POINT-BY-POINT RESPONSE TO REVIEWERS

First we would like to express our gratitude to peer reviewers and editorial staff for their time in reviewing our manuscript and for their comments/suggests for revision. We have addressed each and every reviewer comment to the best of our ability and provide a point-by-point summary of our response/revision below.

Reviewer #1

Comment #1: Overall, the topic of study is interesting. But, poor writing has made the paper to be ineligible in current form for publishing. Most sentences must be written in a better journal style.

Response: We were not entirely sure precisely what the reviewer is referring to here with regard to the writing style, particularly as reviewer #2 evaluated our writing style as "Grade A". None-the-less, we have carefully reviewed the manuscript for grammatical mistakes, improper syntax, and sentence structure. We did find several mistakes that have been corrected in the revised manuscript. We are happy to consider further revision if necessary, but we would respectfully request more specific guidance.

Comment #2: The keywords should be corrected as follows: BDNF; Microglia; Neuroinflammation; Growth factors; Depression

Response: We have revised our key word list according to reviewer suggestion.

Comment #3: The figures are not in high resolution.

Response: In the original submission, figures were specifically requested to be imbedded within the manuscript file. Unfortunately, the high resolution settings were not retained. Original figures/diagrams have been provided in this resubmission per reviewer and editorial request.

Reviewer #2

Comment #1: In line 13, page 3, 'Brain-derived neurotrophic factor (BDNF)' should be 'BDNF' since it has already been mentioned in the main text.

Response: Thank you for pointing this out. We have corrected per reviewer suggestion.

Comment #2: What about the effect of environmental factors on BDNF production and inflammation? Please introduce potential environmental factors. This point should be discussed.

Response: This is an excellent suggestion as several environmental factors are known to impact the BDNF system. While this topic could form the basis of its own review, we agree with the reviewer that introducing some key information would make our manuscript more complete. Therefore, we have added a new paragraph and references on Page 4*: "A number of factors may modulate BDNF expression or function. Prenatal, early life, social, and unpredictable stress are all associated with reduced BDNF expression or protein levels[20]. Exercise increases BDNF expression[21] and environmental enrichment protects against the effects of stress and early life inflammation on BDNF expression[22,23]."* We have added an additional sentence on page 5: *"Further, psychosocial stress, a known precursor to depression and anxiety, reduces BDNF levels[20]."* Finally, the effects of environmental stress, aging, obesity, and alcohol consumption on inflammation are discussed on page 10.

Comment #3: As the authors mentioned, BDNF can be expressed both peripherally and within the central nervous system. In the periphery, BDNF has been detected in the heart and spleen, expressed by myoblasts, dorsal root ganglion cells, vascular endothelial cells, leukocytes and is stored in platelets. In the part of BDNF IN DEPRESSION, however, it is not clear for readers which dysfunctional organs can most contribute to a reduction of BDNF in the blood?

Response: While the relationship between disrupted BDNF system and depression are relatively well established in the literature, the underlying mechanisms remain unclear. In this review article, we focus on one general mechanism that may be involved in the BDNF-depression relationship; interplay between BDNF and inflammation. In this context inflammation is a fairly non-specific context that could include dysfunction of one or multiple organ systems or environmental triggers (described above). Additionally, genetic contributions to BDNF dysregulation exist and are discussed in this review. The literature pointing to a major contribution of a given organ in reducing BDNF levels in depression is inconclusive and, in our estimation, outside the primary focus of this article. Our literature search did reveal a potential contribution of platelets in contributing to reduced BDNF in depression, so we have added the additional text and references as follows. *"Moreover, there is a negative correlation between serum BDNF stored in platelets and depression in humans[66]. BDNF release from platelets may be impaired in depressed patients[67] while antidepressants increase BDNF release from platelets[68], suggesting platelet-derived BDNF is a contributing factor to the interaction between peripheral BDNF levels and depression."*

Comment #4: Can the expression of BDNF (both peripherally and within the central nervous system) be decreased in the elderly people or patients with other diseases?

Response: The short answer is yes and no. There are reports of age or disease-related associations with BDNF levels, and inflammation has been speculated as a potential causative factors. However, the literature is mixed, so we have included additional text and references to acknowledge this concept without going too in depth. We have added new info on Page 4. *"BDNF levels may also decline with age[24,25] and low BDNF levels are associated with age-related neurodegenerative disorders such as Alzheimer's and Parkinson's disease[26,27]. However, some studies suggest BDNF expression does not change with age[28,29]."*

Comment #5: It would be great if the authors point out the microglial brain-BDNF or astrocytic brain-BDNF in the main text, rather than overall expression of BDNF.

