Short Research Article

Mild hyperoxia stimulation increases regional tissue oxygen pressure in rat hippocampus via oxygen radical

ABSTRACT

Aims: The purpose of this study is to confirm whether mild hyperoxia could activate nervous system in the hippocampus.
Study design: Study was an animal experiment with rat.
Place and Duration of Study: Department of Department of Life Science and Applied Chemistry, Nagaya Institute of Technology, between January 2014 and January 2018
Methodology: Rats were exposed to air or mild oxygen gas. At the same time, Local tissue oxygen pressure in hippocampus (Hip-pO_2) were measured for 20 min with or without treatment of two type of radical scavengers.
Results: The Hip-pO_2 levels were significantly increased by mild hyperoxia exposure (50-60% above resting level). The mild hyperoxia-induced enhancement of the Hip-pO_2 levels were inhibited by MnTMPyP (radical scavenger), but not by NADPH oxidase (NOX) inhibitor Apocynin.
Conclusion: These findings suggested that mild hyperoxia could activate hippocampus through generation of oxygen radicals.

Keywords: Mild Hyperoxia, Oxygen gas, Reactive oxygen species, MnTMPyP, Apocynin, Hip-pO_2, neural activation, Clark-type electrode

1. INTRODUCTION (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

Severe hyperoxia produces excessive amount of reactive oxygen species (ROS) and it damages to cell by peroxidation [1-3]. It is reported that 5 days of 80% oxygen gas exposure decreases neuronal density in neonatal rat hippocampal CA1 and DG via apoptosis pathway [4]. In addition, exposure to 95% oxygen gas for 2 hours increased expression of Bcl-X in the cerebral cortex and cell death in the cortex in neonatal rat [5]. Moreover, hyperbaric oxygen irritation induces hyperventilation by brain stem dysfunction caused by damage from ROS. [6,7]. From the above, it seems that oxygen concentration and duration time of hyperoxia are positively related to ROS damage to brain. Meanwhile, other results from mild hyperoxia such as 30 to 40% oxygen concentration or short-term duration of exposure show beneficial effects on biological functions [8-11]. In human studies, Chung S. C. et al. [8, 9] reported that inhalation of 30 to 40% oxygen gas improves spatial recognition. Also, Moss.MC and Scholey A.B [10, 11] reported that inhalation of 100% oxygen gas for 1 to 2 minutes improves learning and memory function. These reports suggest that relatively mild condition of hyperoxia may positively effect on the brain, especially through the hippocampus. In vitro experiments using hippocampal slices showed that exposure of 2.84 ATA (atmosphere absolute) or 4.54 ATA oxygen induces neuronal excitation in CA1 [12]. Similar nerve excitement was also observed when switching from 0 ATA or 0.6 ATA oxygen exposure to 0.95 ATA oxygen [13]. At this time, tissue oxygen content in the hippocampal slice has been observed to increase as the pressure increases. Also, P. D'Agostino D [14] observed that high pressure oxygen exposure increases ROS production with

oxygen concentration dependent manner in hippocampal slices. In addition, it is reported that the amount of SOD mRNA in hippocampal slices increases with 100% oxygen gas exposure [15]. From these results, it is considered that hyperbaric oxygen exposure could increase neuronal excitement in hippocampal tissue. However, there is no report showing this causal relationship. In addition, it is still elusive that inhalation of high concentration of oxygen gas could increase hippocampal oxygen pressure (Hip-pO_2) and activate its functions. In this study, we measured Hip-pO_2 in live rats with mild hyperoxia condition to find causality of hyperbaric oxygen treatment and hippocampal functions.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

2.1 Animals

All animal procedures were performed according to protocols approved by Nagoya Institute of Technology. Male SD rats (Sprague-Dawley rat, Japan SLC) were housed under a 12 hours light/dark cycle and maintained at 23±1℃ with free access to standard rodent chow and water. 8 weeks old rats were used for all experiments.

