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ORIGINAL ARTICLE

Long-term adaptation of cerebral hemodynamic response to somatosensory stimulation during chronic hypoxia in awake mice

Hiroyuki Takuwa[1], Kazuto Masamoto[1,2], Kyoko Yamazaki[1,3], Hiroshi Kawaguchi[1], Yoko Ikoma[1], Yousuke Tajima[1,4], Takayuki Obata[1], Yutaka Tomita[5], Norihiro Suzuki[5], Iwao Kanno[1] and Hiroshi Ito[1]

Effects of chronic hypoxia on hemodynamic response to sensory stimulation were investigated. Using laser-Doppler flowmetry, change in cerebral blood flow (*CBF*) was measured in awake mice, which were housed in a hypoxic chamber (8% O_2) for 1 month. The degree of increase in *CBF* evoked by sensory stimulation was gradually decreased over 1 month of chronic hypoxia. No significant reduction of increase in *CBF* induced by hypercapnia was observed during 1 month. Voltage-sensitive dye (VSD) imaging of the somatosensory cortex showed no significant decrease in neural activation over 1 month, indicating that the reduction of increase in *CBF* to sensory stimulation was not caused by cerebrovascular or neural dysfunction. The simulation study showed that, when effective diffusivity for oxygen in the capillary bed (*D*) value increases by chronic hypoxia due to an increase in capillary blood volume, an increase in the cerebral metabolic rate of oxygen utilization during neural activation can occur without any increase in *CBF*. Although previous study showed no direct effects of acute hypoxia on *CBF* response, our finding showed that hemodynamic response to neural activation could be modified in response to a change in their balance to energy demand using chronic hypoxia experiments.

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INTRODUCTION

The oxygen supply to the brain plays an important role in energy metabolism in tissues, and long-term low oxygen environments cause several adaptation mechanisms.[1,2] In human studies, hypobaric hypoxia at high altitudes has been shown to cause cerebral vasodilatation[3] and increase the cerebral blood flow (*CBF*), blood pressure, and hematocrit.[2,4] In rodent, several long-term adaptations in systemic and cerebral hemodynamics to chronic hypoxia have been observed. Baseline *CBF* increased during the first several days during hypoxia (10% O_2 concentration), and then began to decrease, finally returning to the prehypoxia baseline level after 3 weeks.[2,5] In addition, increases in hematocrit and respiratory rate were observed during 3 weeks of chronic hypoxia. Capillary density in the brain significantly increased,[5,6] and cerebral vasodilatation occurred[7] after several days of chronic hypoxia. These effects on the cerebral vasculature were thought to be associated with hypoxia-inducible factor-1 and angiopoietin-2, whose gene expression levels were activated after several days of hypoxia.[1,8,9]

Although effects of chronic hypoxia on baseline *CBF* and capillary density have been investigated, effects of the hemodynamic response under chronic hypoxia remain unclear. The hemodynamic response to neuronal activation under acute hypoxia was investigated in several studies,[10–13] but it is clear that the animal condition in acute hypoxic experiments is quite different from that in chronic hypoxic experiments. Especially, increases in capillary density, baseline *CBF*, and the diameter of cerebral vessels occur during chronic hypoxia, and such adaptations might affect the hemodynamic responses to neuronal activation. To clarify the long-term changes of *CBF* response to evoked neural activity in the mouse exposed to chronic hypoxia, we measured the cerebrovascular responses to neural activation and hypercapnia in awake mice under chronic hypoxia. The hemodynamic responses were evaluated by laser-Doppler flowmetry (LDF) experiment repeatedly over 1 month of chronic hypoxia in the same mouse somatosensory cortex. Voltage-sensitive dye (VSD) imaging was also performed to assess the effects of chronic hypoxia on neuronal activity. Furthermore, a simulation study was performed to demonstrate the relation between *CBF* and the cerebral metabolic rate of oxygen utilization ($CMRO_2$) during neural activation under the condition of chronic hypoxia.

