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BILATERAL ALTERATIONS IN DENDRITIC MORPHOLOGY AFTER UNILATERAL NEONATAL CEREBRAL HYPOXIA-ISCHEMIA

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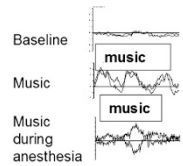
Background: Lasting motor and cognitive impairments can be a consequence of hypoxic-ischemic (HI) injury to the immature brain. Functional deficits could be due to neuronal loss or altered synaptogenesis. Little is known about the effect of neonatal HI brain injury on synaptic indices, either in injured or unaffected brain tissue. Quantitation of dendritic morphology, e.g. dendritic spine density, branching and length in Golgi-stained tissue, is frequently used to evaluate the effects of brain injury on synaptic organization. We hypothesized that unilateral neonatal cerebral HI elicits distinct ipsilateral and contralateral dendritic abnormalities.

Methods: We evaluated hippocampal dendritic trees in seven-day-old (P7) Long-Evans male rats (n=12) that underwent either unilateral cerebral HI (n=6, right carotid ligation followed by 1.5h in 8% O2) or a sham procedure (neck incision followed by 1.5h in 8% O2). These conditions elicit mild to moderate neuronal loss in ipsilateral striatum, cortex and hippocampus, with no visible contralateral abnormalities. Rats were weaned at P21 and were housed in standard cages until P60. Brains were perfused with saline followed by 1% paraformaldehyde, placed in Golgi-Cox solution for 2 wk, then sectioned at 200 μm. Neurons (10 CA1 pyramidal neurons/side/rat) were considered suitable for analysis if they were well impregnated and not obscured by blood vessels, astrocytes, or dendrites of other neurons, and their branching was intact within the section. Cells were drawn via camera lucida. Total dendritic length/neuron was estimated by counting dendritic intersections with a series of concentric spheres at 20 μm intervals centered on the soma. Spine density was calculated by tracing a length of third order terminal basilar dendrite ($\geq$70 μm long) at 2000X. The exact length of the dendritic segment was calculated, and the number of spines along the entire length counted and expressed as spines/10 μm.

Results: Bilateral hippocampal reductions in dendritic length, branch number and spine density were associated with HI. CA1 pyramidal spine density was reduced both ipsilaterally (Spines/10μm, mean$\pm$SD: Sham 10.6$\pm$0.6 vs. HI 8.4$\pm$0.3, p<0.001, t-test) and contralaterally (Sham 10.7$\pm$0.4 vs. HI 9.1$\pm$0.7, p<0.001, t-test).

Conclusion: These findings indicate that abnormalities of synaptic organization persist into adulthood after neonatal HI brain injury, and these abnormalities affect regions of the brain distant from the site of HI neuronal injury.



	Base1	Base2	Music1	Music2	P value
Right HbO2 (SD) iM/L	0.05(0.17)*	0.03(0.10)#	1.54(0.87)*#	-0.35(0.32)*#	*, #<.001
Left HbO2 (SD) iM/L	0.08(0.34)*	0.04(0.2)#$	1.70(1.05)*#	1.46(0.32)$	*,#, $<.001

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SUBVENTRICULAR ZONE PROLIFERATION AFTER AMPA RECEPTOR-MEDIATED NEONATAL BRAIN INJURY

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Background: The forebrain subventricular zone (SVZ) contains oligodendroglial (OL) stem cells. Although some studies suggest acute loss of SVZ OL precursors after hypoxic-ischemic (HI) neonatal brain injury, recovery of myelin basic protein (MBP) immunostaining in the white matter 2 weeks after HI raises the possibility that OL stem cells in the SVZ might proliferate to repopulate injured white matter. As an alternative to using the HI model to study the SVZ response to neonatal brain injury, we developed a model of neonatal brain injury that focuses on a unique mechanism of increased vulnerability of immature OLs, AMPA-receptor mediated excitotoxicity. Intracerebroventricular (icv) injection of the synthetic glutamate analog AMPA in seven-day-old (P7) rats caused reduced expression of two OL-specific genes, proteolipid protein and MBP. We hypothesized that the immature SVZ would respond to injury with cellular proliferation; we used the AMPA icv injection model to test this.

