SUPPLEMENTAL MATERIAL

Dichotomous effects of chronic intermittent hypoxia on focal cerebral ischemic injury

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Supplemental Methods

Resting CBF by ASL-MRI

CBF was assessed quantitatively using arterial spin labeling magnetic resonance imaging (ASL-MRI), performed on a 7.0 Tesla 70/30 Bruker Biospec small animal MRI system with 450 mT/m gradient amplitude and a 4500 T/m/s slew rate, as previously described[1]. Briefly, a volume coil was used for transmission and a surface coil for reception. Anatomical localizer images were acquired to find the transversal slice at the level of bregma. One axial slice was acquired with a field of view of 15 × 15 mm, spatial resolution of 0.117 × 0.117 × 1 mm, TE of 5.368 ms, effective TE of 48.32 ms, recovery time of 10 s, and a RARE factor of 72. 22 TIR values ranging from 30 to 2300 ms were used, and the inversion slab thickness was 4 mm. For computation of CBF, the Bruker ASL perfusion processing macro was used. The masked CBF images were exported and further processed using customized software. Data was spatially despiked and the average value over the slice is reported as CBF (ml/100g/min).

Fluoro-Jade B staining for degenerating neurons

Fluoro-Jade B (FJB) is a polyanionic fluorescein derivative that specifically binds degenerating neurons. FJB staining was performed in coronal sections (14 μm) isolated from the forebrains of paraformaldehyde (4%) perfused naïve mice. Briefly, sections were placed in 1% sodium hydroxide/80% ethanol solution, followed by 70% ethanol, 0.06% potassium permanganate solution and 0.0004% FJB staining solution. Fluorescence (excitation 495 nm; emission 519 nm) was visualised on a confocal laser microscope (Leica) equipped with an argon laser in the region of the cortex. For the positive control, the ipsilateral cortex of a mouse subjected to cerebral ischemia (MCAO) was stained with FJB and imaged.

Supplemental Tables

Table I: Physiological parameters

10% O_2		Body weight (g)	SBP (mmHg)	Blood glucose (mg/dl)	Hematocrit (%)
14 d					
	Sham	22.6±0.5 (29)	124.6±3.0 (20)		
	CIH	22.9±0.5 (30)	127.9±3.3 (20)		
21 d					
	Sham	22.6±0.6 (13)	118.8±3.5 (20)	176.8±15.1 (8)	49.6±2.8 (8)
	CIH	22.7±0.5 (14)	128.9±4.0 (20)	148.2±13.6 (10)	49.2±2.2 (10)
35 d					
	Sham	24.0±0.3 (30)	120.0±2.8 (20)	133.7±20.0 (10)	49.5±3.3 (9)
	CIH	23.7±0.2 (30)	124.8±2.8 (20)	151.4±9.9 (7)	54.0±2.7 (8)
6% O_2		**Body weight (g)**	**SBP (mmHg)**	**Blood glucose (mg/dl)**	**Hematocrit (%)**
14 d					
	Sham	21.1±0.3 (20)	121.8±2.1 (20)	187.8±8.0 (9)	50.8±1.3 (10)
	CIH	19.1±0.3 (19)	118.2±2.7 (19)	181.9±9.8 (7)	52.9±2.8 (7)
21 d					
	Sham	21.6±0.3 (25)	135.8±5.0 (20)	217.9±5.0 (8)	48.5±1.8 (9)
	CIH	20.6±0.3* (25)	137.4±5.8 (20)	166.3±23.8* (7)	58.7±2.9* (7)
35 d					
	Sham	24.5±0.4 (24)	133.8±4.1 (20)	139.9±6.4 (8)	45.5±1.4 (8)
	CIH	21.2±0.4* (24)	134.1±5.4 (20)	150.2±10.7 (10)	49.5±1.3 (10)