MATERIALS AND METHODS

Animal Preparation

All experiments were performed in accordance with the institutional guidelines on the humane care and use of laboratory animals and were approved by the Institutional Committee for Animal Experimentation. A total of 24 male C57BL/6J mice (20 to 30 g, 7 to 11 weeks; Japan SLC, Hamamatsu, Japan) were housed in hypoxic chambers at 8% to 9% O_2 concentration and used in two experiments as follows. In the first experiment (experiment I; Figure 1A), LDF measurement during whisker stimulation was performed before ($N = 12$) and at 7 days ($N = 7$), 14 days ($N = 7$) during, and 1 month ($N = 7$) after chronic hypoxia. In five animals selected from these animals, LDF measurements during CO_2 inhalation and VSD imaging during whisker stimulation were performed before and

[1]Biophysics Program, Molecular Imaging Center, National Institute of Radiological Sciences, Chiba, Japan; [2]Center for Frontier Science and Engineering, University of Electro-Communications, Tokyo, Japan; [3]Department of Biomedical Engineering, Tokyo University, Tokyo, Japan; [4]Department of Neurological Surgery, Chiba University Graduate School of Medicine, Chiba, Japan and [5]Department of Neurology, Keio University School of medicine, Tokyo, Japan. Correspondence: Dr H Ito, Biophysics Program, Molecular Imaging Center, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan.
E-mail: hito@nirs.go.jp
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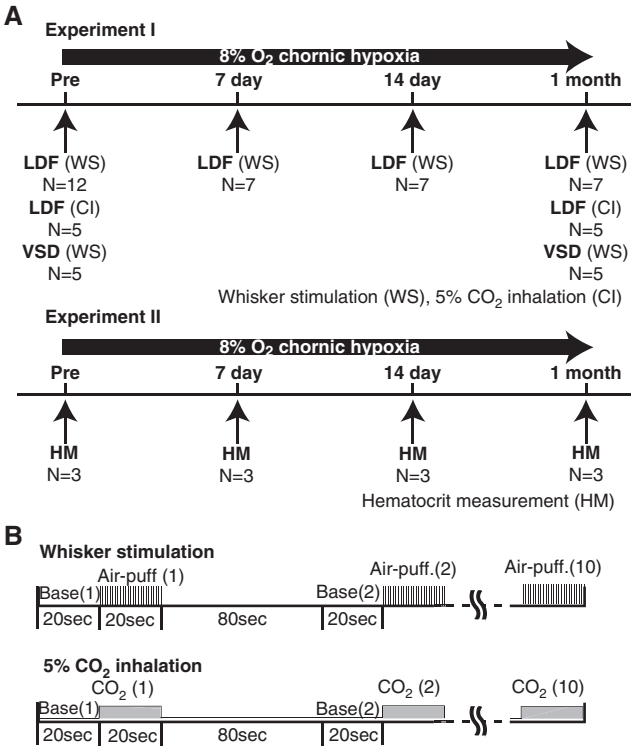

Figure 1. (**A**) Experiment I (laser-Doppler flowmetry (LDF) measurement and voltage-sensitive dye (VSD) imaging in awake animals) and experiment II (hematocrit measurement). LDF measurement during whisker stimulation was performed before ($N = 12$) and 7 days ($N = 7$), 14 days ($N = 7$), and 1 month ($N = 7$) after the start of chronic hypoxia. In five of these animals, LDF measurements during CO_2 inhalation and VSD imaging during whisker stimulation were performed before and 1 month after chronic hypoxia. In experiment II, hematocrit measurement was performed in a total of 12 animals (each measurement used three animals) before, at 7 days and 14 days during, and 1 month after chronic hypoxia. Hematocrit was estimated with a blood analyzer (I-STAT; Abbott). (**B**) Experimental protocols of whisker stimulation and 5% CO_2 inhalation. In whisker stimulation, 20 seconds of rectangular pulse air-puff stimulation (50-milliseconds pulse width and 100-milliseconds onset-to-onset interval, i.e., 10 Hz frequency) was given to the right whisker region of mice. Ten consecutive trials were repeated with an onset-to-onset interval of 120 seconds in each experiment. In CO_2 inhalation, 5% CO_2 gas was given to mice continuously for the same duration (20 seconds) and interval (120 seconds) as the sensory stimulation.

1 month after chronic hypoxia. In the second experiment (experiment II; Figure 1A), hematocrit measurement was performed in a total of 12 animals (each measurement used three animals) before, at 7 days, 14 days during, and 1 month after chronic hypoxia. Anesthesia was only used in this experiment II for pain avoidance.

