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ID 0. Visual tracking performance in very preterm infants at 4 months predicts cognition and behavior at 6.5 years

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Background: Visual tracking of moving objects requires sustained attention and prediction of the object's trajectory. We hypothesized that visual tracking performance in infancy has long-term implications for neurodevelopment in very preterm infants.

Methods: Visual tracking was assessed at 4 month's corrected age in 57 infants with gestational ages 22–31 (mean 28.1) weeks. During the tracking assessment, an object moved back and forth in front of the infant with sinusoidal (predictable) or triangular (abrupt) turns of the direction, while eye and head movements were recorded. Gaze gain, smooth pursuit gain, and timing of gaze to object were analysed. At 6.5 years the children had visual examinations, cognition was assessed with the Wechsler Intelligence Scale for Children (WISC-IV) and attention by the Brown Attention Deficit Disorder (Brown ADD) scale. Univariate and multiple regression analyses were performed and included adjustments for neonatal risk factors: severe brain injury (IVH 3–4/PVL), retinopathy of prematurity stage 3 or more, bronchopulmonary dysplasia, and gestational age. A p value <0.05 was considered significant.

Results: For both motion patterns, gaze gain was strongly related to all WISC-IV parameters and smooth pursuit gain to full-scale IQ and processing speed. For the sinusoidal pattern, smooth pursuit gain was also significantly related to working memory. Both motion patterns also related to several Brown-ADD parameters. For the sinusoidal motion pattern both timing of gaze to object and gaze gain related most strongly to "Focusing sustaining and shifting attention" ($R^2 = 0.17$, $p = 0.004$; and $R^2 = 0.17$, $p = 0.016$, respectively). For the triangular motion pattern, smooth pursuit gain associated to "Regulating alertness, sustaining effort and processing speed" ($R^2 = 0.16$, $p = 0.006$). A visual acuity <0.8 at 6.5 years was associated with lower full-scale IQ but not to the visual tracking parameters.

Conclusion: The ability of very preterm infants to visually track and attend to a moving object at 4 month's corrected age is closely related to cognition and attention at 6.5 years.

ID 59. Disrupted functional brain organization in children born extremely preterm

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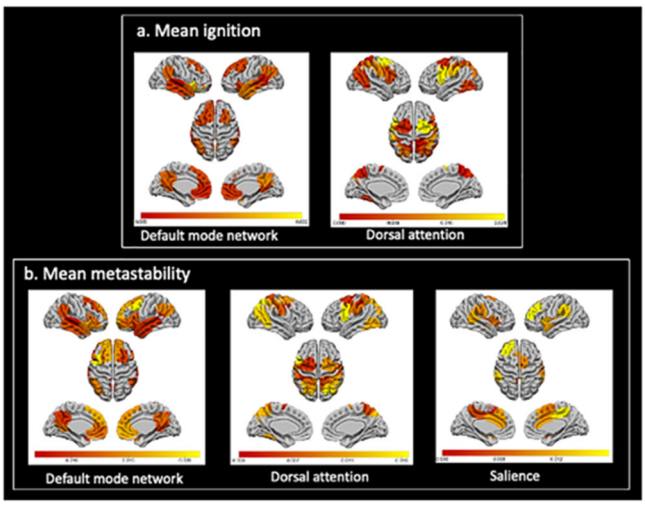
Background: The brain's functional organization is dynamic and reconfigures across time implying the capability of brain regions to propagate neural activity (ignition events) and to switch between brain states (metastability). This ensures that the information processing function operates at an optimal level supporting cognition and behaviour. Any disturbance to these dynamics, such as extreme prematurity, can have a significant impact on healthy brain functioning. Here, we aimed to investigate how extreme prematurity (born below 28 weeks of gestation) disturbs the functional organization of the brain at 10 years of age by adopting an intrinsic ignition and metastability network-based framework using resting state functional MRI. Our second aim was to explore the relationships between brain ignition and metastability measures and cognitive function.

Methods: Sample: 33 extremely preterm (EPT) children (23.5–26.6 weeks of gestation) and 28 term controls (37.3–41.5 weeks of gestation) scanned at 10 years of age and assessed with WISC-V at 12 years. Intrinsic ignition and metastability were calculated using an appropriate algorithm on each functional network. We defined seven networks (default mode network-DMN, limbic, motor, visual, dorsal attention network-DAN, salience and fronto-parietal) based on a functional brain atlas. Differences were tested between EPT and term using Monte-Carlo permutations corrected for multiple comparisons. Spearman's correlation between brain data and developmental scores were performed.

Results: Compared to the term group, EPT children showed reduced mean ignition and metastability in the DMN ($p = 0.002$ and $p = 0.00001$ respectively) and DAN ($p = 0.011$ and $p = 0.00001$ respectively). Metastability was also reduced in the salience network ($p = 0.02$) (Fig. 1). Significant correlations between brain data and developmental scores were found only in the term group. Metastability in the DAN was positively correlated with processing speed ($r's = 0.49$, $p = 0.02$), visuospatial ($r's = 0.50$, $p = 0.02$), and IQ ($r's$ 0.56, $p = 0.008$). All survived FDR correction.

Conclusions: EPT birth disturbs the signatures of functional brain organization at rest involving three core higher-order networks (DMN, salience and DAN). This may have a key role in related cognitive impairments described in this population.



(ID 59) Fig. 1. Ignition and metastability in the extremely preterm brain

Comparisons between extremely preterm and term children (yellow–red areas). a Reduced ignition. b Reduced metastability.

None declared.

ID 110. Neuroprotective effect of remote ischemic postconditioning combined with hypothermia in a piglet model of moderate to severe hypoxic-ischemic encephalopathy

