,15 and 16 state/reference them.<br>Page 5 line 58: Considering mentioning that neuroimaging tools are also objective which can be considered a benefit over subjective self-report measures.<br>Page 6 line 21-22: Change to ‘…brain axis, to potentially help develop therapeutic methods for central nervous system diseases in the future.’<br>Throughout the document it should be clearer whether a study was investigating humans or was animal research. While most of the research referenced is investigating human participants, reinforcing this for the reader provides more clarity, especially because a lot of research involving the gut-brain axis takes place in animals.<br>Beginning at page 10 line 56: It may help to number the three primary outcome measures when explaining how the systematic reviews results will be narratively synthesised. I.e. 1)….. 2)…. and 3)…<br>Beginning at page 12 line 9: While it is important to mention the annual growth in production of probiotic containing foods, I would encourage authors to state that the current number of individuals affected by neurological conditions, and the projected increase in individuals suffering from these conditions, would be an important factor to consider as to why there is increased interest in elucidating how the gut microbiota may be utilised, through probiotics, as alternative treatment options. |

**VERSION 1 – AUTHOR RESPONSE**

**Reviewer #1 (Remarks to the Author (Required)):**

**The topic of this systematic review is interesting and novel and results would be practical. There are some comments to improve the study:(1) I think it is better to include Scopus database in search strategy. The results will be more comprehensive.(2) Regarding to search terms, Acidophilus and prebiotic have not been selected correctly, please omit these words and add important terms like microbiome, intestinal bacteria and other related words to gut microbiota.(3) Since probiotics and prebiotics have different underlying mechanisms, results of articles in these fields should be reported separately or prebiotic related studies should be eliminated.(4) Regarding to selection criteria, explain more about the randomization in trial. What is your decision about non-randomized clinical trial?(5) What is your plan for meta-analysis of the results?**

**1) I think it is better to include Scopus database in search strategy. The results will be more comprehensive.**

**Response:** Thanks for the suggestions. To make the results more comprehensive, we have included Scopus database in our search strategy.

**2) Regarding to search terms, Acidophilus and prebiotic have not been selected correctly, please omit these words and add important terms like microbiome, intestinal bacteria and other related words to gut microbiota.**

**Response:** Thanks for the kindly suggestions. We have already omitted Acidophilus ,

prebiotic and Synbiotics . Meanwhile, we have added gastrointestinal microbiome,gut microbiome, gut microflora, intestinal microflora, intestinal bacteria,intestinal microbiome, microbiome, microbiota, flora and bacteria which were related to gut microbiota. Furthermore, we updated the fMRI-related terms to make the results more comprehensive.

**3) Since probiotics and prebiotics have different underlying mechanisms, results of articles in these fields should be reported separately or prebiotic related studies should be eliminated.**

**Response:** Thanks for the helpful suggestions. We chose to eliminate prebiotic related studies after reviewing the relevant literatures.

**4) Regarding to selection criteria, explain more about the randomization in trial. What is your decision about non-randomized clinical trial?**

**Response:** Thanks for the comment. The process of randomization plays important role in modern controlled clinical trials[1]. It consists of two components:generation of random allocation scheme and concealment of random allocation sequence in implementation.

The commonly used random allocation methods in randomized controlled trials include: looking up the random number table, using SAS or SPSS to generate random sequence. And proper randomization relies on appropriate allocation concealment. Inadequate allocation concealment will lead to exaggerated estimates of treatment effect, even subverting the random allocation sequences[2].We will exclude non-randomized clinical trail.

*[1] Higgins JPT, Altman DG, Sterne JAC (editors) (2011) Chapter 8: Assessing risk of bias in included studies. In:Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updatedMarch 2011). The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.*

*[2] Schulz KF, Grimes DA. Allocation concealment in randomised trials: defending against deciphering. Lancet 2002; 359(9306): 614-618.*

**5) What is your plan for meta-analysis of the results?**

**Response:** Thanks for the comment. The results of the systematic review will be synthesized narratively in the domains of the three primary outcome measures:1) increased/decreased activity in brain regions or altered functional connectivity of brain detected by fMRI and their association with changes in behaviour,gastrointestinal/emotional symptoms after using probiotics;2) changes in composition and diversity of the gut microbiota and their association with changes in behaviour,gastrointestinal/emotional symptoms after using probiotics;3) increased/decreased activity in brain regions or altered functional connectivity of brain detected by fMRI and the changes in composition or diversity of the gut microbiota after probiotics' administration.