A surgical procedure was applied to prepare a chronic cranial window and fixation to the heads of mice for reproducible stereotaxic measurement for up to 1 month. The animals were anesthetized with a mixture of air, oxygen, and isoflurane (3% to 5% for induction and 2% for surgery) via a facemask. The animals were fixed in a stereotactic frame, and rectal temperature was maintained at 38°C using a heating pad (ATC-210, Unique Medical, Tokyo, Japan). The methods for preparing the chronic cranial window have been reported in detail by Tomita *et al.*[14] A midline incision (10 mm) was made to expose the skull. Craniotomy was performed over the left somatosensory cortex, keeping the dura intact (3 to 4 mm diameter, centered at 1.8 mm caudal, and 2.5 mm lateral from bregma). The brain surface was sealed with a quartz coverslip using dental cement (Ionosit, DMG, Hamburg, Germany) to make the preparation waterproof. A custom metal plate was affixed to the skull with a 7-mm diameter hole centered over the cranial window. After completion of the surgery, the animals were allowed to recover from anesthesia and housed for at least 7 days before initiation of the experiments.

Exposure to Chronic Hypoxia

From 1 week after the cranial window surgery, the mice were kept for up to 1 month in hypoxic chambers (8% to 9% O_2 in N_2) with food and water provided *ad libitum*. The chamber was a fully sealed plastic box (200 mm long, 150 mm wide, and 100 mm high) with two nozzles. One nozzle, attached to the lateral side of the chamber, was connected to a gas blender (GB-2C, KOFLOC, Kyoto, Japan) to deliver the hypoxic gas mixture into the chamber. The second nozzle was used to flush out the gas mixture. The hypoxic gas was regulated by gas blender, and the O_2 levels in the chamber were monitored every day using an oxygen sensor (OPA-5000E, KITAGAWA, Kanazawa, Japan). During chronic hypoxia, the mean oxygen concentration in the chamber was 8.6% ± 0.2%, indicating that the hypoxic chamber was maintained within our target range (8% to 9% O_2). The temperature in the chamber was controlled at ~23°C (range 22°C to 24°C) with a room air conditioner. Two animals per chamber were housed in each experiment for a maximum of up to 1 month. The chamber was opened for 10 minutes every 3 days for cleaning and animal care.

Laser-Doppler Flowmetry Measurement

The animals were moved from a hypoxic chamber to a recording room, and the measurement was conducted under normoxic condition. The body weight of the animal was measured, and then the head was fixed to a custom-made stereotactic apparatus with a floating ball device that allowed the animal to move freely during the recording of LDF.[15] Evoked *CBF* was measured with laser-Doppler flowmetry (FLO-C1, OMEGAWAVE, Tokyo, Japan), as described previously.[16] The tip of the LDF probe (Type NS, OMEGAWAVE) was positioned over the whisker stimulation-induced activated cortex on the cranial window while avoiding large blood vessel areas. The activated hot spot was preliminarily determined by screening the response to sensory stimulation at several points in the somatosensory area. Then, the *X*–*Y* position of the LDF tip was marked on the edge of the cranial window for reproducible placement of the LDF tip. The angle of the LDF probe to the cortex was fixed by manipulator, perpendicular to the cranial window surface. Also, the distance between the LDF tip and the surface of the cranial window was maintained among the different experiments. On each day of the experiments, the level constancy of the reflected light signal for the LDF measurements was confirmed before initiation of the recording.

The time course of the LDF signal changes was recorded using a polygraph data acquisition system (MP150, BIOPAC Systems, Goleta, CA, USA) at a sampling rate of 200 Hz and analyzed offline. For each trial of the experiment, the LDF signal was normalized by the 20-second prestimulus baseline level, and averaged across 10 trials. For the whisker stimulation experiments, the magnitude of evoked *CBF* was calculated as the mean percentage change for 20-seconds stimulation periods relative to baseline. In the case of CO_2 inhalation, the mean percentage increase in *CBF* was calculated from 10 to 20 seconds of the stimulation period, because the increase in *CBF* usually started 5 to 10 seconds after inhalation. Statistical analysis was performed to compare the evoked *CBF* across different experimental days using one-way analysis of variance followed by Tukey's test.

Voltage-Sensitive Dye Imaging

The cerebral cortex was stained with RH1691 (Optical Imaging, Rehovot, Israel) via transdura delivery for 2 hours and rinsed with dye-free saline for 30 seconds. Dye and saline were injected through a metal tube (500-μm inside-diameter), which was connected to a space between the cranial window and dura through one side of the cranial window. Then, the mice were fixed onto the apparatus while keeping an awake-state. The excitation light was 632 ± 10 nm, and a fluorescent light from the stained cortex was passed through a dichroic mirror and long-pass filter (>660 nm).[15] The image was obtained using a 128-channel photodiode array at a rate of 1 kHz. The *X*–*Y* in-plane resolution was 250 × 250 μm^2.

