Differential Recovery in Immature and Juvenile Rats After Cerebral-Hypoxia-Ischemia Assessed with functional MR Imaging, Behavioural Testing and with BrdU for Cell Proliferation

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Introduction

The recovery and plasticity of the brain following an injury is often variable. Indeed, young or immature subjects can recover remarkably well from a stroke compared to older or more mature individuals with a similar infarct. We hypothesized that there are differences in functional recovery between rats undergoing a cerebral hypoxic-ischemic insult when they are neonates (e.g. 1 week of age) compared to when they are more mature juveniles (e.g. 4 weeks of age). Specifically we investigated whether these differences can be detected using several complementary methods: 1. as differences in neurological deficits or behavioural recovery, 2. as a differential reorganization of the brain as assessed with functional MR imaging (fMRI) and 3. as a difference in neurogenesis or cell proliferation and finally whether these measures of recovery are inter-related.

Material and Methods

Rats (n=34) were subjected to cerebral HI (unilateral carotid artery occlusion + exposure to 8% oxygen) or sham surgery (controls) at 1or 4 weeks of age (n=8 or 9 /group). Rats were scanned using a 9.4T Bruker Avance system at 24 hr, confirming the presence of an infarct in T2 weighted images. Behavioural assessments, which included a neurological score (0-6, for circling/body rotation and abnormal forepaw extension) were performed weekly after the HI insult. A subgroup of animals were also injected with BrdU (50 mg/kg, I.P) for 2 days each week after the insult. At 9 weeks of age, fMRI in response to electrical stimulation of the forepaw was performed in the rats anesthetized with alpha-chloralose (1). For each experiment a set of 32 gradient echo T2* images were acquired during an electrical stimulation off/on/off/on/off paradigm and the data were analyzed using EvIdent^{TM}. After the fMRI study, brains were perfusion fixed, cryoprotected and frozen. Frozen sections were stained immunohistolochemically for BrDU and NeuN (a neuronal marker). Neurogenesis was assessing by counting cells labelled with BrDU and NeuN.

Results

Cerebral HI resulted in an infarct volume of $313\pm98mm^3$ and $146\pm26mm^3$ in the right hemisphere, in 1-week and 4-week old rats, respectively ($p<0.05$). The behavioural deficit assessed from the neurological score was greater in 4-week than 1-week old animals, despite the smaller infarct size in this group (Fig.1). As expected, in sham control animals, the sensory-motor cortex was activated contralateral to the paw being stimulated in all sham animals (Fig. 2). Brain damage due to an earlier episode of cerebral HI, had similar effects on the activation produced by forepaw stimulation, irrespective of the age at which the insult occurred. There was a marked decrease in activation in the right (infarcted) sensory-motor cortex in response to left forepaw stimulation ($p<0.001$). There was also recruitment of contralateral cortical regions and an enhanced response to stimulation of the paw projecting to the uninjured hemisphere in 25-30% of the animals ($p<0.05$). Cell proliferation in the subventricular zone also increased by about 3 fold in the 1-week old group and 2-fold in the 4-week old group of animals exposed to HI compared to age matched sham animals where the difference between age groups was significant. However, the number of BrdU and BrdU/NeuN labelled cells in the cortex surrounding the infarct appeared similar in the two age groups and was increased bilaterally.

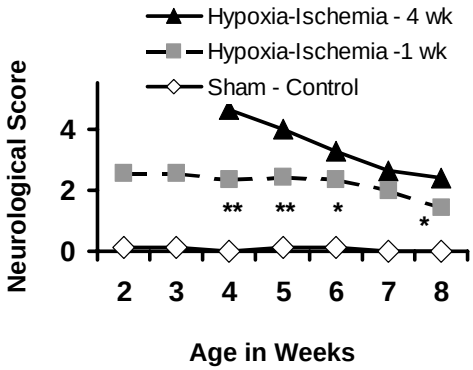

Fig. 1. Neurological score following an episode of cerebral hypoxia-ischemia in rats at either 1 or 4 weeks of age. Some recovery occurs in both age groups. ** $p<0.002$, *$p<0.05$, different from 4 week old group.

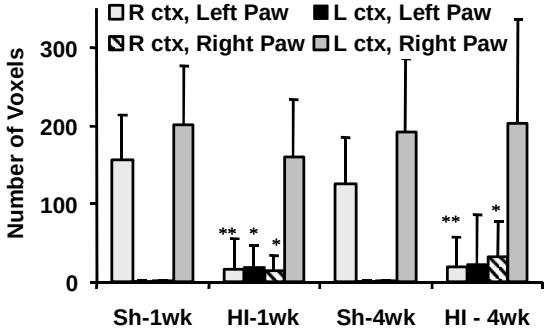

Fig. 2. Activation response within either the right (R) or left (L) cortex in response to electrical stimulation of either the right or left fore-paw. Sham (Sh) control animals without cerebral hypoxia-ischemia (HI) had a response that differed from animals that had undergone cerebral HI at either 1 or 4 weeks of age resulting in an infarct in the right hemisphere (**$p<0.001$, *$p<0.05$, different from respective sham).

Conclusions

Despite a difference in behavioural recovery between the two age groups, there were no major differences in the activation response to electrical stimulation of the forepaw detected with fMRI. This suggests that the greater behavioural recovery or maintenance of sensory/motor function in neonatal animals compared to older animals is not achieved by a differential plasticity or reorganization of direct sensory-motor cortical pathways. Instead, the improved functional recovery may be achieved by a plasticity or reorganization of other pathways, as suggested by the greater overall cell proliferation in the subventricular granular zone occurring in 1-week compared to 4-week old rats. *Supported in part by a grant from the Canadian Institutes for Health Research.*

Reference: 1.Tuor, U.I., T. Hudzik, K. Malisza, S. Sydserff, P.Kozlowski, M.R. Del Bigio. Exp. Neurol 167:272-281, 2001.