**Reviewer #2 (Remarks to the Author (Required)):**

**This manuscript reports the protocol for a systematic review exploring the effects on brain activity of the administration of probiotics. The topic is original and worth of investigation. The results of the systematic review will be very useful for designing future studies to test the effects of probiotics on brain function in different neuro-psychiatric disorders. I have the following comments: 1) The authors focus almost exclusively on probiotics. However, growing**

**evidence suggests that the best strategy for modulating gut microbiota may instead be the administration of prebiotics (functional foods), that can stimulate the growth of bacterial populations not limited only to one or few species. A focus also on prebiotics could represent an important point of strength of the study. 2) The clinical context of the studies that will be selected for the systematic review is unclear. I think that it is useful to specify in the criteria of selection whether the studies were performed in healthy subjects or in patients with neuro-psychiatric diseases. The type of diseases should be also specified. 3) More in general, the authors should focus more on the clinical context and clinical implications of the studies they will consider for the systematic review. 4) Outcome measures are someway unclear. What do the authors mean by "changes in clinical symptoms"? This point should be better detailed. Again, a better focus on the clinical context of studies is needed. 5) Outcomes are mentioned twice in the text. Repetitions should be avoided.**

**1) The authors focus almost exclusively on probiotics. However, growing evidence suggests that the best strategy for modulating gut microbiota may instead be the administration of prebiotics (functional foods), that can stimulate the growth of bacterial populations not limited only to one or few species. A focus also on prebiotics could represent an important point of strength of the study.**

**Response:** Thanks for the important comments. Indeed, as you said, the best strategy to regulate gut microbiota may be the administration of prebiotics. Unfortunately, few researchers focus on brain activity changes detected by fMRI after using prebiotics so far. Since probiotics and prebiotics had different underlying mechanisms[1-5],we finally decided to focus on probiotics only in the present study.

*[1] Hill C, et al. Expert consensus document. The international scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol.2014;11(8):506-14.*

*[2] Gibson, G. R. et al. Expert consensus document:The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat. Rev.Gastroenterol. Hepatol. 14, 491–502 (2017).*

*[3] Nagpal R, et al. Probiotics, their health benefits and applications for developing healthier foods: a review. FEMS Microbiol Lett. 2012;334(1):1-15.*

*[4] Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. Markowiak P et al. Nutrients. (2017)*

*[5] Sanders ME, Merenstein DJ, Reid G, Gibson GR, Rastall RA. Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. Nat Rev Gastroenterol Hepatol. 2019 Oct;16(10):605-616.*

**2) The clinical context of the studies that will be selected for the systematic review is unclear. I think that it is useful to specify in the criteria of selection whether the studies were performed in healthy subjects or in patients with neuro-psychiatric diseases. The type of diseases should be also specified. More in general, the authors should focus more on the clinical context and clinical implications of the studies they will consider for the systematic review.**

**Response:** Thanks for the helpful suggestions. We specified in the criteria of selection that we included studies which were performed in healthy subjects. Furthermore, we clarified the clinical context of the studies that will be selected for this systematic review. We will focus on the patients with neuro-psychiatric diseases in the future study.

**3) Outcome measures are someway unclear. What do the authors mean by "changes in clinical symptoms"? This point should be better detailed. Again, a better focus on the clinical context of studies is needed.**

**Response:** Thanks for the important comments. We have updated outcome measures and we have changed “changes in clinical symptoms” into “changes in behaviour, gastrointestinal/

emotional symptoms ”.

**4) Outcomes are mentioned twice in the text. Repetitions should be avoided.**

**Response:** Thanks for the helpful comments. We have deleted the repeated one.

**Reviewer #3 (Remarks to the Author (Required)):**

**This paper provides the protocol for a systematic review examining the effects of probiotics on functional magnetic resonance imaging outcomes. This is the first planned systematic review to cover this topic.**

**Major Concerns:**

**Throughout the manuscript it should be made clearer what conditions will be included in your systematic review. Are you only looking at studies that investigated anxiety disorders, depression or are you also including studies that looked at cognitive changes (related to specific cognitive tasks) in healthy individuals? The inclusion criteria should also be updated to specifically reflect the conditions you are interested in. It needs to be clearer what brain activity changes will be investiaged, as ‘brain activity’ by itself is not a very specific term. In addition, authors state they will evaluate clinical symptoms, but clinical symptoms of what**