For the analysis of VSDI signals, independent component analysis was applied to exclude systemic physiological noise originating from the heartbeat and respiration.[15] Then, all of the VSDI signals were normalized to the maximal response measured over 128 channels. The whisker stimulation-induced activated region was determined by measuring the number of pixels at which the normalized VSD signal was greater than an intensity threshold of 0.5 (maximum pixel intensity 1.0). Comparison of the activated region was made between pre- and posthypoxia conditions, and statistical analysis was performed by paired *t*-test.

Whisker Stimulation

Compressed air (up to 10 psi) was generated with an air compressor (NUP-2, AS ONE, Osaka, Japan), and the pressure was controlled with a Pico Pump (PV830, WPI, Osaka, Japan). The compressed air pulse was delivered to the entire right whisker region from a nozzle placed ~1 cm away from the mouse.[16] Twenty seconds rectangular pulse stimulation (50-milliseconds pulse width and 100-milliseconds onset-to-onset interval, i.e., 10 Hz frequency) was generated with Master-8 (A.M.P.I, Jerusalem, Israel). In each experiment, 10 consecutive trials were repeated with an onset-to-onset interval of 120 seconds (Figure 1B).

Hypercapnia (5% CO_2 Inhalation)

A hypercapnic gas mixture of 5% CO_2, 21% O_2, and residual N_2 was inhaled by awake-behaving mice via a facemask (300 mL/min). At all times except during the CO_2 inhalation, the mice inhaled room air (300 mL/min). CO_2 gas was given to the mice for the same duration (20 seconds) and interval (120 seconds) as the sensory stimulation using the Master 8 (Figure 1B). CO_2 inhalation was repeated 10 times, and the LDF signals were averaged offline.

Experiment II: Hematocrit Measurement

Hematocrit measurement was performed in total 12 animals (each measurement used three animals) before, at 7 days, 14 days, and 1 month after the start of chronic hypoxia. Because mice under hematocrit measurement, which severely influenced the physiological state, were unsuitable for the long-term LDF and VSDI experiment, those in experiment II were different from those in experiment I. In the experiment, the mice were moved from the hypoxic chambers and exposed to room air. They were anesthetized with isoflurane (3% to 5% for induction and 2% for surgery) using facemasks. Body temperature was monitored with a rectal probe and maintained at ~37.0°C with a heating pad. Heart blood samples were obtained with a needle (23 gauge) before and after 7 days, 14 days, and 30 days of hypoxic chamber exposure (Figure 1A). The hematocrit level was analyzed with a blood analyzer (I-STAT; Abbott, Chicago, IL, USA).

Simulation Study

To evaluate the relation between *CBF* and $CMRO_2$ during neural activation under the condition of chronic hypoxia, a simulation study was performed. The effective diffusivity for oxygen in the capillary bed (*D*) was defined as $OEF = 1 - e^{(-D/CBF)}$, where *OEF* is the oxygen extraction fraction.[17] Cerebral metabolic rate of oxygen utilization can be calculated as $CMRO_2 = C_a \bullet CBF \bullet OEF$, where C_a is the total oxygen content in arterial blood. Thus, the relation between changes in *CBF* and $CMRO_2$ during neural activation should depend on *D*. Assuming the baseline *CBF* for mouse as 100 mL per 100 mL per minute[17] and baseline *OEF* to be 0.2,[18] *D* can be calculated to be 0.223 mL per mL per minute. The *D* value is proportional to the capillary blood volume,[19] and a 40% to 70% increase in capillary diameter by chronic hypoxia has been reported in mice using two-photon laser microscopy,[7] corresponding to a 96% to 189% increase in capillary blood volume and, therefore, in *D*. Thus, the relation between changes in *CBF* and $CMRO_2$ during neural activation was simulated for *D* values of 0.223 (baseline), 0.245 (10% increase), 0.268 (20% increase), 0.335 (50% increase), 0.446 (100% increase), and 0.669 (200% increase) mL per mL per minute.

RESULTS

Change in Systemic Hematocrit and Body Weight During Chronic Hypoxia

The body weights before and after 7 days, 14 days, and 1 month of exposure to chronic hypoxia were 23.3 ± 2.3 g, 22.2 ± 2.2 g, 20.7 ± 1.6 g, and 22.0 ± 1.4 g, respectively. There was no significant difference in body weight among the respective measurement days. On the other hand, when control mice were housed in a normoxic chamber, body weights significantly increased over one month (day 0: 23.8 ± 2.2 g, day 30: 26.4 ± 2.4 g, $P < 0.01$, $N = 6$). Therefore, it was possible that chronic hypoxia inhibited the weight gain of mice over the month. Hematocrit was significantly higher ($P < 0.01$) at 7 days (51.5%), 14 days (59.0%), and 1 month (68.5%) from the start of exposure to chronic hypoxia as compared with control mice (34.6%). These results were in good agreement with previous studies conducted in rats and mice under chronic hypoxia.[10]

