Learning and Behavior in adult mice following neonatal hypoxic-ischemic brain injury

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Introduction: Unilateral carotid ligation followed by hypoxia is a widely used model to study cerebral hypoxia-ischemia (HI) in the neonatal animal. Application of this technique in the postnatal day 7 mouse leads to an injury pattern seen in premature humans while the injury pattern in the postnatal day10 mouse approximates that seen in a full term newborn. (1) Although acute injury histology and behavior following H-I in P10 mice have been described (2), the long-term effects on learning and behavior in adult animals that had H-I as neonates, as in the clinical situation, remains unknown.

Methods: Following IACUC approval the litters born to 5 mating pairs (129T2/SvEv males x C57Bl6/J females) were randomized (block on mating pair, male and females randomized independently) to receive one of four treatments in P10 mice. 1) sham R carotid ligation followed by room air; (n=32) R carotid ligation followed by either 45 (n=32),60 (n=33)or 75 minutes (n=33) of hypoxia. Eight of the 75 min animals died during hypoxis and 3 died before weaning. On postnatal day50 (n=114) mice were subjected to behavioral tests including zero maze, gross locomotion, narrow bridge crossing, novel object recognition, cued water maze, hidden platform water maze with probe trial, reversed platform water maze with probe trial, a two week delayed probe trial and apomorphine challenge. After testing, mice were sacrificed and brains removed. Brain sections were cut at the striatum, thalamus and hippocampus. On postnatal day17 (n= 20) mice were sacrificed and brains removed to assess acute histology injury. Behavioral testing data was analyzed using the general linear models with post hoc analysis of multiple comparisons. The time required for the mice to find the platform (latency) was used as the primary variable for the analysis of the water maze data.

Results: The adult animals that had H-I at P10 showed no gross neurological deficits. However, there is clear evidence of cognitive and learning impairment as shown in Table A. The animals require increasing time to successfully complete the water mazes as the duration of H-I time increases. Evaluation of gross locomotion indicated that the 75 min H-I group had hyperkinetic behavior and made frequent counter-clockwise rotations. They also showed extensive clock-wise circling on apomorphine challenge, indicating serious striatal injury(4). The adult histology showed remodeling of the CA1 in the 45 and 60 min groups compared to the acute histology. Damage was noted in the hippocampus (minimal in 45 min increasing to cystic degenation at 75 min), the striatum (75 min only) but not in the cortex in any group. Persistent defects were noted in CA2-CA3 in the 60 min animals but some evidence of remodeling compared to the acute injury was seen. 75 min of H-I resulted in total destruction of the ipsilateral hippocampus and dentate gyrus.

Discussion: Mice subjected to H-I brain injury at P10 exhibit persistent learning and behavior deficits despite lack of gross neurological abnormalities and extensive histologic damage. Despite early histologic severe injury in the hippocampus, remodeling occurred over the ensuing three months. This system provides a model to study late effects of neonatal injury.

Table A

Test	**Cued WM**	**Hidden WM**	**Reversal MW**
GLM (p for H-I time)	1.41E-11	4.00E-11	7.72E-10
Post hoc comparisons			
0 vs. 45 min (p-value)	0.013	0.029	0.022
0 vs. 60 min (p-value)	9.25E-05	0.8±0.30*	1.71E-07
0 vs. 75 min (p-value)	6.27E-06	1.7±0.50	9.23E-09
45 vs. 60 min (p-value)	NS	NS (#)	0.052
45 vs. 75 min (p-value)	0.0004	0.00003	0.038
60 vs. 75 min (p-value)	0.048	0.007	NS

GLM p-values for the significance of H-I time as a determinant of latency for the water maze test. Below p-values for comparisons among groups. # The 45 and 60 minute H-I time groups separate on probe trial (p= 0.011 45 vs 60 min)

References:

1. RA Sheldon et al, Brain Res, 810:114, 1998
2. RC Vannucci et al, J Neuro Sci Res. 55:158, 1999
3. DC Montgomery Design and Analysis of Experiments (4th Ed) John Wiley &Sons, 1996
4. Balduini et al, Brain Res. 859:318,2000