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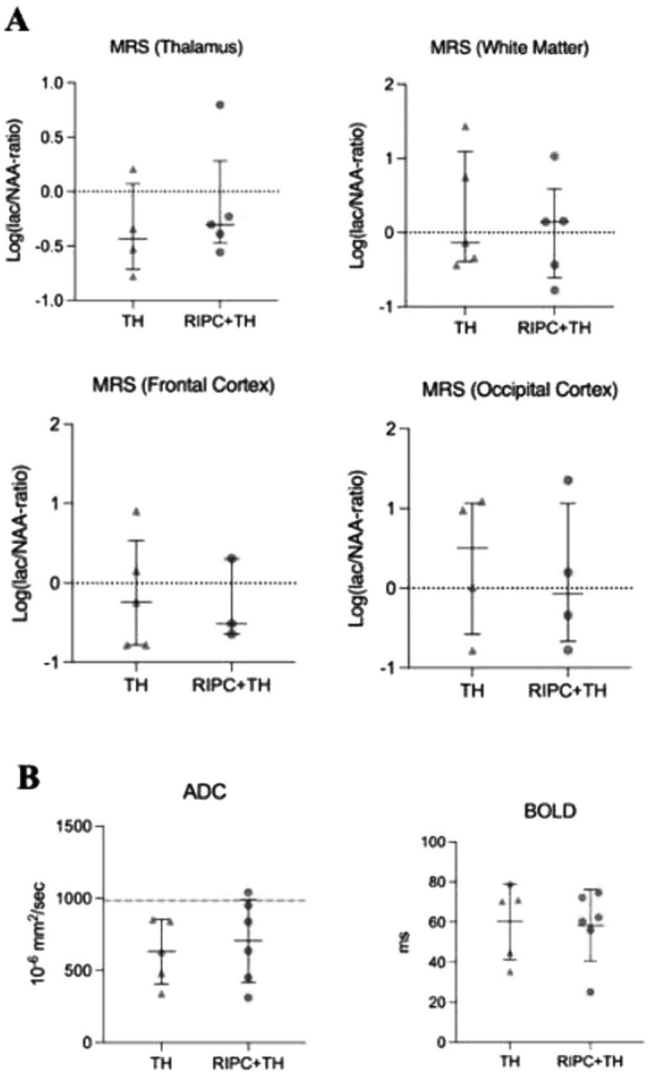
Background: Hypoxic–ischemic encephalopathy (HIE) is a major cause of mortality and neurological disability in newborns. Therapeutic hypothermia (TH) is the only neuroprotective treatment for HIE. However, TH is only partly neuroprotective and additional neuroprotective treatments to supplement TH are needed. Remote ischemic postconditioning (RIPC) is brief, repeated non-lethal ischemia to one or more extremities. RIPC has shown neuroprotective properties in rats and piglets. It is still unknown whether RIPC adds to the neuroprotective effect of TH. Our primary objective is to investigate the effect of RIPC combined with TH in a piglet model of moderate to severe hypoxia and ischemia.

Methods: A global hypoxic–ischemic insult will be induced in 34 newborn piglets. The piglets will be randomized to TH+RIPC, TH alone, or supportive care only. RIPC will be conducted by occluding blood flow to both hind limbs for 5 min, followed by 5 min of reperfusion in four cycles. RIPC will be repeated after 12 and 24 h. Our primary outcome will be a composite endpoint of death or severe CNS outcome, defined as the upper quartile of adverse thalamic lactate/n-acetylaspartate ratio measured by magnetic resonance spectroscopy. Secondary outcomes will be brain histology, amplitude integrated encephalogram, and various magnetic resonance imaging measures.

Results: All baseline- and insult characteristics were similar between the two groups. A moderate to severe insult was induced; duration of aEEG suppression (median; 42 vs 41 min), duration of hypotension (5 vs 6 min), and post-insult metabolic acidosis (median; 6.7 vs 6.9). RIPC piglets first in all brackets. One piglet died in the TH+RIPC group and two piglets died in the TH group. No difference between groups was found in Lac/NAA ratio in any brain regions (Fig. 1A). Furthermore, we found no difference between groups in MRI measures of cerebral edema (ADC), and cerebral oxygenation (BOLD) (Fig. 1B).

Conclusion: Our preliminary data showed no additional neuroprotective effect of RIPC when combined with TH.



(ID 110) - Piglets subjected to a standardized hypoxic-ischemic insult and randomized to either TH (n=5) or RIPC+TH (n=6). Magnetic resonance spectroscopy (A) and -imaging data (B) performed 42 hours after the insult. MRS data are median with interquartile range tested with Mann-Whitney U test and MRI data are mean with standard deviation. Tested with Student's t-test. Lac, lactate; NAA, N-AcetylAspartate, ADC: apparent diffusion coefficient, BOLD: blood oxygenation level dependent.

None declared.

ID 113. The role of MicroRNAS in the development of retinopathy of prematurity

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Introduction: Retinopathy of prematurity (ROP) develops after abnormal proliferation of retinal vessels and is one of the main reasons of preventable childhood blindness. A large number of angiogenic factors are effective in the pathogenesis of ROP. MicroRNAs (miRNAs) are small RNA molecules that are approximately 20–22 nucleotides in length, not encoded, in a single chain structure, and are involved in many cellular events and regulate gene expression posttranscriptionally. In the development of ROP, microRNAs may be effective in the balance of factors that inhibit and activate angiogenic factors and in the process of regulating vascular integrity and angiogenesis, In this study, we aimed to determine the changes in the blood levels of miR-146a, miR-143, miR-210, miR-126, miR-211, miR-221, miR-106 and let 7f which control pathological angiogenesis and apoptosis, and to investigate their role in predicting prognosis in cases of ROP.

Materials and methods: The study was conducted prospectively in preterm infants with the diagnosis of ROP at every stage. Serum levels of 8 miRNAs were measured by real-time PCR. Gender, gestational age, birth weight, delivery pattern, morbidity and ROP stages were recorded and compared. The relationship between disease stage and progression with miRNA gene expression was analysed. Preterm infants without ROP were taken as the control group.

Results: A total of 63 infants, including 48 patients with ROP and 15 controls, were included in the study. Of the infants who had ROP, 29 (60.4%) were boys. The final analysis was performed in 61 babies, since sufficient data could not be obtained in two samples among the miRNAs. In the ROP group, miR-210, miR-146a, miR-21 were statistically significantly lower. In the ROP group the expression level of miR-143 was insignificantly lower, miRNA-221 was insignificantly higher, and miR-106, miR-126 and let 7f were variable.

Conclusion: In our study, it was observed that miR-210, miR-146a, miR-21 and miR-143 were significantly lower in patients with ROP compared to the control group. These miRNAs may be used as biomarkers of early diagnosis and to determine the severity of ROP.

None declared.

ID 123. Association of preterm birth and deprivation together as risk factors for learning difficulties

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Background: 1 in 10 children globally are born preterm1 leading to a large proportion of morbidity and mortality in children representing a major target for intervention for benefit to public health. Preterm birth is associated with learning difficulties and impaired school performance in later life. Increasing risk of intellectual disability has been demonstrated with increasing social deprivation. We sought to identify if children born preterm to families with higher levels of deprivation are disproportionately more likely to have a learning difficulty.

Methods: Data from the RANOPS (Respiratory And Neurological Outcomes in children born Preterm Study) a cross sectional survey of children in Wales was used to assess the prevalence of learning difficulties, behavioural problems and need for an educational statement by parental report by exposure. The effects of exposures of prematurity (gestation of less than 37 weeks) and deprivation (measured using the Welsh Index of Multiple Deprivation (WIMD)) were reviewed. Logistic regression models adapted for random effects of age at time of the survey were used to examine if gestational age and deprivation impacts interacted after adjustment for possible confounders. Primary outcome measure was parentally reported learning difficulty. Secondary outcome measures were parentally reported behavioural problems and need for a statement of special educational need Ethical approval was given for the original study by the South Wales Ethic Committee.