2.2 Habituation

Before the surgery, rats were habituated to gas chamber for 4 consecutive days to minimize the effect of stress from environment (60, 90, 120 and 120 minutes at each day). Rats were placed on the gas chamber (cylindrical acrylic chamber (43 cm × 24 cm × 18 cm, 4 slit with 25 cm x 1.5 cm) in an acrylic cage (50 cm × 30 cm × 20 cm)) refluxed with air. Air (oxygen concentration, 21±0.5%) was supplied to the cage at a flow rate of 8 l/min using an air charger (α1500, manufactured by Nippon Tankan Industrial Co., Ltd. and HIBLOW AIR POMP, manufactured by Techno Takatsuki and MS-X 2, National), and oxygen gas (oxygen concentration, 32±0.5%) was delivered at a same flow rate to air.

2.2 Stereotaxic surgery for cannulation

After habituation period, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and a stainless steel guide cannula (O.D. 0.8mm, Unique Medical Co., Tokyo, Japan) was stereotaxically implanted into the left dorsal hippocampal region (co-ordinates: anteroposterior +1.5mm, mediolateral 3.6 mm from the bregma, and dorsoventral -2.0 mm from the dura). The guide cannula was fixed to the skull with an anchor screw using dental cement (Shofu Co., Tokyom, Japan). After surgery, rats were housed individually and allowed to recover for several days.

2.3 Hip-pO_2 measurement

Hip-pO_2 was measured by using improved Clark-Type electrodes (U0E-04TS, Unique Medical Co., Tokyo, Japan) composed with a sensor at the tip (diameter 0.4 mm, length 10 mm of Teflon tube coating) and followed by a 35 mm stainless steel coating. Each electrode was connected to a digital pO_2 monitor (POG-203, Unique Medical Co., Tokyo, Japan). Details are described in previous our report [16]. Rats were stabilized in acryl chamber cage for 10min, meantime, the electrode sensor was calibrated in water that was saturated with 20.9%O2-N2 balance, air and 0% O2-N2 gas. After calibration, the electrode sensor tip was heparinized, then inserted into the hippocampal region through the guide cannula and fixed with rocking nut. The tip of sensor protruded 1.0 mm from the end of the guide cannula.

2.3.1 Experiment 1: Hip-pO_2 changes during oxygen gas exposure

Rats were placed on the gas chamber flowing with air (rate, 1.0 L/min) for 10 minutes and the heparinized electrode was inserted through the cannula. After wait for stabilization, Hip-pO_2 level was measured for 80 minutes flowing schedule: air (10 min) – 30% oxygen gas (20 min) – air (20 min) – 30% oxygen gas (20 min) – air (20 min).

2.3.1 Experiment 2: Effect of ROS scavenger and NOX inhibitor on oxygen gas exposure

Overall experimental conditions were identical to experiment 1. MnTMPyP was prepared in physiological saline to a concentration of 5 mg/kg.B.W. Apocynin was prepared in physiological saline and ethanol to a concentration of 4 mg/kg.B.W (0.5% ethanol). Each reagent was administered by i.p. 20 minutes before the experiment. Hip-pO_2 level was measured for 45 minutes flowing schedule: air (15 min) – 30% oxygen gas (15 min) – air (15 min)

2.4 Statistics

The data were analyzed by one- or two-way ANOVA, followed by a post-hoc test (Fisher's PLSD) for comparison among means. All data were expressed as means ± SD.

