

# HIF-1 $\alpha$ DEFICIENT MICE HAVE INCREASED BRAIN INJURY AFTER NEONATAL HYPOXIA-ISCHEMIA

R. Ann Sheldon<sup>1</sup>, Christina L. Lee<sup>1</sup>, Xiangning Jiang<sup>1</sup>, Dezhi Mu<sup>1,3</sup> and Donna M. Ferriero<sup>1,2</sup>

Depts. of Neurology<sup>1</sup> and Pediatrics<sup>2</sup>, Univ. of California, San Francisco, CA 94143, USA; Dept. of Pediatrics, West China Second Univ. Hospital, Sichuan Univ., Chengdu, China<sup>3</sup>

**Background:** Hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) is a transcription factor that is essential for the activation of over 40 hypoxia-inducible genes like erythropoietin, glucose transporters, glycolytic enzymes, and vascular endothelial growth factor. HIF-1 $\alpha$  activation may promote cell survival in hypoxic or ischemic brain. Reports from studies in adult ischemia have shown conflicting evidence in regard to the role of HIF.

**Objectives:** To understand the role of HIF-1 $\alpha$  in neonatal hypoxic-ischemic (HI) brain injury.

**Methods:** Mice with conditional neuron-specific inactivation of HIF-1 $\alpha$  were generated using Cre/Lox technology. Briefly, mice heterozygous for CAMCRE were bred with homozygous “floxed” HIF-1 $\alpha$  transgenic mice. The resulting litters produced mice with a forebrain predominant deletion of HIF-1 $\alpha$  (HIF-1 $\alpha^{\Delta\Delta}$ ), as well as littermates without the deletion. All mice negative for the CAMCRE gene were considered ‘wildtype (Wt)’. Genotyping was carried out by PCR on tail DNA samples.

Litters were subjected to the Vannucci procedure of HI at P7: unilateral ligation of the right common carotid artery followed by 30 min of hypoxia (8% oxygen). Five days after HI, mice were perfused with paraformaldehyde, brains were processed histologically and analyzed for degree of injury using a scoring system. Some mice were perfused with saline and a piece of contralateral cortex was obtained for Western blot to confirm the tail DNA PCR results. These brains were then immersion-fixed in PFA prior to histological preparation. We also determined HIF-1 $\alpha$  expression with and without a hypoxic stimulus. P7 HIF-1 $\alpha^{\Delta\Delta}$  and Wt mice were exposed to hypoxia (8% oxygen) or room air for 1 hour. Western blots were performed on brain cortices collected immediately after the hypoxic stimulus.

**Results:** HI injury is increased in the neuronally knocked down HIF-1 $\alpha^{\Delta\Delta}$  mouse brain ( $P < 0.05$ ). This injury is more severe in the cortex of the HIF-1 $\alpha$  deficient brains compared to Wt littermates ( $p < 0.01$ ), than in the hippocampus, where there was no difference in degree of injury ( $p > 0.96$ ) Mann-Whitney.

Results of Western blotting of mouse cortices exposed to hypoxia stimulus or room air confirms that HIF-1 $\alpha^{\Delta\Delta}$  cortex expresses a minimal amount of HIF-1 $\alpha$  protein compared to Wt, and both HIF-1 $\alpha^{\Delta\Delta}$  and Wt have increased HIF-1 $\alpha$  with hypoxic stimulus (Fig. 1). Preliminary results of HIF-1 $\alpha$  protein expression after HI suggest a large increase of HIF-1 $\alpha$  in the cortex of Wt after HI compared to hypoxic (contralateral) cortex and a smaller increase in the cortex HIF-1 $\alpha^{\Delta\Delta}$  after HI (Fig. 2).

Fig. 1: HIF1 protein expression after Hypoxia

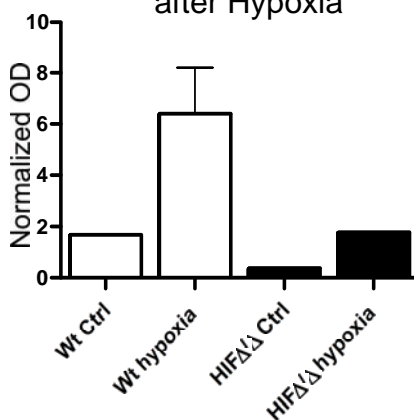
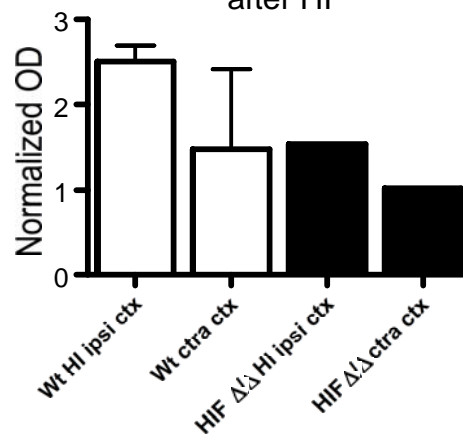


Fig 2: HIF1 protein expression after HI



**Conclusions:** Genetic reduction of neuronal HIF-1 $\alpha$  results in a worsening of injury after neonatal HI. There may also be a region-specific role for HIF-1 $\alpha$  in the setting of neonatal brain injury.