Response: We agree with the reviewer suggestion and have added additional text to the revised manuscript accordingly. New sentence and references have been added on page 4. *"In the brain, BDNF is expressed by neurons, astrocytes[15], and microglia[16]. BDNF is highly expressed in the hippocampus and is found in lower concentrations in the cerebral cortex and brainstem[17]. TrkB is expressed in neurons, microglia, and astrocytes throughout the brain[18,19]."* On page 13, we have added the following sentence and reference. *"Astrocytes likewise express BDNF when stimulated by TNFα[15] and increase expression of BDNF, TNFα, and IL-6 after LPS treatment[167]"* Finally, we have added new information on page 6. *"In the brain, anti-depressant treatment induces BDNF mRNA expression in neurons[86], astrocytes[87–89], and microglia[89]."*

Comment #6: Microglia serve important physiological functions in learning and memory by promoting learning-related synapse formation through BDNF signaling (Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor, Cell, 2013). Emerging data has convincingly

demonstrated the existence of sex-dependent structural and functional differences of microglia (Uncovering sex differences of rodent microglia, Journal of Neuroinflammation, 2021). Sex should be considered as a biological variable in the condition of depression (for example, sex hormones and the immune system). This point should be discussed in the revised manuscript.

Response: The question of sex as an important biological variable is undoubtedly an important consideration in, and sex hormones represent yet an additional factor that should be integrated into the context with which any discussion of biological basis for depression is taking place. As is the case in many of the reviewer's comments, the role of sex hormones in modulating the BDNF system or inflammation is a topic in itself worthy of review. In an effort to acknowledge the point without unduly expanding the focus of our manuscript, we have added a new paragraph and references on page 13. *"Additionally, investigating the interaction between BDNF-TrkB system and inflammation may be relevant for addressing the sex differences in the presentation of depression. Women report experiencing depression at up to twice the rate of men. BDNF is expressed differentially in various regions of the CNS between males and females and environmental conditions modulate BDNF expression differentially between males and females, although circulating levels of peripheral BDNF appear consistent between sexes[165]. Female BDNF conditional KO mice display more depressive-like behaviors and attenuated anti-depressant response than male BDNF conditional KO mice[166]. Women may also be more vulnerable to developing inflammation-induced depression. Females tend to have higher baseline levels of inflammation than males[167] and have a larger pro-inflammatory and depressive response to endotoxin exposure[168]. In the brain, while male microglia appear to be more reactive early in life than female microglia, female microglia may be reactive and inflammatory later in life, when neuropsychiatric disorders tend to manifest[169]. Estrogen may also play a role: rodent models of estrogen deficiency results in increased depressive-like behaviors, pro-inflammatory cytokine expression, and increased levels of kynurenine pathway enzyme IDO in the hippocampus[170]. There is also evidence that estrogen regulates expression of BDNF and that the estrogen receptor may be necessary for the protective effects of TrkB activation[165]. These findings suggest the relationship between BDNF, inflammation, and sex warrants further investigation."*

Comment #7: In Figure 2, BDNF plays a negative regulatory role in resolving neuroinflammation, and high inflammation reduces BDNF expression. Which is the cause and consequence in your perspective?

Response: In our view, the cause and consequence could go either direction, depending on the context. The main focus of our manuscript is to present evidence and underlying mechanisms for a bidirectional relationship. There is a sub-section of the manuscript devoted specifically to this concept and we include a figure to illustrate our view. Page 13: "*Mounting evidence suggests that the connection between BDNF expression and neuroinflammation regulation is bi-directional in nature (Figure 2).*"

Comment #8: In the conclusion, the authors only mentioned anti-inflammatory effects of BDNF on microglia. However, BDNF can also be expressed by neurons and astrocytes.

Response: We thank the reviewer for pointing out this overly precise statement. We intended to communicate the more general relationship within the brain without elaborating on individual cell types. The sentence has been revised to read, "*the anti-inflammatory effects of BDNF in the brain*"

LANGUAGE QUALITY

Response: We have conducted a careful review of the original manuscript and corrected any errors that were identified. Both authors are native English speakers.

ABBREVIATIONS

Response: We have reviewed the entire manuscript for adherence to journal abbreviation rules.

Science Editor (additional comments not mentioned from above)

Comment #1: The self-referencing rates should be less than 10%. Please keep the reasonable self-citations (i.e. those that are most closely related to the topic of the manuscript) and remove all other improper self-citations. If the authors fail to address the critical issue of self-citation, the editing process of this manuscript will be terminated

Response: We have carefully reviewed our entire citation list. In the revised manuscript, we include 13 self-citations out of 200 total citations (6.5%). We feel that each of these self-citations is appropriate.

Comment #2: The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s).

Response: We are unable to locate this form for completion and are unsure what the comment is referring to. Please provide additional guidance.

Comment #3: The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor.

Response: Original pictures are provided in powerpoint in this resubmission per editorial request.

Comment #4: PMID and DOI numbers are missing in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. Please revise throughout.

Response: The reference list has been revised accordingly. Of note, reference #43 is not indexed in PubMed and has no PMID number associated with it. All references now include the doi.