3. RESULTS AND DISCUSSION

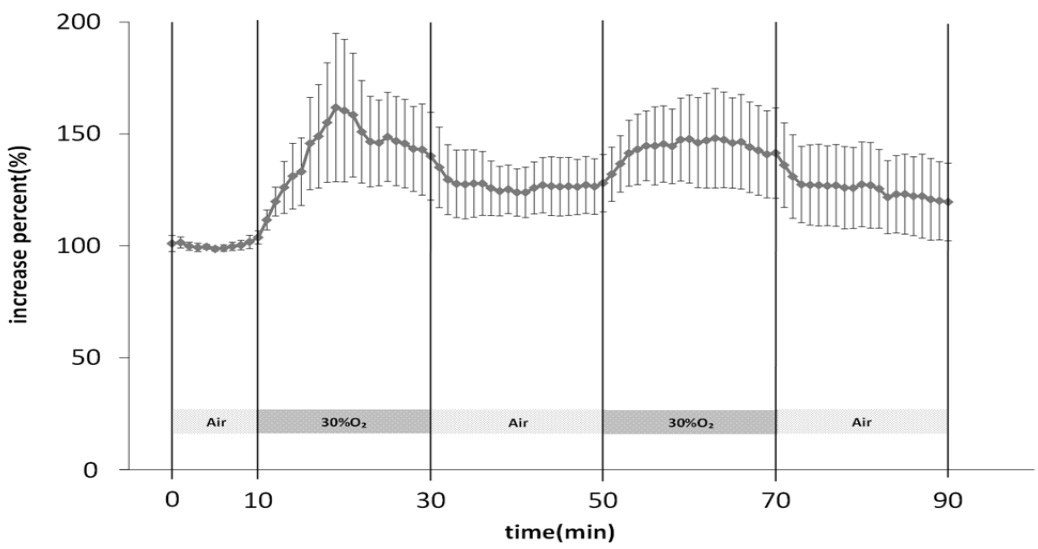

Fig. 1. Mild hyperoxia increases hippocampal tissue oxygen pressure with sustained pattern.
Rats in gas chamber were exposed to 32% of oxygen gas and air according to following schedule: Air (10 min) – O2 gas (20 min) – Air (20 min) – O2 gas (20 min) – Air (20 min). The Hip-pO2 was introduced to pre-implanted cannula, and measured during all gas exposure experiment. Data are mean ± SD.

3.1 Mild hyperoxia increases Hip-pO_2.

After switch air to 30% oxygen gas, Hip-pO_2 was increased to 60% above resting level. Surprisingly, this high level was maintained after switch to air again. In addition, 48% increase of Hip-pO_2 was observed in the second 30% oxygen gas exposure and maintained after switch to air again (Figure 1.). Since rats were restrained in the chamber during experiment, possibility that restraint stress could affect our results remained. However, we did not observe over-excitement of animals. Therefore, the change of Hip-pO_2 by 30% oxygen gas exposure was due to mild hyperoxia.

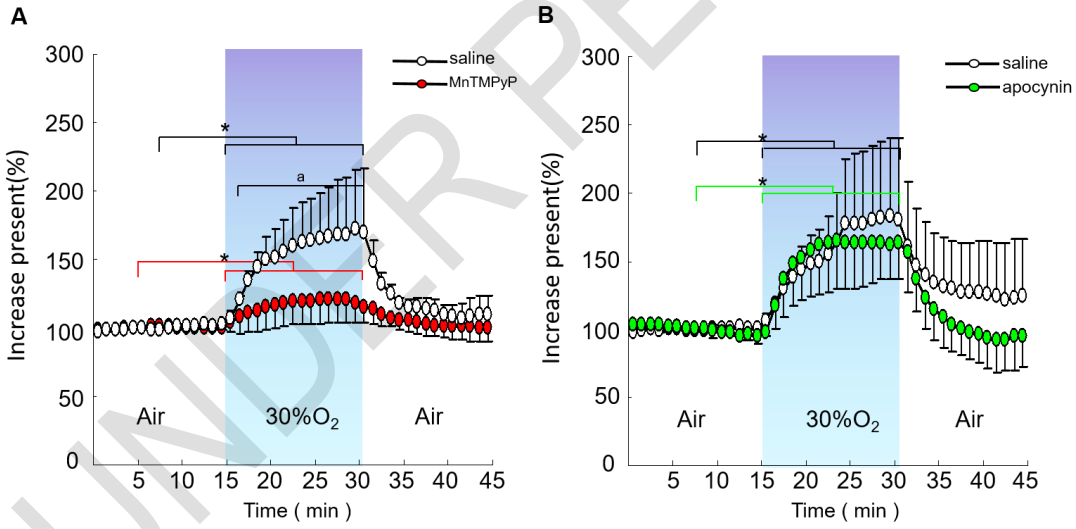

Fig. 2. Effect of the inhibitor or scavenger administration on pO_2 changes induced by mild hyperoxia.
*Drug was applied during 30% oxygen gas exposure: (A) MnTMPyP (5mg/kg I.P) (n=5), (B) apocynin (4mg/kg I.P) (n=4). Data are mean ± SD. *: P<0.01 vs Air control, a: P<0.05 MnTMPyP vs saline control*