Results: 6691 infants were investigated. Deprivation measured by decile (OR 1.08 (1.03–1.12), adjusted) and prematurity (OR 2.67 (2.02–3.53) adjusted) were both associated with occurrence of learning difficulty. The population attributable risk fraction (PAF) for learning difficulty following preterm birth was 4.89%. There was little evidence a model with interaction between prematurity and deprivation was superior to one without on likelihood ratio testing ($p = 0.298$, adjusted).

Conclusion: Deprivation and preterm birth both have significant associations with learning difficulties. While deprivation does not appear to have potentiated the impact of preterm birth, preterm infants in the most deprived areas have the highest risk of learning difficulties with almost one in three extremely premature born infants with a learning difficulty in the most deprived areas.

Neurodevelopmental Measure	Unadjusted model			Adjusted for demographics factors*			Adjusted for demographics* and clinical factors**		
	OR (95% CI)	p	Pinteraction	OR (95% CI)	p	Pinteraction	OR (95% CI)	p	Pinteraction
Learning Disability	N=6691			N=5443			N=4563		
Prematurity	2.52 (2.04-3.12)	<0.001	0.393	2.64 (2.07-3.37)	<0.001	0.517	2.67 (2.02-3.53)	<0.001	0.298
WIMD decile	1.08 (1.04-1.11)	<0.001		1.07 (1.03-1.11)	0.001		1.08 (1.03-1.12)	0.001	
Educational Statement	N=3356			N=2594			N=2062		
Prematurity	2.99 (2.04-4.38)	<0.001	0.196	2.68 (1.75-4.11)	<0.001	0.578	2.44 (1.50-3.98)	<0.001	0.463
WIMD decile	1.09 (1.03-1.15)	0.002		1.11 (1.05-1.19)	0.001		1.14 (1.06-1.23)	0.001	
Behavioural problems	N=6672			N=5429			N=4550		
Prematurity	2.01 (1.57-2.44)	<0.001	0.1425	2.14 (1.73-2.67)	<0.001	0.1417	2.07 (1.62-2.65)	<0.001	0.255
WIMD decile	1.19 (1.15-1.22)	<0.001		1.14 (1.11-1.19)	<0.001		1.11 (1.07-1.16)	<0.001	

(ID 123) - Logistic regression analysis of learning difficulties for preterm birth and increasing deprivation. (*Adjusted for maternal age, sex and ethnicity** smoking, multiple birth, mode of delivery, birthweight and breastfeeding).

None declared.

ID 152. Maternal melatonin administration leads to improved brain myelination in growth restricted fetal lambs

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Background: Fetal growth restriction (FGR) is a serious pregnancy complication associated with increased risk of adverse neurodevelopment in the offspring. Melatonin can be safely given to the mother during pregnancy, and it readily crosses the placenta and fetal blood brain barrier. We investigated the effects of maternal melatonin administration on fetal brain structure in early-onset FGR, and assessed the presence and distribution of melatonin receptors, MT1 and MT2.

Methods: Surgery was performed on twin-bearing pregnant ewes. Placental insufficiency and subsequent FGR was induced via single umbilical artery ligation in one of the twin fetuses at 88 days (0.6 gestation). Melatonin was administered intravenously (6 mg/day) to a group of ewes from the time of surgery until 125 days (0.8 gestation), at which point the ewe and fetuses were euthanased, and fetal brains collected.

Results: Study groups included control (n = 5), FGR (n = 5), control melatonin (control+MLT; n = 6) and FGR melatonin (FGR+MLT; n = 6). Melatonin administration did not alter fetal body or brain weights and was well tolerated. There were no significant differences seen in oligodendrocyte (Olig-2+) counts across all brain regions examined. Myelin (CNPase+) fibre density was reduced in FGR vs. control animals in most brain regions ($p < 0.05$) and melatonin treatment restored CNPase fibre density. A similar but less pronounced trend was seen with mature myelin (MBP+). Significantly increased astrocyte (GFAP+) immunoreactivity was seen in the intragyral white matter and cortex of FGR vs. control animals, while melatonin decreased immunoreactivity in corpus callosum and external capsule (EC). Significant differences in activated microglia (Iba-1) activity were seen between FGR vs. FGR+ MLT groups in periventricular white matter (PVWM), subventricular zone and EC ($p < 0.001$). MT1 receptors were found only in white matter; however, no significant differences were noted between groups. MT2 receptors were increased in PVWM in FGR animals, and reduced in FGR+MLT animals.

Conclusions: Maternal melatonin administration in an early onset model of FGR led to improved myelination of white matter brain regions, possibly mediated by decreased inflammation.

None declared.

ID 155. Parental bonding effects on cognitive outcomes after moderate and late preterm birth

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Background: Moderate and late preterm (MLPT) neonates are born during a sensitive period for brain development, accounting for more than 80% of preterm deliveries. For this reason, the aim of this study was to assess the cognitive functioning after MLPT birth during childhood. Besides, it sought to evaluate the potential role that parental bonding may play in MLPT children's cognition.

Methods: A total of 97 participants partook in this study: 40 moderate preterm-born children (Mage = 11.63 years; SDage = 1.61), 32 late preterm-born children (Mage = 12.22 years; SDage = 0.79), and 25 full-term peers (Mage = 11.08 years; SDage = 1.75). All participants underwent a cognitive assessment and parental bonding measures of care and overprotection were registered through The Parental Bonding Instrument. Cognitive functioning was assessed using a composite score (Cronbach's alpha = 0.88) obtained from the following psychometric tests: Peabody Picture Vocabulary Test-III, Modified Wisconsin Card Sorting Test, Wechsler Intelligence Scale for Children-V, Verbal Fluency Test, Stroop Test, Color Trail Making Test, and Rey Auditory Verbal Learning Test.

Results: Significant differences were found in cognitive functioning among groups with a large effect size ($F = 8.65$, $p < 0.001$, $\eta_p^2 = 0.22$). Despite parental bonding´s care and overprotection measures did not significantly differ between groups, care measure moderated the relation between the degree of maturity/immaturity at birth and cognitive functioning ($F(4,92) = 6.64$, $p < 0.001$, $R^2 = 0.22$). In terms of different degrees of care (i.e. low = 24.00; medium = 29.50; high = 33.00), for only lower care measure there was a significant relation between the degree of maturity/immaturity at birth and cognitive functioning; that is, having a high-up care (i.e. higher than 29.08) did not further moderate this relation across childhood.

Conclusions: Findings showed different cognitive functioning during childhood, with MLPT children reporting lower values. Additionally, higher parental care in this study seems to have a protective effect on cognition only in those with the lowest gestational age. In fact, while those born at term would perform worse cognitively in the face of higher degrees of care, those late preterm-born children will obtain similar outcomes regardless of the degree of care received. Nevertheless, cognitive functioning of those who are moderately preterm-born children is negatively affected by an unfavorable care level at this stage.