**disease(s)? And what rating(s) of clinical symptoms will be used? Or, if a range of methods to determine clinical symptoms of the same condition are used how will authors collate and compare this information? In relation to the above points, the title could be changed to be clear on what 'brain activity' is exactly being investigated. There is no clarity regarding the fMRI outcomes or why fMRI was selected as the imaging modality. Does this review include task based fMRI and resting state functional connectivity? fMRI outcomes need to be clearly defined. "Functional alteration area of the brain" is a vague outcome, does not map to specific fMRI techniques, and will be influenced by the fMRI study design.The introduction could be restructured to better show why the topic of gut bacteria and how it may relate to brain activity is important. Perhaps authors could first mention the meta-analysis showing the levels of Lactobacillus, Bifidobacterium and Faecalibacterium prausnitzii in irritable bowel syndrome (IBS) patients compared with healthy controls, then mention the clinical trials showing neurological disorders being linked to gastrointestinal dysfunction and then move on to introducing how probiotics were shown to alleviate anxiety symptoms in healthy individuals, and how they improved depression severity in individuals with major depressive disorder. Collectively this information shows that gut bacteria is related to various diseases, and that modulating gut bacteria through probiotics/prebiotics/synbiotics may offer a potential treatment option. Then the introduction can cover more specifically how prebiotics have been shown to relate to brain activity.On page 5 line 39: The authors state "The above results show that probiotics affect central nervous system diseases and this effect may be exerted through the microbiota-gut-brain axis." This is followed by "A study has systematically reviewed the effects of probiotics on central nervous system function in animals and humans suggesting that more research using both behavioral and neuroimaging measures on healthy volunteers and patients is needed in the future.17" These two sentences are somewhat contradictory as it is stated that probiotics do affect central nervous system diseases (as indicated by studies 14,15 and 16), but then state how a systematic review determined more research is needed. Please clarify. Page 3, line 43 and on page 7, line 17: The authors indicate that the search has already been conducted. Why have databases already been searched if this is a systematic review protocol?**

**Minor Concerns:**

**The abstract requires further English review:Page 3, line 32: '…aim to summarize the literature…"Page 4, line 29: 'meta-analyses'**

**Other Minor concerns:Page 5, line 50: Specifically, what studies do the authors mean by '…clinical trials…', if this is study 14,15 and 16 state/reference them. Page 5 line 58: Considering mentioning that neuroimaging tools are also objective which can be considered a benefit over subjective self-report measures. Page 6 line 21-22: Change to '…brain axis, to potentially help develop therapeutic methods for central nervous system diseases in the future.'Throughout the document it should be clearer whether a study was investigating humans or was animal research. While most of the research referenced is investigating human**

**participants, reinforcing this for the reader provides more clarity, especially because a lot of research involving the gut-brain axis takes place in animals.Beginning at page 10 line 56: It may help to number the three primary outcome measures when explaining how the systematic reviews results will be narratively synthesised. I.e. 1)….. 2)…. and 3)…Beginning at page 12 line 9: While it is important to mention the annual growth in production of probiotic containing foods, I would encourage authors to state that the current number of individuals affected by neurological conditions, and the projected increase in individuals suffering from these conditions, would be an important factor to consider as to why there is increased interest in elucidating how the gut microbiota may be utilised, through probiotics, as alternative treatment options.**

**1) Throughout the manuscript it should be made clearer what conditions will be included in your systematic review. Are you only looking at studies that investigated anxiety disorders, depression or are you also including studies that looked at cognitive changes (related to specific cognitive tasks) in healthy individuals? The inclusion criteria should also be updated to specifically reflect the conditions you are interested in.**

**Response:** Thanks for the helpful suggestions. We have updated the inclusion criteria, and we will include studies which were performed in healthy subjects.