Cerebral Blood Flow Response to Sensory Stimulation

Time–response curves of the increase in *CBF* during whisker stimulation during 1 month of chronic hypoxia are shown in Figure 2. The mean percentage increases were $20.3\% \pm 6.8\%$, $13.1\% \pm 3.3\%$, $9.9\% \pm 4.2\%$, and $3.9\% \pm 4.0\%$ before and after 7 days, 14 days, and 1 month of chronic hypoxia, respectively (Figure 3). Statistically significant differences were found at day 7 ($P < 0.05$), day 14 ($P < 0.01$), and 1 month ($P < 0.01$) of chronic hypoxia, compared with that of prehypoxic control.

Cerebral Blood Flow Response to 5% CO_2 Inhalation

The time–response curve of the increase in *CBF* during 5% CO_2 inhalation before chronic hypoxia was almost identical to that after 1 month of hypoxia (Figure 4A). The mean percentage increase in *CBF* induced by 5% CO_2 inhalation was $14.8\% \pm 3.5\%$ and $16.3\% \pm 4.0\%$ before and 1 month after chronic hypoxia, respectively (Figure 4B). There was no significant difference in the increase in *CBF* between the measurements before and 1 month after chronic hypoxia.

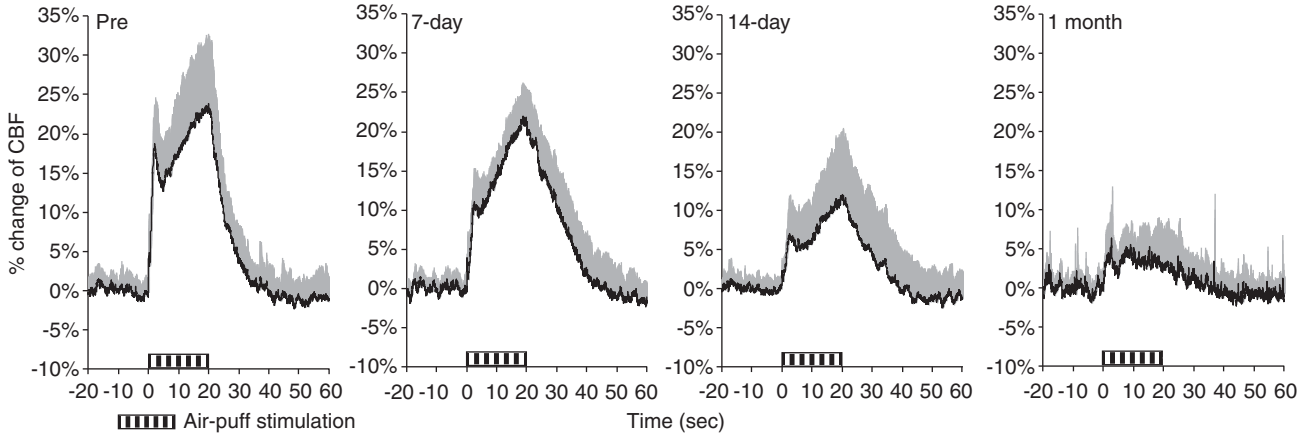

Figure 2. Time–response curves for normalized increase in cerebral blood flow (*CBF*) response to sensory stimulation during chronic hypoxia. Horizontal bars indicate the stimulation period. These data were normalized to baseline level (20 seconds before sensory stimulation). Each response curve represents the mean of all animals at each measurement day. Error bars indicate s.d.

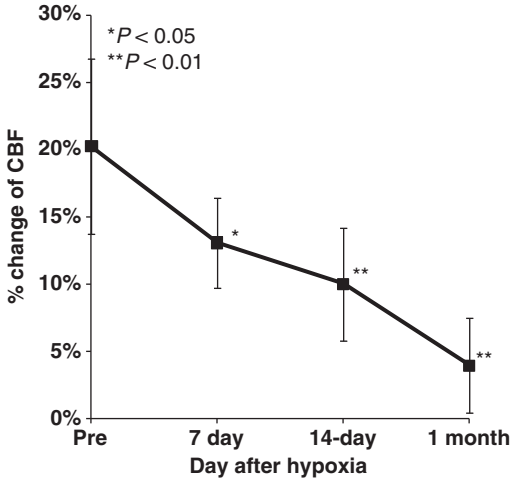

Figure 3. Longitudinal cerebral blood flow (*CBF*) measurements under chronic hypoxia. Increase in *CBF* response to sensory stimulation was consistently observed at pre, 7 days, 14 days, and 1 month. Bold squares and line represent the mean of all animal data of average values at each measurement day. Error bars indicate s.d. $^{*}P<0.05$, $^{**}P<0.01$.