None declared.

ID 159. Regulatory T cells contribute to sexual dimorphism in neonatal hypoxic–ischemic brain injury

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Background: Neonatal encephalopathy caused by hypoxia–ischemia (HI) is a major cause of death and disability in newborns. Clinical and experimental studies suggest a sexual dimorphism in HI induced brain injury and therapy responses. A major hallmark of HI pathophysiology is the infiltration of peripheral immune cells into the injured brain. However, the specific role of regulatory T cells (Tregs) is still unknown.

Methods: Nine-day-old mice were exposed to HI by ligation of the right common carotid artery followed by 1 h hypoxia (10% oxygen). Using immunohistochemistry, flow cytometry and microarray analyses, Tregs were investigated in the brain, spleen and blood 24 h post HI. The functional role of Tregs was evaluated by acute Treg depletion in DEREG mice. Brain injury, neuroinflammatory responses and vascular injury were analyzed via immunohistochemistry and western blot 48 h and 7 days after HI.

Results: Females revealed an increased cerebral Treg infiltration, coinciding with elevated chemokine receptor expression. Treg depletion in females aggravated HI-induced brain tissue injury, associated with enhanced microglia and endothelial activation and leukocyte infiltration. Treg depletion in males resulted in neuroprotection, associated with reduced astrogliosis and vascular injury. Ex vivo isolated female Tregs displayed an increased immunosuppressive activity associated with an altered transcriptional profile compared to male Tregs.

Conclusion: The present findings demonstrate that Tregs from female mice provide endogenous neuroprotection, whereas Tregs from male mice enhance secondary neurodegeneration. As potential mechanisms, we identified intrinsic transcriptional differences associated with enhanced anti-inflammatory activity of female Tregs and non-immunological detrimental effects of male Tregs, related to vascular damage. Our study emphasizes the urgent need for sex-stratified clinical and pre-clinical analyses.

None declared.

ID 186. Exposure to fetal growth restriction and consequence on neonatal microglia proteome in rat

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Background: Intra-uterine growth restriction (IUGR) is a leading cause of ante/perinatal stress and brain injury, responsible for neurocognitive and behavioral disorders. Perinatal inflammation and the consequent microglia activation are key factors of brain vulnerability in neonates born with IUGR. However, the consequences of IUGR on microglia development and on microglia proteome are still unknown. The aim of this study was thus the characterization of microglia proteome in response to IUGR and the description of the effect of IUGR on microglia proteome during development. In addition, a concordance analysis between proteome and transcriptome was performed.

Methods: We used a rat model of IUGR induced by a gestational low-protein diet (LPD). Microglia cells were magnetically sorted from control and LPD animals at postnatal day 1 (P1) and 4 (P4) and a proteomic analysis was performed using the label free technology. Proteomic data were analyzed using both ORA and GSEA analysis and additional biochemical approach was used to confirm proteomic results.

Results: Significant changes of microglia proteome were observed early after birth in growth-restricted pups previously exposed to gestational LPD. Expression of protein sets associated with fetal growth were significantly enriched in LPD microglia at both P1 and P4. An upregulation of protein sets associated to inflammation and immune response was observed confirming the pro-inflammatory effect of LPD on microglia cells. Interestingly, an upregulation of protein sets associated with oxidative stress response and to ROS production was observed only at P4. This results were further supported by a exacerbated ROS production observed in primary microglial cell culture. During the development, inflammation-associated proteins were found to be upregulated between P1 and P4 both in control and LPD microglia. In contrast, proteins associated with DNA repair and senescence pathways were upregulated only in LPD microglia. Similarly protein sets involved in the protein retrograde transport were significantly downregulated only in LPD.

Conclusions: Overall, these data demonstrate significant effect of LPD-induced IUGR on developmental program of microglial cells leading to abnormal proteome within the first postnatal days.

None declared.

ID 267. Neurodevelopmental outcomes at 2 years after extremely preterm birth in Sweden. A comparison between EXPRESS (2004–2007) and EXPRESS2 (2014–2016) cohorts

Ulrika Ådén[1], Aijaz Farooqi[2], Karin Sävman[3], Abrahamsson Thomas[4], Lars Björklund[5], Magnus Domellöf[2], Anders Elfvin[3], Fredrik Serenius[6], Stellan Håkansson[2], Karin Källén[5], David Ley[5], Erik Normann[6], Petra Um-Bergström[1], Lena Hellström-Westas[6], Mikael Norman[1]*

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Background: Survival for extremely preterm infants has increased in Sweden and other countries. The latest study cohort (EXPRESS2, births in 2014–2016) showed significantly higher 1-year survival without major neonatal morbidities among live births at 22–26 weeks than EXPRESS (births in 2004–2007)(1). We tested the hypothesis that neurodevelopmental outcome at 2 years of age improved between the study periods.

Methods: All births at 22–26 weeks gestational age (n=2205) between April 1, 2004, and March 31, 2007 and between January 1, 2014, and December 31, 2016, in Sweden were studied (1). Follow-up data from EXPRESS2 (2014–2016) collected according to the national guidelines at 2 years corrected age were obtained from the Swedish neonatal quality register. Out of 695 eligible survivors, neurosensory impairment (NSI; CP, visual and hearing impairment) was scored in 616 (89%) and neurodevelopmental impairment (NDI; CP, cognitive, language, motor, visual and hearing impairment) in 608 (87%) and compared with data from the EXPRESS 2004–2007 cohort where 456 out of 491 (93%) were assessed. Groups were compared with chi-square test.

Results: At 2 years in EXPRESS2, 35% had moderate-severe neurodevelopmental impairments compared to 27% in EXPRESS and 12% in EXPRESS2 had moderate-severe neurosensory impairments compared to 7.5% in EXPRESS (see Table 1).

Conclusion: Improvements in neonatal survival and 1 year morbidity free survival were not paralleled by reduced rates of neurosensory or neurodevelopmental impairments at 2 years.

(ID 267) - REFERENCES

(1) Norman, M. et al. *JAMA* PMID: 30912837 (2019).

(2) Serenius, F. et al. *JAMA* PMID: 23632725 (2013).

N(%)	NSI EXPRESS (2004-2007) N=456	NSI EXPRESS2 (2014-2016) N=616	NSI EXPRESS vs EXPRESS2	NDI EXPRESS (2004-2007) N=456	NDI EXPRESS2 (2014-2016) N=608	NDI EXPRESS vs EXPRESS2
No	409 (89.7)	486 (78.8)	P<0.001	192 (42.1)	275 (45.2)	P<0.001
Mild	13 (2.9)	54 (8.9)		140 (30.7)	121 (19.9)	
Moderate	25 (5.5)	44 (7.1)		74 (16.2)	107 (17.6)	
Severe	9 (2.0)	32 (5.2)		50 (11.0)	105 (17.2)	

None declared.