**2) It needs to be clearer what brain activity changes will be investiaged, as 'brain activity' by itself is not a very specific term.In addition, authors state they will evaluate clinical symptoms, but clinical symptoms of what disease(s)? And what rating(s) of clinical symptoms will be used? Or, if a range of methods to determine clinical symptoms of the same condition are used how will authors collate and compare this information? In relation to the above points, the title could be changed to be clear on what 'brain activity' is exactly being investigated.**

**Response:**Thank you for raising this question.Both increased/decreased activity in brain regions and altered functional connectivity of brain will be investigated by us.We have updated the inclusion criteria that we will include studies which were performed in healthy subjects.So,we will evaluate clinical symptoms including behaviour,gastrointestinal symptoms and emotional symptoms in healthy subjects.All the scales assessing these symptoms will be used.And we have changed the title to "Effect of regulating gut microbiota using probiotics on functional changes in the brain: protocol for a systematic review"

**3) There is no clarity regarding the fMRI outcomes or why fMRI was selected as the imaging modality. Does this review include task based fMRI and resting state functional connectivity? fMRI outcomes need to be clearly defined. "Functional alteration area of the brain" is a vague**

**outcome, does not map to specific fMRI techniques, and will be influenced by the fMRI study design.**

**Response:**This review includes both task based fMRI and resting-state fMRI.fMRI outcomes include increased/decreased activity in brain regions or altered functional connectivity of brain detected by fMRI.We have clarified the reason of selecting fMRI as the imaging modality in the uploaded manuscript.

**4) The introduction could be restructured to better show why the topic of gut bacteria and how it may relate to brain activity is important. Perhaps authors could first mention the meta-analysis showing the levels of Lactobacillus, Bifidobacterium and Faecalibacterium prausnitzii in irritable bowel syndrome (IBS) patients compared with healthy controls, then mention the clinical trials showing neurological disorders being linked to gastrointestinal dysfunction and then move on to introducing how probiotics were shown to alleviate anxiety symptoms in healthy individuals, and how they improved depression severity in individuals with major depressive disorder. Collectively this information shows that gut bacteria is related to various diseases, and that modulating gut bacteria through probiotics/prebiotics/synbiotics may offer a potential treatment option. Then the introduction can cover more specifically how prebiotics have been shown to relate to brain activity.**

**Response:**Thanks for the important suggestions.We have restructured the introduction,

however,few researchers focused on brain activity changes detected by fMRI after using prebiotics.Since probiotics and prebiotics had different underlying mechanisms[1-5],we finally decided to focus on probiotics only and delete the related studies of prebiotics.

*[1]Hill C, et al. Expert consensus document. The international scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol.2014;11(8):506-14.*

*[2]Gibson, G. R. et al. Expert consensus document:The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat. Rev.Gastroenterol. Hepatol. 14, 491–502 (2017).*

*[3]Nagpal R, et al. Probiotics, their health benefits and applications for developing healthier foods: a review. FEMS Microbiol Lett. 2012;334(1):1-15.*

*[4]Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. Markowiak P et al. Nutrients. (2017)*

*[5]Sanders ME, Merenstein DJ, Reid G, Gibson GR, Rastall RA. Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. Nat Rev Gastroenterol Hepatol. 2019 Oct;16(10):605-616.*

**5) On page 5 line 39: The authors state "The above results show that probiotics affect central nervous system diseases and this effect may be exerted through the microbiota-gut-brain axis." This is followed by "A study has systematically reviewed the effects of probiotics on central nervous system function in animals and humans suggesting that more research using both behavioral and neuroimaging measures on healthy volunteers and patients is needed in the future.17" These two sentences are somewhat contradictory as it is stated that probiotics do affect central nervous system diseases (as indicated by studies 14,15 and 16), but then state how a systematic review determined more research is needed.**

**Response:**Thanks for the helpful suggestions.We have deleted "A study has systematically reviewed the effects of probiotics on central nervous system function in animals and humans suggesting that more research using both behavioral and neuroimaging measures on healthy volunteers and patients is needed in the future.17" to avoid contradiction in our uploaded manuscript.

**6) Please clarify. Page 3, line 43 and on page 7, line 17: The authors indicate that the search has already been conducted. Why have databases already been searched if this is a systematic review protocol?**

**Response:**Thank you for raising this question.We used the inaccurate English expression,and we have modified the tense in the uploaded manuscript.

**7) The abstract requires further English review:Page 3, line 32: '…aim to summarize the literature…"Page 4, line 29: 'meta-analyses'**

**Response:**We thank the reviewer for this suggestion. We have modified these words in the uploaded manuscript.

**8) Page 5, line 50: Specifically, what studies do the authors mean by '…clinical trials…', if this is study 14,15 and 16 state/reference them.**

**Response:**Thanks for the kindly suggestions.We have added references to clarify what clinical trials specifically refer to.

**9) Page 5 line 58: Considering mentioning that neuroimaging tools are also objective which can be considered a benefit over subjective self-report measures.**

**Response:**Thanks for the kindly suggestions.We have mentioned that neuroimaging tools are also objective which can be considered a benefit over subjective self-report measures in our uploaded manuscript.