Methods: P7 rats received either left (n=4) or right (n=3) stereotaxic icv injections of S-AMPA, 2.5 nmol in 5 μl sterile water (pH adjusted to 7.2). Controls included right icv injections of sterile PBS 5 μl (n=3) and non-injected littermates (n=3). 4h prior to sacrifice, all animals received an intraperitoneal injection of bromodeoxyuridine (BrdU), 100 mg/kg, to label cells entering the cell cycle. On P14, animals were perfused with 4% paraformaldehyde, cryoprotected in sucrose and sectioned serially at 60 μm. A series of every fourth section was stained immunohistochemically for the novel proliferation marker Ki67. A second series was immunolabeled with anti-BrdU. Bilateral SVZ was outlined and Ki67+ or BrdU+ cells were counted stereologically using the Optical Disector method.

Results: The density of Ki67+ cells was increased in ipsilateral SVZ of AMPA injected rats relative to PBS-injected and normal controls (Mean ($\pm$SD) density/mm3 AMPA ipsilateral 111,261$\pm$37,197*†, AMPA contralateral 93,698$\pm$32,248†, PBS 63,572$\pm$18,793, Normal 36,697$\pm$15,169; p<0.05 ANOVA with Fisher LSD post-hoc test, compared to * PBS and † Normal). The number of Ki67+ cells/SVZ was increased both ipsi- and contralateral to the icv injection, compared to normals, but Ki67+ cell number was also increased to a lesser, but not significant extent in PBS controls.

Conclusion: This preliminary study suggests that there is a proliferative response in the immature SVZ in response to an AMPA receptor-mediated excitotoxic stimulus.

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AUDITORY AWARENESS OF MUSIC DURING SLEEP AND ANESTHESIA. A NEAR INFRARED SPECTROSCOPY STUDY OF INFANTS

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Background: The level of sensory awareness during anesthesia is difficult to monitor. Near infrared spectroscopy NIRS, is a non-invasive bedside tool which allows monitoring cerebral cortical haemodynamic changes in response to different kinds of sensory stimulation.

Aim: To assess the cortical response to auditory stimulation during anaesthesia in infants undergoing elective surgery.

Methods: Water music, by Handel was presented via earphones to three infants aged between 18 and 22 months. NIRS was recorded in different conditions: baseline with no music when the child was asleep (base 1), during the music with the child sleeping before anesthesia (music 1), baseline in deep anesthesia (base 2) and during the music when the child was in deep anesthesia (music 2). HR, satO2, BP, eCO2, did not change during the study. Only small fluctuations of HbO2, HbH and Hbtot where observed during baseline conditions (these fluctuations were slightly more pronounced in base1 than base2, but not significantly different). When the music was played to the infant asleep before anaesthesia, an increase in HbO2 in both hemispheres above the illuminated cortical area (i.e. auditory cortex) was observed. After the anaesthesia was induced and the music played, there was an increase in HbO2 on the left side and a decrease on the right in all three infants (see table and figure 1).

Conclusions: NIRS has been easily used in the operation setting to monitor haemodynamic cortical responses to auditory stimulation. The observed pronounced bilateral HbO2 increase during sleeping is similar to that previously observed in awaked subjects and suggests that the infant perceives the auditory stimulus and likely process it. When the infant is under anesthesia, and many neuronal circuits are not functioning, the auditory stimulus can still be perceived as suggested by the increase of HbO2 on one hemisphere, but processing might be altered.

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SEVOFLURANE EFFECT ON CEREBRAL HAEMODYNAMICS DURING INDUCTION OF ANESTHESIA IN YOUNG CHILDREN ASSESSED BY NEAR INFRARED SPECTROSCOPY. PRELIMINARY RESULTS

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Background: Induction of anaesthesia with anaesthetic gas might be a potential cause for cerebral impairment in the newborn and young infant. Near infrared spectroscopy - NIRS - is a continuous, non-invasive bedside technique that has been shown to a reliable to monitor cerebral oxygenation status in different conditions. Aim: To assess non-invasively by means of NIRS, cerebral oxygenation during the different steps of induction of anesthesia by sevoflurane. Study population: Children below 2 years of age undergoing elective surgery, without CNS and cardiac malformations were eligible for the study. Three infants aged from 20 to 24 months (20, 22 and 18-month older) have completed the experiment and their data were analysed, so far.