ID 277. Levetiracetam or phenobarbitone as a first line anticonvulsant in asphyxiated term newborns?: an open label, single centre, randomised controlled pragmatic trial

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Background: Neonatal seizures are one of the most difficult conundrums for experts across the globe. Although there is no consensus on the 'ideal' treatment of neonatal seizures, phenobarbitone has been the drug of choice for decades. Levetiracetam, though extensively studied in adults and children, lacks rigorous evaluation in the neonatal population, despite its frequent use as an off-label drug.

Methods: The study was designed as an open-label, randomized active control, single-center, pragmatic trial. The objective was to compare the efficacy of levetiracetam to phenobarbitone in term asphyxiated babies as a first-line drug. Inborn term asphyxiated babies with seizures in the first 48 h of life were included. Babies satisfying the inclusion criteria were randomized to receive levetiracetam (20 mg/kg) or phenobarbitone (20 mg/kg). Clinical seizure control was noted. Babies who failed to respond to the primary drug were crossed over to receive the other group drug.

Results: Out of 103 eligible babies, 82 were randomized (44 levetiracetam group, 38 phenobarbitone group). Clinical seizure control with the primary drug and maintenance of the same for 24 hs was observed in 29 babies (65.9%) in the levetiracetam group and 13 babies (34.2%) in the phenobarbitone group ($p < 0.05$, RR 0.52, 95% CI 0.32–0.84). In all, 57.8% of babies in the phenobarbitone group were controlled after cross over to levetiracetam ($p < 0.05$) (9 ref Table 2).

Conclusion: Levetiracetam can be used with good efficacy as a first- and second-line drug in asphyxiated term babies. A larger study on pharmacokinetics and optimal regimen is required.

	Levetiracetam (n = 44)	Phenobarbitone (n = 38)	Relative risk	95% CI	P value
Seizure control after primary drug	29 (65.9%)	13 (34.2%)	0.52	0.34 to 0.66	**<0.05**
Seizures controlled after cross over	14 (31.8%)	22 (57.8%)	0.54	0.32 to 0.91	**<0.05**
Abnormal neurological examination at discharge	6 (13.6%)	11 (28.9%)	0.47	0.19 to 1.15	>0.05

(ID 277) - Table 1: Outcomes in the two groups.

None declared.

ID 278. Movement difficulties at the age of 5 years in children born extremely preterm: prevalence and risk factors in the European EPICE-SHIPS cohort

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Background: Children born extremely preterm (EPT <28 weeks of gestational age (GA)) have a higher risk for movement difficulties (MD) compared to their term-born peers. However, estimates of the prevalence of MD vary greatly between studies. In addition, while male sex has been consistently associated with increased risk for MD in the literature, knowledge about other perinatal, neonatal and sociodemographic risk factors is needed to identify the children who could benefit from early intervention.

Methods: We used data from a European population-based cohort of children born EPT in 2011–2012 in 19 regions in 11 European countries. 772 children, without cerebral palsy (CP) or severe neurosensory impairment (NSI), were assessed with the Movement Assessment Battery for Children—2nd edition (MABC-2) at 5 years of age and then classified as having no MD, at risk of MD (6th to 15th percentile) or significant MD (SMD) (≤5th percentile). We assessed associations of MD with perinatal, neonatal and sociodemographic factors using multinomial logistic regression models. Inverse probability weighting (IPW) was used to account for loss to follow-up.

Results: 47.4% of the children had no MD, 23.7% were at risk of MD and 28.9% had SMD. Wide variations existed between countries (range of SMD: 11.6–74.2%). Children born with a lower GA, severe brain lesions, and who received postnatal steroids were more likely to have SMD, while small for GA (<3rd percentile), male sex and BPD were associated with being at risk of MD and SMD. Children with younger and less educated mothers were more likely to have SMD, whereas children with at least one parent unemployed were more likely to be at risk of MD.

Conclusion: This study confirms a very high prevalence of MD among EPT children. In addition to perinatal and neonatal risk factors, we show that social factors affect the risk for MD. Persistent differences between countries after case-mix adjustment suggest that contextual factors play a role which may explain widely varying prevalence estimates from study to study.

None declared.

ID 279. Severe IVH with PHVD alters brain white and gray matter structure, leading to aberrant corticogenesis in the preterm rabbit pup

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Background: Up to 60% of infants with severe intraventricular hemorrhage (IVH) develop post-hemorrhagic ventricular dilatation (PHVD), resulting in neurodevelopmental impairment. There are scarce data available on long-term neurodevelopmental outcomes of IVH in animal models limited mainly to term-born animals. We aimed to establish a long-term model of PHVD in preterm rabbit pups and characterize the pattern of white and gray matter injury, and cortical impairment.

Methods: IVH in preterm rabbit pups was induced by intraperitoneal injection of glycerol at postconceptional day 29 (full term = 31). The presence of IVH was confirmed by high-frequency ultrasound at 24 h of age. The preterm rabbit pups were raised by a wet-nurse until postnatal day 33. Immunostainings were applied to investigate neurogenesis, synaptogenesis, astrogliosis, myelination, and corticogenesis. The assessment of the orientation and directionality of myelinated fibers was performed.

Results: The occurrence of IVH in the study was 58% (45/77). Survival of pups with IVH/PHVD (17.8%; 8/45) was significantly reduced compared to control pups (40.6%; 13/32). Twenty pups (IVH/PHVD = 7) were used for analysis. The pups with IVH/PHVD had globally reduced myelin content compared to controls ($p = 0.0009$), an aberrant cortical myelination microstructure, and thinner upper cortical layers (I–III) (mean diff −3.31; 95% CI −6.53 to −0.09; $p = 0.04$). We observed lower number of parvalbumin (PV)-positive interneurons in deeper cortical layers (IV–VI) in IVH/PHVD animals (mean diff −4.28; 95% CI −8.25 to −0.31; $p = 0.03$). IVH/PHDV inhibited the normal process of maturation of PV-positive interneurons, characterized by a reduction of the numbers of PV-positive perineuronal network-negative cells (mean diff −3.91; 95% CI −5.52 to −0.85; $p = 0.007$). Pups with IVH/PHVD had overall reduced neurogenesis and synaptogenesis compared to the controls ($p = 0.008$ and $p = 0.0003$, respectively). We observed signs of reduced microglial activation in pups with IVH/PHVD in all studied brain regions ($p = 0.08$) except the hypothalamus and internal capsule.