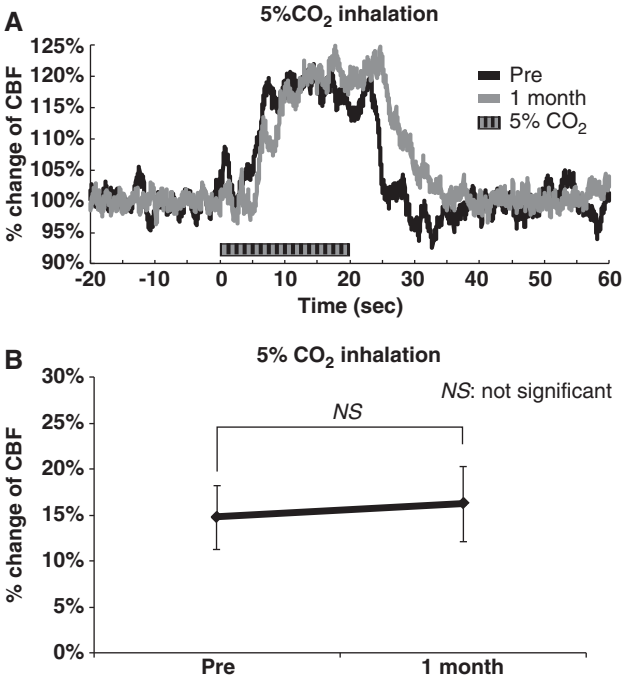

Figure 4. Increase in cerebral blood flow (*CBF*) evoked by 5% CO_2 inhalation. (**A**) Time–response curve of increase in *CBF*. Time–response curves were normalized to the baseline level (20 seconds before sensory stimulation) and shown for one representative animal. Horizontal bars indicate the stimulation period. (**B**) Mean percentage increase in *CBF* within 10 to 20 seconds 5% CO_2 inhalation ($N=5$). Error bars indicate s.d.

Neural Response to Whisker Stimulation

Figure 5 shows the representative fluorescent VSD signals evoked by a single sensory stimulation after 1 month of chronic hypoxia (Figure 5). The region of neuronal activity before chronic hypoxia was almost identical to that after 1 month of hypoxia (Figure 5A). There were no significant differences in the number of pixels between before and after 1 month of chronic hypoxia (Figure 5B).