Methods: Induction of anesthesia: under control condition an air oxygen mixture via circle system (face mask connected to a semiclodsed anaesthetic circuit) with an increasing dose (1% - 2% - 3%) of sevoflurane was administered to the child. NIRS was continuously recordedng with a 2-channel instrumentation. Each channel was placed in order to illuminate the tempo-parietal region on each side of the head. After a 60-s baseline (before the exposure to the gas), the stepwise increases of the gas concentration were marked on the NIRS recording. Haemodynamic responses to the different gas concentration have been assessed by NIRS. Oxygenated (O2 Hb), de-oxygenated (H Hb), total haemoglobin (tot Hb) and the derived CBV and TOI have been calculated. Heart rate (HR), blood pressure (BP), peripheral oxygen saturation (satO2) and carbon dioxide (CO2) have been monitored.

Results: In all infants we have observed a clear increase in the cerebral blood volume during the administration of sevoflurane. There was an initial increase in the cerebral tissue oxygenation index. Particularly during step 1 and 2 we have seen a more pronounced increase, while during step 3 the increase was quite slow and not statistically significant. HR, BP and satO2 did not change significantly. CO2 slightly increased, but not significantly.

Conclusion: In the present study, cerebral blood volume increases during the induction of anaesthesia with sevoflurane. Cerebral tissue oxygenation showed a significant increase especially during the first phase of the induction. As approximately 70% of the blood in the cerebral vascular bed is venous blood we can speculate that sevoflure related effect of decreasing venous return may have repercussion on the cerebral blood volume.

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PARENTAL INVOLVEMENT IN PRENATAL COUNSELING FOR INFANTS AT THE MARGIN OF VIABILITY: AN INTERNATIONAL COMPARISON

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Background: The inclusion of parents in the difficult decision-making inherent in prenatal counseling for infants at the margin of viability is internationally debated. However, there is little information on how much parental decision-making is incorporated into these conversations. This survey explores the extent of parental involvement in prenatal consults in two countries.

Objective: To compare prenatal consultation attitudes and practices toward parental decision-making of New England (NE), USA and Swedish neonatologists for a hypothetical case of an infant at the margin of viability (23.5–24.5 weeks gestation, imminent delivery).

Methods: An anonymous, self-administered survey was developed and distributed in English to all practicing neonatologists in 6 New England states and the country of Sweden (S).

Results: For the New England portion, 148/175 surveys were completed (response rate 85%). For the Swedish portion, 88/128 surveys were completed (response rate 69%). Compared to their US counterparts, Swedish neonatologists less frequently report the belief that parents should be involved in making resuscitations decisions (NE 77% v S 26%, p<.0001). The practice of actually including the parents in the final decision regarding resuscitation is also reported less frequently in Sweden than in New England (NE 41% v S 10%, p<.0001). When asked to rank their most important role as a neonatologist, both countries ranked the provision of factual data first (NE 58% v S 55%). In Sweden communicating that resuscitation decisions will be made in the delivery room was more frequently ranked second (NE 28% v S 62%, p<.0001), while in New England assisting parents in weighing the risks and benefits of treatment options was more frequently ranked second (NE 56% v S 16%, p<.0001). New England and Swedish neonatologists report similar rates of asking about prior experiences with death and dying (NE 70% v S 68%) and prior experiences with premature or handicapped children (NE 91% v S 87%). However, different rates are reported when asking about parental interpretation of good quality of life (NE 73% v S 55%, p<.06) and desired parental role in the decision-making process (NE 89% v S 69%, p<.0001)

Conclusion: Neonatologists in the US and Sweden report differences in the amount of parental input they seek in making decisions regarding resuscitation of infants at the border of viability.