Conclusions: At 1 month of age, IVH/PHVD in the preterm rabbit pups resulted in alterations of cortical myelination microstructure and cortical organization with a reduction and maturational delay of PV-positive interneurons and a global decrease of neurogenesis and synaptogenesis.

None declared.

ID 283. Nature and nurture: neonatal and social risk profiles in extremely and very preterm born children and the impact on developmental outcomes at 5.5 years

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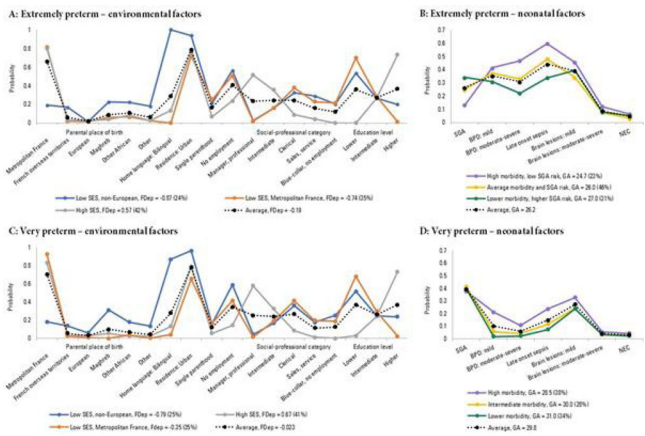
Background: To identify extremely preterm (EP; <28 weeks' gestation) and very preterm (VP; 28–31 weeks) born children with an increased risk for neurodevelopmental impairments, research has mostly focused on neonatal characteristics. However, developmental outcomes result from the interplay of biological and environmental factors. Despite this awareness, there has been little research interest in environmental factors to identify high-risk preterm infants. We aimed to address heterogeneity in the EP/VP population by describing environmental/social and neonatal risk profiles in a French population-based cohort and the relation with developmental outcomes at 5.5 years.

Methods: The sample included 553 EP and 1497 VP children from the population-based EPIPAGE-2 cohort. Latent class analysis was used to distinguish risk groups based on eight environmental factors (parental place of birth, language, neighborhood deprivation, urbanicity, single motherhood, profession, employment status, parental education level) and six neonatal factors (gestational age, small for gestational age, bronchopulmonary dysplasia, late-onset sepsis, brain lesions, necrotizing enterocolitis). Multivariable regression was used to test the relation between environmental and neonatal classes and intelligence (WPPSI-IV), motor skills (MABC-II), and behavior problems (SDQ).

Results: Three environmental/social classes were distinguished in both EP and VP samples (see Figure): low socioeconomic position with parent(s) born outside Europe, low socioeconomic position with both parents born in Metropolitan France, and high socioeconomic position. Based on neonatal characteristics, three classes were distinguished: high risk, moderate risk, and low risk, but probabilities for specific morbidities differed between EP and VP samples (see Figure). Environmental/social class had a large effect on IQ and small effects on motor skills, overall behavior, and externalizing problems in EP and VP children. Neonatal characteristics had small effects on motor skills, overall behavior, and externalizing problems in EP children and small effects on IQ and motor skills in VP children.

Conclusion: The EP/VP population is highly heterogeneous in terms of environmental/social and neonatal characteristics and outcomes. By identifying children with similar characteristics we gained more insight in which children are at risk for which difficulties. The findings emphasize that the role of the environment should not be underestimated in the identification of infants at risk for long-term impairments.



(ID 283) - Profiles of environmental/social factors (A, C) and neonatal characteristics (B, D) in the extremely (A, B) and very preterm sample (C, D).

None declared.

ID 348. The impact of preterm birth on the brain connectivity dynamics in 8-to-9-year-old children

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Background: Preterm infants are cerebrally vulnerable due to a combination of alterations in brain development and growth, and a propensity for brain injuries. There is a need for further data on long-term brain dynamics and trajectories. Recent advances in analytical frameworks bridging functional and structural brain connectivity are promising tools allowing such explorations. We hypothesized that, compared to full-term born children, preterm children would show durable changes in spatial and temporal brain dynamics, primarily in sensory, attentional, and executive functional systems.
Methods: In a prospective cohort study of 51 children born prematurely <30 gestational weeks in a Swiss level-III center, 43 children aged 8–9 years were recruited for a multimodal brain magnetic resonance imaging (MRI) session. We leveraged anatomical, diffusion-weighted, and resting-state functional imaging data to investigate the spatial and temporal reorganization of brain activity over time. So far, we compared data from 15 children to those of a reference group of 15 similar age full-term children recruited in the same period and area in a parallel study using identical MRI protocol. We computed two metrics of brain connectivity (i.e., systems diversity and spatio-temporal diversity) for each group. The between-group comparisons were based on 50,000 permutations of subjects for each metric at both levels.
Results: Compared to full-term children aged 8–9 years, preterm children showed no significant differences in spatial and temporal brain dynamics at the whole-brain level ($p > 0.05$). However, a comparison of functional systems revealed that prematurity was associated with lower system integration over time in systems related to sensory processing ($p < 0.05$), executive and attentional controls ($p < 0.01$). Furthermore, functional systems implied in cognitive flexibility (default and executive networks) were less stable across time (both $p < 0.05$).
Conclusion: This is one of the first evidence that prematurity affects the long-term development of children's brain connectivity, investigated using spatio-temporal brain dynamics studies. These patterns are coherent with clinical outcomes, such as attentional deficits, or diminished cognitive flexibility. Future studies include long-term longitudinal assessment of the brain dynamic development and its correlation with functional outcomes to identify early preventive interventions and assess their long-term impact.
None declared.

ID 372. The impact of increased maternal sFlt-1/PlGF ratio as a biomarker for preeclampsia on the motor outcome of preterm infants

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Background: The sFlt-1 (soluble fms-like tyrosine kinase-1)/PlGF (placental growth factor) ratio serves as a biomarker to predict preeclampsia. Elevated levels also increase the risk for prematurity and intrauterine growth restriction. However, its predictive value for subsequent neurological outcome has not yet been examined. This study aims to evaluate the correlation between maternal sFlt-1/PlGF ratios and early motor outcome of preterm infants.
Methods: 82 preterm infants (gestational age ≤34+4) born between February 2017 and August 2020 at the Department of Obstetrics and Gynecology, University Hospital Essen, Germany were included. The following variables were available: maternal sFlt-1 and PlGF levels and general movement assessment (GMA) of the infant at the corrected age of 10–16 weeks. The infants were stratified into high and low ratio groups according to maternal sFlt-1/PlGF cut-off of 85. To investigate the early motor repertoire and quality of spontaneous movements of the infant, the Motor Optimality Score (MOS-R) based on antigravity movements and posture patterns was applied. This approach allows for early identification of severity and type of neurological dysfunctions (Einspieler et al., 2019).
Results: Linear regression analysis shows that the sFlt-1/PlGF ratio does not predict the MOS-R score ($\beta = -0{,}09$; $p = 0.424$). However, children with birth weight below the 10th percentile scored significantly lower (mean 21.0 vs 22.7; $p = 0.05$). These children were in 84% in the group with an increased sFLT1/PLGF ratio, which in turn is a predictor of low birth weight ($\beta = -0{,}389$; $p = <0.001$).
Conclusion: In this cohort, low birth weight correlates with an elevated sFLT-1/PLGF ratio and had a negative effect on the outcome in the MOS-R. A direct correlation between an increased ratio and a worse motor outcome was not demonstrated.
None declared.