3.2 Administration of MnTMPyP, but not Apocynin, suppressed mild hyperoxia-induced Hip-pO2 increases.

The increase of Hip-pO_2 might be a consequence of increase of ROS activity. Therefore, MnTMPyP (active oxygen scavenger) and apocynin (NOX inhibitor) were treated to investigate whether ROS was involved in the rise in Hip-pO_2 at 30% oxygen gas exposure. MnTMPyP is a widely used reagent as an active oxygen scavenger and has an effect of

reducing oxidative stress [17, 18]. Also, apocynin is a reagent that specifically inhibits NOX, and it has been found that the effect of reducing nerve cell death and oxidative stress upon NOX activation [19]. Before the experiment, we intraperitoneally injected MnTMPyP or apoxynin and measured change of Hip-pO_2 with 30% oxygen gas exposure (Figure 2.). At the first, administration of MnTMPyP suppressed increase of Hip-pO_2 by 32% oxygen gas exposure to 10-20% above from resting level (control groups, 50-60% above from resting level). However, apocynin showed no suppressive effect on Hip-pO_2 increase by 30% oxygen gas exposure (both of control and apocynin group, 50-60% above from resting level).

3.3 Excitement of hippocampal neurons increases Hip-pO_2.

The reasons for the increase of Hip-pO_2 are as follows: 1) the blood oxygen amount increases after mild hyperoxia, and 2) increases of blood flow by neuronal excitation. Hemoglobin carries the most of oxygen in blood. Because 98% of hemoglobin already has oxygen, increases of Hip-pO_2 are not due to increase of hemoglobin-bound oxygen. Also, dissolved oxygen changes by 0.003 mL / dL every 1 mmHg change of oxygen exposure. Therefore, the increase of Hip-pO_2 by mild hyperoxia means increase of hippocampal neuronal excitement.

Local cerebral blood flow changes by alteration of the regional neuronal activity. For example, it has been reported that local cerebral blood flow in the rat striatum increases when striatum neuronal cells are excited [20, 21]. In addition, cerebral blood flow in the hippocampus is increased by the treadmill running exercise, and reports suggesting that this increase in blood flow is due to an increase in neural activity in the hippocampus [22, 23]. For these findings, the main reason for the increase in Hip-pO_2 by mild hyperoxia is increase of hippocampal cerebral blood flow from increase of neuronal activity.

3.4 Increase of Hip-pO_2 by mild hyperoxia is related to ROS production change.

MnTMTpy suppressed effect of mild hyperoxia in increase of Hip-pO_2. In vitro experiments using hippocampal slices reported that ROS increases in a concentration dependent manner with 40 to 60% of oxygen gas. It is considered that active ROS were generated from increase 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, 24,25,26]. Even with a slight increase in blood or tissue oxygen level, ROS production occurs, and as a result of this ROS causing hippocampal nervous excitation, accompanied by an increase in blood flow, it is surmised that the Hip-pO_2 rise.

Four possible sources of ROS production are mitochondria, NADPH oxidase (NOX), Monoamine oxidase (MAO), and NO synthase (NOS) [24]. Mitochondria is an important organelle as a place of energy production in aerobic respiration. ROS generation of mitochondria is mainly caused by leakage of electrons of reductase of complex I (NADH ubiquitin oxidoreductase) and complex III (ubiquinol-cytochrome-reductase) of mitochondrial respiratory chain [27]. Complex I, also known as NADH ubiquinone oxidoreductase, is a trans-mitochondrial membrane complex that oxidizes previously reduced NADH using coenzyme Q 10 as the electron acceptor. Complex I is a major site where electrons leak to oxygen at an early stage, thereby producing superoxide (O_2-). The reaction mechanism of complex III consists of the reaction of cytochrome c by participation of coenzyme Q and its accompanying pumping out of 4 protons from the mitochondrial matrix into the intermembrane space, also called the Q cycle, where electrons Leak out and participate in O2- formation [28]. On the other hand, in complex IV, cytochrome c is oxidized and reduces to water by transferring electrons to oxygen molecules. It is easily anticipated that increased oxygen intracellularly is taken up into the mitochondria immediately and causes an increase in energy production and consequently an increase in O2- production. However, there are no drugs that suppress superoxide from mitochondria or drugs capture electrons leaked from complex I and III, and it is impossible to verify this hypothesis at the moment.