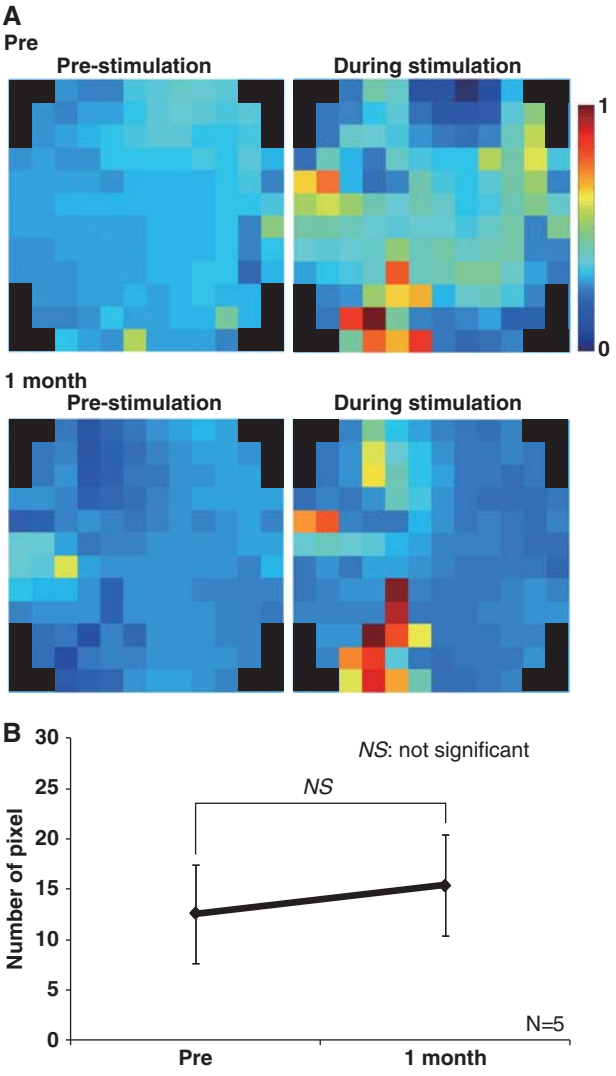

Figure 5. Neuronal activities evoked by sensory stimulation under chronic hypoxia condition. (**A**) Activation maps of voltage-sensitive dye (VSD) imaging experiment performed before (top) and 1 month (bottom) after chronic hypoxia, shown for one representative animal. Right and left frames showed VSD imagings before and immediately after sensory stimulation, respectively. (**B**) Summary of VSD results in five animals. There was no significant difference in the number of pixels between pre and 1 month.

Effects of Changes in Effective Diffusivity for Oxygen in the Capillary Bed (D) by Chronic Hypoxia

The relation between changes in *CBF* and $CMRO_2$, corresponding to *CBF* multiplied by *OEF*, during neural activation for each *D* value was simulated using Hyder's model[19] as shown in Figure 6. Based on the simulation results in Figure 6, a 690% increase in *CBF* during neuronal activation is required for a 10% increase in $CMRO_2$ when *D* remained at 0.223 in spite of chronic hypoxia. As mention above, the *D* values increased from 0.223 to 0.466 to 0.669 after 3 weeks of chronic hypoxia as a result of a 96% to 189% increase in capillary blood volume.[7] The results shown in Figure 5 indicated that neuronal activation at somatosensory cortex was quite stable throughout the 1 month of chronic hypoxia, indicating that $CMRO_2$ was also stable. Thus, as a result of the increase in *D* (0.466 to 0.669) caused by chronic hypoxia, no increase in *CBF* is required for the 10% increase in $CMRO_2$ (Figure 6) because, in the case of $D=0.466$ to 0.669, the % change in $CMRO_2$ was already above 10%, even if *CBF* did not increase.

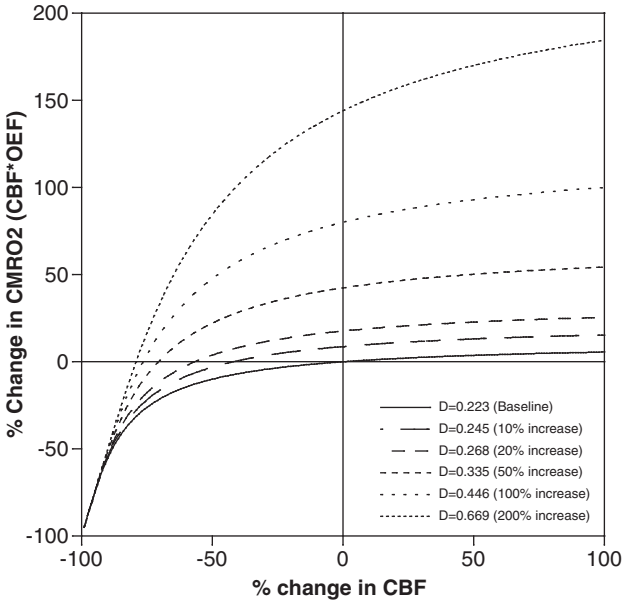

Figure 6. Simulation result of Hyder's model. Solid and dashed lines indicated the relationship between cerebral metabolic rate of oxygen utilization ($CMRO_2$) and cerebral blood flow (*CBF*) among the respective *D* values.

DISCUSSION

We performed *LDF* measurements in awake mice maintained under chronic hypoxic conditions. Although adaptation of the baseline *CBF* to chronic hypoxia has been previously investigated using human and animal models, this study represents the first observation of the effects of chronic hypoxia on the hemodynamic responses in awake mice. In this work, we observed that chronic hypoxia causes a significant reduction of the increase in *CBF* evoked by sensory stimulation. Previous study using BOLD fMRI (blood oxygen level-dependent functional magnetic resonance imaging) showed reduced cerebrovascular response to visual stimulation in native-born high-altitude residents as compared with native-born sea-level residents.[20] These results might be related to our results of changes in *CBF* during neural activation.