ID 401. Music impacts brain cortical microstructural maturation in very preterm infants

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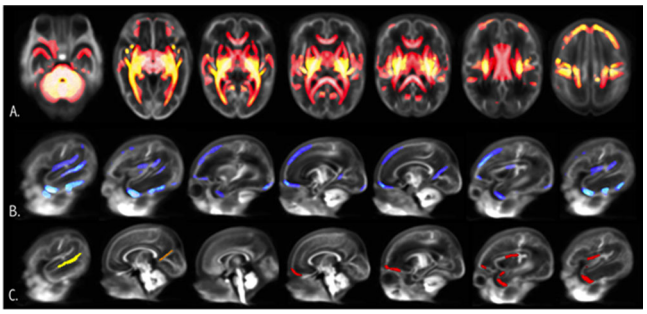
Background: Preterm birth disrupts important micro- and macrostructural neurodevelopmental processes taking place from mid-fetal stage to birth, a critical period of activity dependent plasticity. Music interventions have been used in neonatal intensive care units (NICU), aiming to modulate early brain networks development during this critical period. Such interventions have been shown to impact early brain functional networks' known to be negatively affected by prematurity, but literature is still scarce regarding its impact on brain structure.
Methods: In order to evaluate if a music intervention might induce brain macro- or microstructural changes, multi-shell diffusion imaging data were acquired longitudinally in 54 very preterm infants (VPT), randomized into a music or control group and undergoing an MRI before the intervention (during the 33th week gestational age) and at its end, at term-equivalent-age (TEA). Data were analysed using a longitudinal whole-brain fixel-based analysis (FBA) complemented by NODDI microstructural analysis.
Results: Between the 33th week and TEA, a longitudinal increase of fiber density (FD), fiber cross-section (FC) and fiber density cross-section (FDC) in all major cerebral white matter (WM) fibers in VPT. In cortical grey matter (GM) regions, while FC and orientation dispersion index (ODI) increase longitudinally, the FD and FDC decrease. When comparing VPT music and control groups, the early music intervention resulted in a longitudinal significantly superior increase of FC and ODI in certain cortical regions, namely in the right middle temporal gyrus, the right precuneus/posterior cingulate gyrus and the left insulo-orbito-temporopolar complex.
Conclusion: Important macro- and microstructural changes, measured by multi-shell diffusion imaging, are taking place in human WM and GM between the 33th week and TEA, corresponding to tissue-specific developmental maturation processes. Early music intervention, as an activity stimulating process, can increase the complexity of important cortical regions implied in auditory, cognitive and specially socio-emotional processing.



(ID 401) - WM fibers with increased FD/FC/FDC (A) and cortical GM regions with increased FC (B) in VPT from 33th week GA to TEA; GM regions with increased FC in music group.

None declared.

ID 408. Creative music therapy and neurodevelopmental outcomes in preterm infants at age two years: results of a randomized controlled pilot trial

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Background: Creative music therapy (CMT) aims to prevent and reduce neurobehavioral deficits in high-risk neonates using musical stimulation and socio-emotional co-regulation, integrating both the infant and parent(s) in meaningful infant-directed singing. The present trial's primary outcome analysis provide evidence that CMT beneficially affects brain connectivity as assessed by cranial magnetic resonance assessment in very preterm born infants (VPT) at term equivalent age.
Methods: This randomized, clinical pilot trial was conducted to test the feasibility and the effect of CMT on structural brain development in VPT, i.e., born <32 weeks' gestation. Here, we present the prespecified secondary outcomes at 2 years of corrected age. Eighty-two infants were randomized to either CMT or standard care. A specially trained music therapist provided CMT 2–3 times a week during hospitalization. The secondary outcomes were: the cognitive, language, and motor scores of the Bayley Scales of Infant and Toddler Development, 3rd edition; cerebral palsy; hearing and vision problems; and somatic growth parameters. Outcomes were compared between groups according to the treatment assigned to randomization using the independent t- and the Fisher's exact test for continuous and nominal variables.
Results: Fifty-six (68%) randomized infants underwent follow-up examination. Baseline characteristics of participants and non-participants in the 2-year follow-up were similar except for of a lower gestational age ($p < 0.001$), longer supplemental oxygen therapy ($p = 0.001$), and longer hospital stay ($p < 0.001$) in infants assessed at age 2 years than in dropouts. No evidence for a difference in the secondary outcomes was observed between groups.
Discussion: While in this randomized pilot trial, a positive effect of CMT on brain connectivity was demonstrated in VPT infants at term equivalent age, no evidence of a treatment effect on cognitive, language, motor, and neurosensory outcomes was reported at 2 years of corrected age.
Conclusion: Long-term neurodevelopmental follow-up of larger cohorts of VPT exposed to CMT are recommended to elucidate possible effects of music on more sensitive outcomes such as executive function, detailed language processing, and social-emotional development since the small size of the collective and the short-term neurodevelopmental measurements limit these findings' generalisability.
None declared.

ID 412. Obstructive cholestasis compromises balance and alters brain lipid composition in neonatal piglets

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Background: Infants with neonatal cholestasis are prone to neurodevelopmental deficits including neuromotor function. They accumulate potentially neurotoxic molecules in the bloodstream including ammonia, bilirubin and bile acids, and are at risk of malabsorbing lipids important for brain development. This study examined neuromotor function and bile acid and lipid composition of the brain in a piglet model of neonatal obstructive cholestasis.

Methods: Eight-day old piglets underwent bile duct ligation (BDL) or sham surgery (SHAM) and were followed for 3 weeks during which neuromotor functional tests (balance beam, open field, neuromotor score, gait analysis) were conducted. Samples were collected for liver histopathology, RNA sequencing of the cerebellum, and bile acid and lipid profiling of the plasma and/or cerebellum.