MAO, NOS are enzymes that do not generate ROS as a by-product or directly use oxygen[24], and are hard to be considered as a source of high oxygen-dependent ROS. Also, in experiments using these inhibitors, direct nervous excitement or suppression effect appears first and the effect by ROS suppression can not be verified. Therefore, it is presumed that the main source is mitochondria, but it can only be said that it is involved other than NOX so far.

4. CONCLUSION

Under the high oxygen gas environment, there is a possibility that the generation amount of active oxygen and cell damage increase according to its concentration / partial pressure and gas inhalation time. Nagatomo. F [36] found that oxidative metabolites in the blood did not increase even if a gas with oxygen concentration of 35% or less was inhaled for 24 hours under atmospheric pressure in rats, and but more than 40% O2 inhalation for 24 hours induced oxidative stress. From this, it is conceivable that relatively mild hyperoxia about 30% (strictly 32 ± 2%) oxygen used in this study generates ROS causing nerve excitement, but it does not greatly damage the brain. In this time, we were able to investigate the reactivity of the Hip-pO2 to O2 gas stimulus in real time. It began to react in one minute after the start of the stimulation, reached the peak after 6 minutes. It is considered that oxygen gas of 40% or more may not receive the oxidative stress

disorder depending on the time. Further study, it is necessary to investigate the stimulation time in detail about the safe and useful use of oxygen.

REFERENCES

References must be listed at the end of the manuscript and numbered in the order that they appear in the text. Every reference referred in the text must also present in the reference list and vice versa. In the text, citations should be indicated by the reference number in brackets [3].

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1. B Halliwell and J M Gutteridge. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J. 219(1) (1984) pp1–14. PMID: 6326753

2. Bickford PC, Chadman K, Williams B, Shukitt-Hale B, Holmes D, Taglialatela G, and Joseph J.Effect of normobaric hyperoxia on two indexes of synaptic function in fisher 344 rats. Free Radic Biol Med 26 (1999) pp817-824. https://doi.org/10.1016/S0891-5849(98)00260-3

3. Torbati D, Church DF, Keller JM, and Pryor WA. Free radical generation in the brain precedes hyperbaric oxygen-induced convulsions. Free Radic Biol Med 13 (1992) pp101-106. https://doi.org/10.1016/0891-5849(92)90070-W

4. Yis U, Kurul SH, Kumral A, Cilaker S, Tugyan K, Genc S, Yilmaz O. Hyperoxic exposure leads to cell death in the developing brain. Brain & Development 30 (2008) pp556-562. https://doi.org/10.1016/j.braindev.2008.01.010

5. Hu X, Qiu J, Grate MR, Rea HC, Rassin DK, Perez-Polo JR. Bcl-2 family members make different contributions to cell death in hypoxia and/or hyperoxia in rat cerebral cortex. Int J Dev Neurosic. 21(7) (2003) pp371-7

6. Dean JB, Mulkey DK, Henderson RA Ⅲ, Potter SJ, and Putnam RW. Hyperoxia, reactive oxygen species, and hyperventilation: oxygen sensitivity of brain stem neurons. J Appl Physiol 96 (2004) pp784-791

7. Mulkey DK, Henderson RA Ⅲ, Putnam RW, and Dean JB. Hyperbaric oxygen and chemical oxidants stimulate CO2/H+-sensitive neurons in rat brain stem slices. J Appl Physiol 95 (2003) pp910-921

8. Soon-Cheol Chung, Gye-Rae Tack,Bongsoo Lee,Gwang-Moon Eom,Soo-Yeol Lee,Jin-Hun Sohn. The effect of 30% oxygen on visuospatial performance and brain activation :An fMRI study. Brain Cogn. Dec;56(3) (2004) pp279-85. https://doi.org/10.1016/j.bandc.2004.07.005