*$p<0.05$

Supplemental Figures & Figure Legends

Figure I

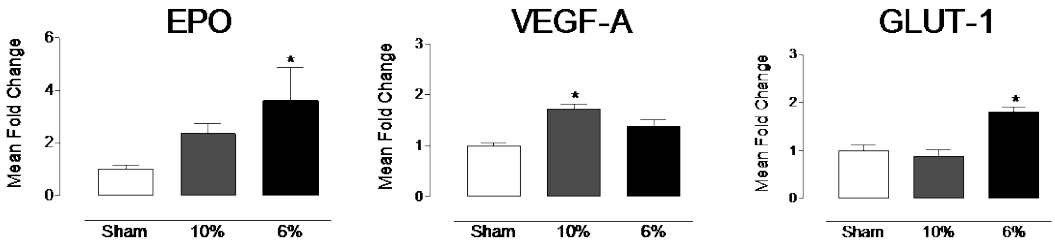

Figure II

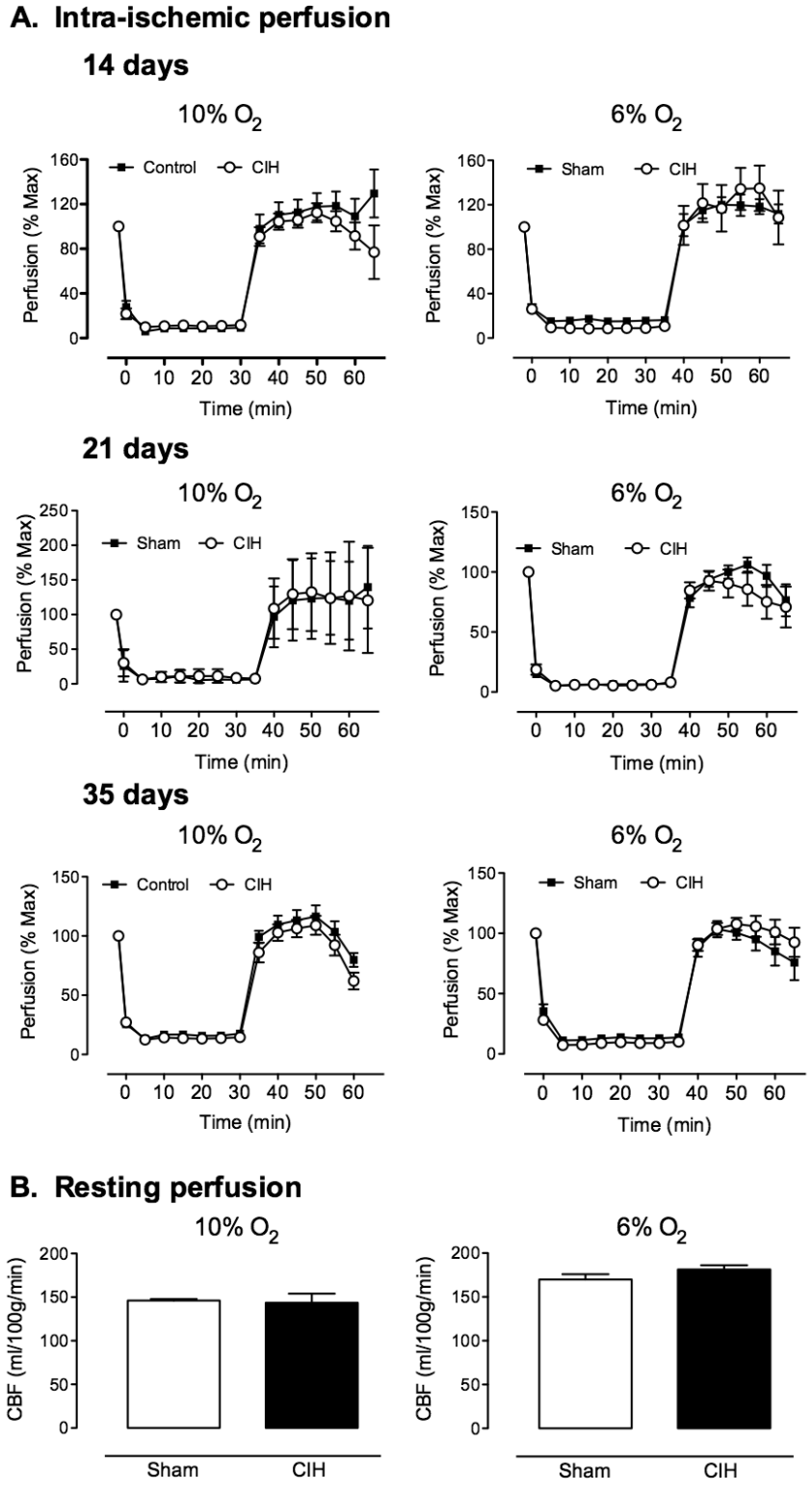
A. Intra-ischemic perfusion
14 days
10% O2
6% O2
Control
CIH
Sham
CIH
Perfusion (% Max)
Time (min)
21 days
10% O2
6% O2
Sham
CIH
Perfusion (% Max)
Time (min)
35 days
10% O2
6% O2
Control
CIH
Sham
CIH
Perfusion (% Max)
Time (min)
B. Resting perfusion
10% O2
6% O2
CBF (ml/100g/min)
Sham
CIH

