Transient cerebral ischemia in rodents exposed to chronic intermittent hypoxia

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Target Audience: Researchers interested in chronic intermittent hypoxia, sleep apnea, or stroke

Purpose: Obstructive sleep apnea is a sleep related disorder in which an individual stops breathing periodically throughout the night. Hallmarks of sleep apnea include snoring, daytime sleepiness, and reduced cognitive function. Severe sleep apnea has been correlated with stroke, arterial hypertension, heart failure, pulmonary hypertension, endothelial dysfunction, vascular remodeling, reduced cerebral autoregulation, and increased sympathetic activity.[1] In addition to increased risk for stroke, there is evidence to suggest that stroke severity is worse in patients with sleep apnea, including poorer short-term and long-term outcomes.[2,3,4] Animal models of sleep apnea include exposure to chronic intermittent hypoxia (CIH). There is controversy in the literature on whether prior exposure to CIH is detrimental to or neuroprotective against transient cerebral ischemia, likely due to differences in CIH protocol.[5] In this study, we will induce transient (60 minute) cerebral ischemia using middle cerebral artery occlusion (MCAO) in controls and animals exposed to CIH of 10% O_2 for 14 days. Ischemic infarct and recovery will be characterized using multimodal MRI.

Methods: Male SD rats exposed to chronic intermittent hypoxia were housed in chambers connected to a custom-built automated system in which ambient levels of oxygen were controlled. Oxygen cycled between 21% and 10% ten times an hour from 8:15am to 4:15pm, during the animals normal sleep time. Chambers were maintained at 21% oxygen from 4:15pm to 8:15am. 60-min MCAO was induced after 14 days of CIH exposure. Rats were intubated, and mechanically ventilated. Rectal temperature, oximetry and heart rates were monitored and maintained within normal physiological ranges. MRI experiments were performed under 1.2% isoflurane at 7T. CBF, ADC, and T2 lesion volumes were longitudinally measured using standard protocols.[6,7] Data analysis was performed using STIMULATE software (University of Minnesota). Stroke lesions were calculated with a threshold of 0.3ml/g/min for CBF and 0.0055 for ADC. Data were excluded for animals that died before 2 days post stroke. One-way ANOVA was used to compare groups with significance at $p<0.05$. All data is presented as mean ± S.E.M.

Results: Figure 1 shows a representative CBF, ADC at 30 minutes and T2 at 2 days from a control rat and a 14 day CIH rat. **Figure 2** shows the group-averaged lesion volumes at different time points. Group averaged CBF stroke lesion volumes at 30 minutes were not statistically different between controls and CIH exposed animals. Successful reperfusion was achieved in all animals. Group averaged ADC lesion volumes at 30, 90 and 180 minutes after stroke were significantly larger in the CIH exposed group as compared to controls. Group averaged T2 lesion volumes on days 2, 7 and 14 were significantly larger in the CIH exposed group as well.

Discussion/Conclusion: Both groups had the same initial lesion volume as measured by blood flow, indicating same insults were achieved in both group of animals. The CIH animals had significantly larger ADC and T2 lesion volumes compared to control animals throughout all time points. Our findings are consistent with data from other CIH exposed rats undergoing MCAO in which 2,3,5-triphenyltetrazolium chloride was used to calculate lesion volume [8], as well as data from clinical stroke patients with prior sleep apnea. Our findings indicate that with exposure to CIH of 10% O_2 for 14 days, there are significant changes in the brain that makes it more susceptible to larger infarct volume following transient ischemic insult. Future studies will investigate treatments to ameliorate negative effects of CIH on transient cerebral ischemia and will include behavioral and functional imaging measures.

References: **1.** Dempsey et al. Physiol Rev. 2010; 90(1):47-112. **2.** Parra et al. Am J Respir Crit Care Med. 2000; Feb(161):375-80. **3.** Good et al. Stroke. 1996; 27(2):252-9. **4.** Ryan et al. Stroke. 2011; 42(4): 1062-7. **5.** Jackman et al. Stroke. 2014; May; 45(5):1460-7. **6.** Shen et al. J Cereb Blood Flow and Metab. 2003; 23: 1479-88. **7.** Duong et al. Magn Reson Med. 2000; 43:383-92. **8.** Wang et al. J Neuroscience. 2011; 31(28):10241-8.

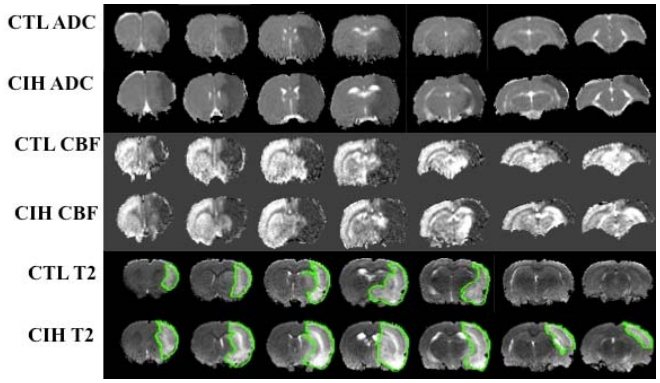

Figure 1: Stroke lesion CBF and ADC at 30 min, and T2 at day 2 for representative control and 14 day CIH animals.

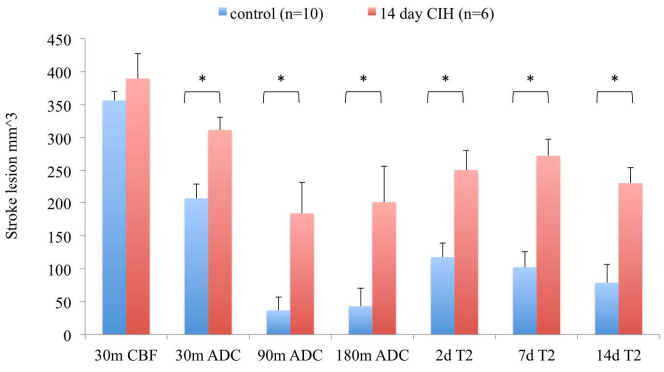

Figure 2: Group averaged lesion volume comparisons. * $p<0.05$