9. Soon-Cheol Chung,Ji-Hun Kwon,Hang-Woon Lee,gye-Rae tack,Bongsoo Lee.Jeong-Han Yi and Soo-Yeol Le. Effects of high concentration oxygen administration on n-back task performance and physiological signals. Physiol Meas. 28(4) (2007) pp389-96. DOI:10.1088/0967-3334/28/4/005

10. Mark C.Moss , Andrew B.Scholey. Oxygen administration enhances memory formation in healthy young adult. Psychopharmacology (Berl). 124(3) (1996) pp255-60. PMID:8740047

11. Scholey AB1, Moss MC, Neave N and Wesnes K. Cognitive performance, hyperoxia, and heart rate following oxygen administration in healthy young adults. Physiol Behav. 67(5) (1999) pp783-9. https://doi.org/10.1016/S0031-9384(99)00183-3

12. Alfredo J.Garcia Ⅲ,Robert W.Putnam,and jay B.Dean. Hyperbaric hyperoxia and normobaric reoxygenation increase excitability and activate oxygen-induced potentiation in CA1 hippocampal neurons. J Appl Physiol. 109(3) (2010) pp804‐819. doi: 10.1152/japplphysiol.91429.2008

13. Alfredo J.Garcia Ⅲ,Robert W.Putnam,and Jay B Dean Hyperoxic stimulation of synchronous orthodromic activity and induction of neural plasticity does not require changes in excitatory synaptic transmission. J Appl Physiol. 109(3) (2010) pp820‐829. doi: 10.1152/japplphysiol.91430.2008

14. Dominic P.D'Agostino,Robert W.Putnam and Jay B,Dean. Superoxide (·O2−) Production in CA1 Neurons of Rat Hippocampal Slices Exposed to Graded Levels of Oxygen. J Neurophysiol. 98(2) (2007) pp1030-41. doi.org/10.1152/jn.01003.2006

15. Freiberger J1, Coulombe K, Suliman H, Carraway M, Piantadosi C. Superoxide dismutase responds to hyperoxia in rat hippocampus. Undersea Hyperb Med. 31(2) (2004) pp227-32. PMID:15485085

16. Gegentonglaga, Yoshizato H, Higuchi Y, Toyota Y, Hanai Y, Ando Y, Yoshimura A. Variable alteration of regional tissue oxygen pressure in rat hippocampus by acute swimming exercise. Life Sci. 93(21) (2013) pp773-777. doi: 10.1016/j.lfs.2013.09.022.

17. Roeser JC1, Brackett DG, van Heerden ES, Young KM, Bavis RW. Potentiation of the hypoxic ventilatory response by 1 day of hyperoxia in neonatal rats. Respir Physiol Neurobiol. 176(1-2)(2011) pp50-56. doi: 10.1016/j.resp.2011.01.004.

18. Sharma SS1, Gupta S. Neuroprotective effect of MnTMPyP, a superoxide dismutase/catalase mimetic in global cerebral ischemia is mediated through reduction of oxidative stress and DNA fragmentation. Eur J Pharmacol. 561(1-3) (2007) pp72-79. https://doi.org/10.1016/j.ejphar.2006.12.039

19. Zhang QG1, Laird MD, Han D, Nguyen K, Scott E, Dong Y, Dhandapani KM, Brann DW. Critical role of NADPH oxidase in neuronal oxidative damage and microglia activation following traumatic brain injury. PLoS One. (4) (2012) e34504. doi: 10.1371/journal.pone.0034504.

20. Lois C, Alvarez-Buylla A. Evidence for uncoupling of oxygen and glucose utilization during neuronal activation in rat striatum. J Physiol. 498(Pt 2) (1997) pp497–501. DOI: 10.1113/jphysiol.1997.sp021875 PMC1159218

21. Lowry JP, Demestre M, and Fillenz M. Relation between Cerebral Blood Flow and Extracellular Glucose in Rat Striatum during Mild Hypoxia and Hyperoxia. Dev Neurosci. 20 (1998) pp52–58. https://doi.org/10.1159/000017298

22. Nishijima T, Soya H. Evidence of functional hyperemia in the rat hippocampus during mild treadmill running. Neurosci Res. 54 (2006) pp186−191. DOI: 10.1016/j.neures.2005.11.005

23. Nakajima K, Uchida S, Suzuki A, Hotta H, Aikawa Y. The effect of walking on regional blood flow and acetylcholine in the hippocampus in conscious rats. Auton. Neurosci. 103 (2003) pp83–92. DOI: 10.1016/S1566-0702(02)00263-1

24. Massaad CA, Klann E. Reactive Oxygen Species in the Regulation of Synaptic Plasticity and Memory. Antioxid Redox Signal. 14(10) (2011) pp2013-2054. doi: 10.1089/ars.2010.3208.

