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Journal Name:	Journal of Applied Life Sciences International
Manuscript Number:	Ms_JALSI_50178
Title of the Manuscript:	Mild hyperoxia stimulation increases regional tissue oxygen pressure in rat hippocampus via oxygen radical
Type of the Article	Short Research Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound. To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	<ol style="list-style-type: none">1. <u>Section 3.3 of the Results.</u> Hyperoxia has been shown to affect microvascular pO₂ as well as haemoglobin affinity to oxygen. Therefore, additional experiments are required to demonstrate that the mentioned above parameters remain unchanged under mild hyperoxic conditions used in the study.2. <u>Section 3.3 of the Results.</u> In the present study authors do not provide any experimental data, but state that “the main reason for the increase in Hip-pO₂ by mild hyperoxia is increase of hippocampal cerebral blood flow from increase of neuronal activity”. However, Lowry et al. (ref. 21 in the manuscript) demonstrated that “Hyperoxia did not lead to significant changes in cerebral blood flow”. Therefore, additional experiments on evaluation of hippocampal cerebral blood flow and detection of hippocampal activity must be performed.3. <u>Section 3.4 of the Results.</u> Hypoxia has been shown to induce intracellular ROS production. Authors should discuss mechanisms of both hypoxia and hyperoxia-mediated induction of ROS.4. <u>Section 3.4 of the Results.</u> There are several scavengers of mitochondrial superoxide available (MitoTEMPO, etc). Authors should perform additional experiments using one of superoxide scavengers to support their statement.5. <u>Section 3.4 of the Results.</u> Inhibition of NADPH oxidase has been shown to boost ROS production. If authors claim that the effects of hyperoxia are superoxide-mediated, why apocynin doesn't affect Hip-pO₂?	<ol style="list-style-type: none">1. The oxygen partial pressure sensor used in this experiment does not measure the degree of hemoglobin saturation, but measures the total oxygen partial pressure of the tissue. In addition, this sensor is capable of linear measurement for 0 to 100% concentration of oxygen. Please refer to Ref. 16.2. As you point out, the increase in blood flow is just a guess. However, this speculation follows that changes in regional blood flow or tissue oxygen tension as shown in previous studies are indicative of neural activity. We changed from section 3.3 to 3.2 and completely rewritten, as follow: The reasons for the increase in local tissue oxygen pressure in brain under high oxygen gas environment are as follows: 1) the blood oxygen amount increases due to an increase in the amount of oxygen in inspiration, and 2) an increase in blood flow due to neuronal activation is considered [17-19]. Regards 1), oxygen present in the blood are divided into hemoglobin-bound oxygen and dissolved oxygen, and most of oxygen exists as hemoglobin-bound oxygen. However, when air is normally inhaled under atmospheric pressure, the oxygen saturation of hemoglobin has already reached approximately 98%, and even when exposed to high oxygen gas, the saturation increase of only 2% can be anticipated. Dissolved oxygen that increases by 0.003 mL / dL every 1 mmHg increases only about 0.2% in the case of inhalation of 32±0.5% oxygen gas. From this it can not be explained that the increase in blood oxygen level alone can increase Hip-pO₂ by more than 50% by exposure to about 30% oxygen gas. Therefore, it is speculated that local blood flow increase is accompanied. Local cerebral blood flow increases as the neuronal activity at that site increases. For example, it has been reported that local cerebral blood flow in the rat striatum increases when striatum neuron cells are active [17]. In addition, cerebral blood flow in the hippocampus is increased by the treadmill running exercise, reports suggesting that this increase in blood flow is due to an increase in neural activity in the hippocampus [18, 19]. For these findings, the main reason for the increase in Hip-pO₂ due to the exposure to oxygen gas of about 30% observed in this experiment is that the hippocampal neurons are activated by a slight increase in blood oxygen amount, and it is inferred that this is due to an increase in the local blood flow caused by it.3. In hyperoxic conditions, an increase in dissolved oxygen and a concomitant increase in mitochondrial respiratory chains may be driving an increase in ROS. completely rewritten section 3.4, as follow: In this study, we showed that the rise in Hip-pO₂ due to mild hyperoxia is mediated by reactive oxygen species (ROS) from experiments using radical scavenger (MnTMPyP). In vitro experiments using hippocampal slices reported that ROS increases in a concentration dependent manner with 40 to 60% oxygen gas [14]. In the culture medium without blood flow, it is considered that active oxygen ROS was generated due to an increase in the amount of tissue oxygen due to an increase in dissolved oxygen. Subsequently, it has been reported that ROS production was induced to excite the hippocampal nerve cells in many cases [14, 23-25]. Even with a slight increase in blood or tissue oxygen level, ROS production occurs, and as a result of this ROS causing neuronal activation in hippocampus, could accompanie by an increase in blood flow. This is surmised to be cause of the greatly Hip-pO₂ rise as our results have shown.



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		<p>Four possible sources of ROS production are mitochondria, NADPH oxidase (NOX), Monoamine oxidase (MAO), and NO synthase (NOS) [23]. NOX is a major ROS production department in blood vessels [26-29], and it is also expressed in the brain [30, 31]. It is thought that oxygen ingested is the first to act due to the fact that the production of ROS (O₂⁻) is the main function and because NOX localized on the cell membrane. However, a NOX inhibitor, apocynin could not suppress the mild hyperoxia-induced Hip-pO₂ increases. Furthermore, MAO and NOS are enzymes that do not generate ROS as a by-product or directly use oxygen [23], therefore, these would be hard to be considered as a source of high oxygen-dependent ROS. Consequently, mitochondria are likely to be the source of ROS production by mild hyperoxia stimulation. Under hypoxic conditions, it is known that ROS is increased by decreasing electron transfer chain by inhibiting oxidative phosphorylation [32-35]. In hyperoxic conditions, an increase in dissolved oxygen and a concomitant increase in mitochondrial respiratory chains may be driving an increase in ROS. However, further studies with mitochondrial superoxide scavengers are needed to clarify the mechanisms of the mild hyperoxia-induced ROS production.</p> <p>4. It is as you pointed out. I would like to consider this experiment in the next study.</p> <p>5. Apocynin is widely used as an inhibitor of ROS production. It mainly inhibits ROS production from blood vessels and immune cells, but it seems to suggest that this study does not contribute to ROS production in the hippocampus.</p>
Minor REVISION comments	<ol style="list-style-type: none"> 1. The introduction should be overwritten to highlight the importance and relevance of the study. 2. The authors should change the title of 3.3 Result section as this statement is not supported by data. 3. In some sentences (neuron) “excitement” should be replaced with “stimulation” or “activation” to avoid unnecessary repeats. 	<ol style="list-style-type: none"> 1. We have completely rewritten the introduction. 2. We changed from section 3.3 to 3.2 and completely rewritten. Described above. 3. We revised, “excitement” → “activation”
Optional/General comments	The study could be of interest, although there are several concerns that need to be addressed before the manuscript can be accepted for publication.	

PART 2:

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Are there ethical issues in this manuscript?	<i>(If yes, Kindly please write down the ethical issues here in details)</i>	<p>We revised Material and Methods, and Competing interests as follow: All animal procedures were approved by the Nagoya Institute of technology's Laboratory Animal Care and Use Committee.</p> <p>as follow: The authors declare that they have no conflict of interests</p>