10) **Page 6 line 21-22: Change to '…brain axis, to potentially help develop therapeutic methods for central nervous system diseases in the future.'Throughout the document it should be clearer whether a study was investigating humans or was animal research. While most of the research referenced is investigating human participants, reinforcing this for the reader provides more clarity, especially because a lot of research involving the gut-brain axis takes place in animals.**

**Response:**Thanks for the kindly suggestions.We have changed the sentence.In addition,we have clarified whether a study was investigating humans or animals throughout the document.

11) **Beginning at page 10 line 56: It may help to number the three primary outcome measures when explaining how the systematic reviews results will be narratively synthesised. I.e. 1)….. 2)…. and 3)…**

**Response:**Thanks for the kindly suggestions.We have numbered the three primary outcomes measures when explaining how the systematic reviews results will be narratively synthesized.

12) **Beginning at page 12 line 9: While it is important to mention the annual growth in production of probiotic containing foods, I would encourage authors to state that the current number of individuals affected by neurological conditions, and the projected increase in individuals suffering from these conditions, would be an important factor to consider as to why there is increased interest in elucidating how the gut microbiota may be utilised, through probiotics, as alternative treatment options.**

**Response:**Thanks for your kindly comments and encouragement.We have stated that "According to the Global Burden of Disease Study, 322 and 264 million people worldwide suffered from depression and anxiety, respectively in 2015. This is an increase of 18.4% and 14.9% over the 2005 figures[1].There were more than 80 million stroke survivors in the world[2],43.8 million people with dementia[3],45.9 million patients with an active epilepsy[4],and 6.1 million individuals with Parkinson's disease[5].Globally, in 2016, neurological disorders were the leading cause of disability (276 million disability-adjusted life-years) and the second leading cause of deaths (9 million) in the world[6]." in the uploaded manuscript.

*[1] Vos, T., Allen, C., Arora, M., et al. (2016). Global, regional,and national incidence, prevalence, and years lived with disability for 310 diseases and injuries,1990–2015: a systematic analysis for the*

*Global Burden of Disease Study 2015. The Lancet,*

*388(10053), 1545–1602.*

*[2] GBD 2016 Stroke Collaborators. Global, regional, and national burden of stroke, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016.*

*Lancet Neurol 2019;18:439–58.*

*[3] GBD 2016 Dementia Collaborators. Global, regional, and national burden of Alzheimer's disease and other dementias, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol 2019;18:88–106.*

*[4] GBD 2016 Epilepsy Collaborators. Global, regional, and national burden of epilepsy, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016.Lancet Neurol 2019;18:357–75.*

*[5] GBD 2016 Parkinson's Disease Collaborators. Global,regional, and national burden of Parkinson's disease,1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol 2018;17:939–53.*

*[6]GBD 2016 Neurology Collaborators. Global, regional, and national burden of neurological disorders, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol 2019;18:459–80.*

**VERSION 2 – REVIEW**

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| **REVIEWER** | Prof. Bagher Larijani<br>Endocrinology and Metabolism Research Institute, Tehran University of Medical Sciences, Tehran, Iran |
| **REVIEW RETURNED** | 14-Apr-2020 |

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| **GENERAL COMMENTS** | Dear Editor<br>Thank you for returning the manuscript "bmjopen-2020-037582.R1" entitled "Effect of regulating gut microbiota using probiotics on functional changes in the brain: protocol for a systematic review" to me. Having carefully examined the manuscript, I believe that all shortcoming I've detected are fully addressed. From my viewpoint, you may proceed and publish the manuscript in its current format.<br><br>Best regards, |

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| **REVIEWER** | Andrea Ticinesi<br>Azienda Ospedaliero-Universitaria di Parma, Parma, Italy |
| **REVIEW RETURNED** | 14-Apr-2020 |

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| **GENERAL COMMENTS** | The authors have satisfactorily responded to my previous comments. I have no further comments. |

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| **REVIEWER** | Helen Macpherson and Nathan Nuzum<br>Deakin University, Australia |
| **REVIEW RETURNED** | 17-Apr-2020 |

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| **GENERAL COMMENTS** | The protocol has been sufficiently amended and the main concern that the search had already been conducted has been addressed. The editor may want to consider further English language review prior to publishing this paper. |