25. Beckhauser TF, Francis-Oliveira J, De Pasquale R. Reactive Oxygen Species: Physiological and Physiopathological Effects on Synaptic Plasticity. J Exp Neurosci. 10(Suppl 1) (2016) 23-48. doi: 10.4137/JEN.S39887.

26. Brennan AM, Suh SW, Won SJ, Narasimhan P, Kauppinen TM, Lee H, Edling Y, Chan PH, Swanson RA. NADPH oxidase is the primary source of superoxide induced by NMDA receptor activation. Nat Neurosci. 12(7) (2009) pp857-63. doi: 10.1038/nn.2334.

27. Stowe DF, Camara AK. Mitochondrial reactive oxygen species production in excitable cells: modulators of mitochondrial and cell function. Antioxid Redox Signal. 11(6) (2009) pp1373-1414. doi: 10.1089/ars.2008.2331

28. St Pierre J, Buckingham JA, Roebuck SJ, and Brand MD. Topology of superoxide production from different sites in the mitochondrial electron transport chain. J Biol Chem. 277(47) (2002) pp44784-44790. DOI:10.1074/jbc.M207217200

29. Cai H. Hydrogen peroxide regulation of endothe- lial function: Orgins, mechanisms, and conse- quences. Cardiovasc Res. 68 (2005) pp26-36. https://doi.org/10.1016/j.cardiores.2005.06.021

30. Bretón-Romero R, Lamas S. Hydrogen peroxide signaling in vascular endothelial cells. Redox Biol. 2 (2014) pp529-34.doi: 10.1016/j.redox.2014.02.005.

31. William M. Nauseef . Biological Roles for the NOX Family NADPH Oxidases. J Biol Chem. 283(25) (2008) pp16961–16965. doi: 10.1074/jbc.R700045200

32. Clempus RE, Griendling KK. Reactive oxygen species signaling in vascular smooth muscle cells. Cardiovasc Res. 71 (2006) pp216-225. doi: 10.1016/j.cardiores.2006.02.033

33. Kim MJ, Shin KS, Chung YB, Jung KW, Cha CI, Shin DH. Immunohistochemical study of p47Phox and gp91Phox distribution in rat brain. Brain Res. 1040(1-2) (2005) pp178-186. https://doi.org/10.1016/j.brainres.2005.01.066

34. Serrano F, Kolluri NS, Wientjes FB, Card JP, Klann E. NADPH oxidase immunoreactivity in the mouse brain. Brain Res. 988(1-2) (2003) 193-198. https://doi.org/10.1016/S0006-8993(03)03364-X

35. Sumimoto H. Structure, regulation and evolution of Nox-family NADPH oxidases that produce reactive oxygen species. FEBS J. 275(13) (2008) pp3249-3277. doi: 10.1111/j.1742-4658.2008.06488.x.

36. Nagatomo F, Fujino H, Kondo H, Ishihara A. Oxygen concentration-dependent oxidative stress levels in rats. Oxid Med Cell Longev. (2012) 2012:381763. doi: 10.1155/2012/381763.

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1. Saha M, Adams ML, Nelson SC. Review of digit fusion in the mouse embryo. J Embryol Exp Morphol. 2009;49(3): *(In press)*.

Note: List the first six authors followed by et al.
Note: Use of a DOI number is encouraged (if available).
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DEFINITIONS, ACRONYMS, ABBREVIATIONS

Hip-pO_2: the local tissue oxygen pressure in hippocampus
ROS: reactive oxygen species
NOX : NADPH oxidase
MAO: Monoamine oxidase
NOS: NO synthase
O_2- : superoxide