Figure III

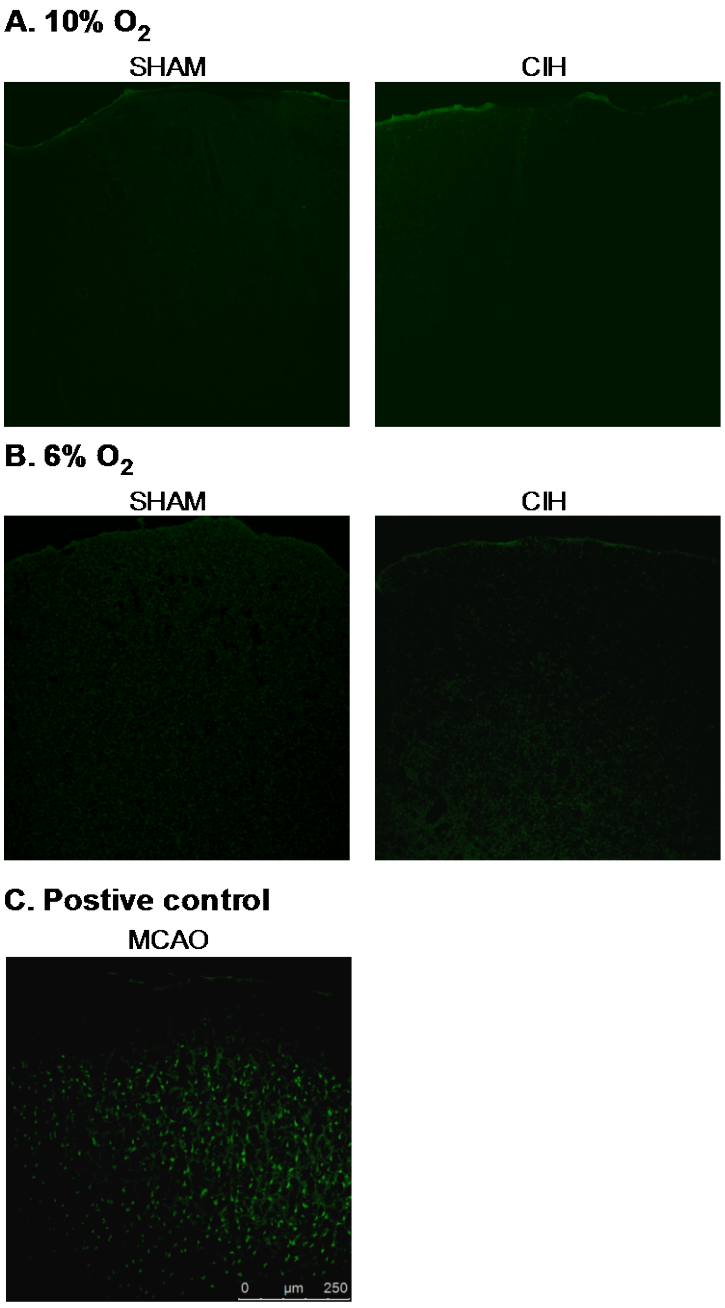

Figure I: CIH induced changes in HIF-1-dependent gene expression. mRNA expression of HIF-1 mediated genes EPO, VEGF-A and Glut-1 tended. Both 10% and 6% CIH increased EPO mRNA expression (35 days; n=5-15/group; *p<0.05 from sham, ANOVA). In addition, modest increases in VEGF-A at 10% CIH and Glut-1 at 6% CIH were observed.

Figure II: Influence of CIH on resting and intra-ischemic CBF. A. The degree of CBF reduction and reperfusion is similar in the ischemic territory in mice exposed to either sham, 10% or 6% CIH (14, 21 and 35 days; n=7-10/group). **B.** Resting CBF (ml/100g/min), measured using ASL-MRI, is similar in sham, 10% and 6% CIH exposed mice (35 days; n=5/group; p>0.05, t-test).

Figure III: A, B. Representative images of cerebral cortex stained with FJB illustrating no evidence of neuronal degeneration in mice exposed to either sham, 10% or 6% CIH. **C.** Cortex from mouse subjected to MCAO (positive control) confirms that FJB detects degenerating neurons. CIH, chronic intermittent hypoxia; FJB, Fluoro-Jade B; MCAO, middle cerebral artery occlusion.

Supplemental References

1. Jackman K, Kahles T, Lane D, Garcia-Bonilla L, Abe T, Capone C, et al. Progranulin deficiency promotes post-ischemic blood-brain barrier disruption. *J Neurosci*. 2013;33:19579–19589.