The reduction in the increase of *CBF* during neuronal activation appeared to lead to a reduction in the increase of the oxygen supply to the brain. To explain the reasons for the reduction of the increase in *CBF* during stimulation under chronic hypoxic conditions, we hypothesized that this reduction was caused by (1) cerebrovascular dysfunction, (2) neuronal dysfunction, and (3) regulation of hemodynamics to adapt to the chronic hypoxic condition. Because the hypercapnic *CBF* response was not reduced, vascular dilatory function was sufficiently sustained in the chronic hypoxia mice. Moreover, VSD imaging showed no attenuation of neuronal activation during sensory stimulation during the 1 month of chronic hypoxia. Based on these findings, it was clear that the reduction in the increase in functional hyperemia under chronic hypoxic conditions was not caused either by cerebrovascular dysfunction or by neuronal dysfunction in the somatosensory cortex.

As mentioned above, we have previously reported that the vasodilation induced by chronic hypoxia occurred mainly in the parenchymal capillaries, indicating the ability to adjust the diameter in response to the oxygen environment.[7] Based on Hyder's model,[19] the relation between changes in *CBF* and $CMRO_2$, corresponding to *CBF* multiplied by *OEF*, during neural activation can be simulated for each *D* value, which is in proportion to capillary blood volume, as shown in Figure 6. This simulation study shows that when *D* increases by chronic hypoxia, an increase in $CMRO_2$ during neural activation can be revealed without any increase in *CBF*. On the other hand, the oxygen content (C_a) of mice might be changed during hypoxic condition. C_a is expressed as follows:

$$C_a = \alpha \times Hb \times SaO_2 + \beta \times PaO_2,$$

where α is the oxygen binding capacity of hemoglobin (1.39 mL/g),[21] *Hb* is the hemoglobin concentration (g/dL), SaO_2 is arterial O_2 saturation, PaO_2 is arterial oxygen partial pressure (mm Hg), and β is the oxygen solubility (0.00315 mL per 100 mL per mm Hg). When the hypoxic condition (8% O_2) decreased PaO_2 from 100 to 40 mm Hg, SaO_2 was decreased from 0.92 to 0.47 (P_{50} of mouse = 41.5 mm Hg; pH = 7.40).[22] The hematocrit results in our study indicated that hemoglobin was increased by about two times during chronic hypoxia. Based on these parameters, C_a remained almost unchanged under hypoxia (19.73 mL/mL blood) as compared with that under normoxia (19.50 mL/mL blood).

The adaptive regulation mechanism of *CBF* responses during chronic hypoxia must be associated with several factors. One possibility is the inhibition of a synthetase of a vasoactive mediator (e.g., nitric oxide, cyclooxygenase-2, and adenosine) released from neurons and glia by neural activity[23–25] or by disturbing astrocyte function, which is associated with neurovascular coupling.[26] Especially, nitric oxide is associated with hypoxia-inducible factor-1 activity,[27,28] suggesting that nitric oxide plays an important role in the cerebrovascular tone in chronic hypoxia and might also affect to the reduction of increase in *CBF*. To explore the mechanism, further experiments using synthetase inhibitors or immunostaining techniques in mice need to be performed.

Using positron emission tomography in human subjects, Mintun *et al*[10] has shown that hemodynamic response to visual stimulation was identical between normoxia and mild acute hypoxia (fraction of inspired oxygen; FiO_2 of 12%) conditions.[11] These findings suggest that the neural activity-induced *CBF* response is determined by factors other than local requirements of oxygen. On the other hand, in the present study, the long-term low oxygen condition could be modified by neurovascular coupling, and the simulation results suggested that the reduction in the increase in *CBF* contributed to the balance between oxygen supply and metabolism in the brain. In other words, the hemodynamic response to neuronal activation can be modified in response to the change in their balance to energy demand. The discrepancy between acute hypoxia and chronic hypoxia could be explained as follows. A short-term exposure to hypoxia may not produce a stabilized baseline state, whereas a long-term exposure produces a newly established baseline state in which the oxygen supply-demand level can be balanced. Further experiments are needed to elucidate this slow adaptation mechanism of neurovascular coupling to changes in the oxygen environment, which involve (1) sensing the tissue oxygen state, (2) monitoring the supply-demand balance, and (3) controlling the magnification factor of *CBF* changes in neurovascular coupling.

In summary, we found a reduction in the increase in *CBF* evoked by neuronal activation in mice occurring during 1 month of chronic hypoxia. The results of the simulation using Hyder's model indicated that a slight increase in *CBF* caused a large increase in oxygen supply to the brain under increased *D* value conditions. The adaptation mechanisms underlying the reduction in the increase of *CBF* under chronic hypoxia remain to be identified. To explore these mechanisms, further experiments using synthetase inhibitors or immunostaining techniques on mice should be performed.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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