Results: BDL piglets had increased liver enzyme levels, liver fibrosis and bile duct proliferation compared to SHAM piglets. Balance was compromised in BDL piglets ($p < 0.05$) but only minor changes were found in other tests of neuromotor function. Plasma and cerebellum bile acid profiles differed between BDL and SHAM piglets with hyocholic acid and conjugated bile acid forms dominating in the BDL group (approximately 89% and 99% of total bile acids, respectively). Total bile acids and levels of the bile acid biosynthesis marker C4 (7α-hydroxy-4-cholesten-3-one) in plasma and cerebellum were increased in the BDL group, while plasma FGF-19 levels were reduced (all $p < 0.001$). In the cerebellum there were lower total lipid, phosphatidylinositol and 18:2 fatty acid levels, while of several low abundance saturated and unsaturated fatty acids of both even and odd chain length were increased in the BDL group relative to SHAM (all $p < 0.05$). RNA sequencing revealed similar gene expression profiles between groups.

Conclusion: In conclusion, surgically induced neonatal obstructive cholestasis compromised aspects of neuromotor function and altered the bile acid and lipid profile of plasma and/or cerebellum in piglets. Longer-term studies are needed to determine these effects at a stage of more advanced liver disease.

None declared.

ID 416. Newborn neurobehavior is related to later neurodevelopment and social cognition skills in extremely preterm born children, a prospective longitudinal cohort study

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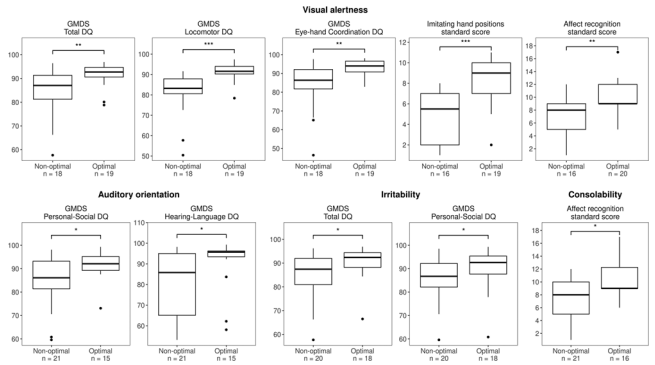
Background: Extremely preterm born children are at high risk for sensorimotor, language, visuocognitive and social impairments. Understanding of neonatal predictors of childhood outcomes is important for early detection of neurodevelopmental impairments and for allocating timely interventions at an early age of the potential brain plasticity. Our aim was to evaluate the ability of the neonatal neurobehavioral characteristics to act as an indicator of later neurodevelopment and neurocognitive performance.

Methods: Sixty-six infants born extremely preterm (<28 gestational weeks) were followed until 6.5 years. Neurobehavior at term age was assessed by the behavior subscale of Hammersmith Neonatal Neurological Examination (HNNE) using dichotomic rating, optimal and non-optimal. The Griffiths Scales (GMDS) at 2 years, and Wechsler Intelligence Scales and a Neuropsychological Assessment at 6.5 years were used to assess neurodevelopment and neurocognitive performance including social cognition skills.

Results: An optimal auditory orientation at term age was associated with better developmental quotients (DQ) in Personal-Social and Hearing-Language GMDS subscale at 2 years ($p < 0.05$). An optimal visual alertness was associated with better Total ($p < 0.01$), Locomotor ($p < 0.001$), and Eye-Hand Coordination DQs ($p < 0.01$) at 2 years, and with sensorimotor function ($p < 0.001$), and social perception ($p < 0.01$) tests at 6.5 years of age (Figure).

Conclusion: Newborn neurobehavior might serve as a precursor of social cognition skills and the HNNE behavior subscale offer a tool to identify infants at risk for later deficits in neurodevelopment and social cognition.



(ID 416) - The relationships between the Hammersmith Neonatal Neurological Examination items and the Griffiths Mental Developmental Scales (GMDS) and a Developmental Neuropsychological Assessment. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ID 420. Language related brain areas and outcomes in extremely preterm children during childhood

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Introduction: Language disabilities are common among preterm children. Both positive and negative correlations between verbal IQ and grey and white matter volumes for very preterm children (<32 weeks) have been reported during childhood and adolescence. We reported altered patterns of brain asymmetry at term age in extremely preterm children with later diagnosis of autism. However, there is a lack of knowledge about the associations between language scores and brain volumetric data in extremely preterm children (<27 weeks) during childhood.

Methods: Brain volumetry was investigated in 50 EPT children (<27 weeks) and 37 controls at 10 years using atlas-based segmentation. Brain volumes of separate regions within the language network (inferior frontal gyrus (opercular and triangular), temporal gyri (superior, middle and inferior), supplementary motor areas, Heschl gyrus, supramarginal and angular cortices) and all regions summed together were compared between EPT children and controls. Results were adjusted for age at scan, intracranial volume, sex and handedness. We used the Bonferroni correction. An asymmetry index for the language network was calculated based on the formula: $AI = (VL–VR)/(VL + VR)$. The brain volume of the volume and asymmetry score of the language network was correlated with language outcomes at 12 years (similarities scale, vocabulary scale and verbal IQ within WISC V and repetition of sentences within CELF 4) for $n = 40$ EPT children and $n = 29$ controls using Pearson's correlations for normally distributed data, correcting for age at scan, sex and handedness.

Results: All regions within the language network except for the Heschl'gyrus were significantly smaller for the EPT children compared to the controls, also after correcting for multiple comparisons (Table 1). Asymmetry indices were not different between extremely preterm children and controls. We found significant positive correlations between the volume of the language network and the vocabulary subscale within WISC V and repetitions of sentence scale within CELF 4, $p < 0.05$. Asymmetry index of the language network did not correlate significantly with language scores.

Conclusion: These findings demonstrate abnormal development of language-related brain regions in EPT children with an impact on their functional outcome. However, brain asymmetry within the language network seems to be of less importance at this age.

Brain region	Extremely preterm children adjusted mean volumes, cm^3 (SD)	Controls adjusted mean volumes, cm^3 (SD)	Mean difference, (confidence interval), adjusted	*p* value adjusted
Supplementary motor area, right	15.00 (0.47)	15.27 (0.53)	−0.27 (−0.40, −0.14)	**<0.001**
Supplementary motor area, left	13.60 (0.53)	13.84 (0.56)	−0.25 (−0.37, −0.13)	**<0.001**
Inferior frontal gyrus, triangular, right	13.64 (0.66)	13.89 (0.67)	−0.25 (−0.38, −0.11)	**<0.001**
Inferior frontal gyrus, triangular, left	15.82 (0.37)	16.13 (0.51)	−0.31 (−0.47, −0.15)	**<0.001**
Inferior frontal gyrus, opercular, right	9.04 (0.35)	9.18 (0.32)	−0.13 (−0.22, −0.05)	**0.002**
Inferior frontal gyrus, opercular, left	6.39 (0.34)	6.49 (0.4)	−0.10 (−0.15, −0.05)	**<0.001**
Heschel gyrus, right	1.53 (0.057)	1.55 (0.061)	−0.025 (−0.042, −0.008)	0.005
Heschel gyrus, left	1.36 (0.028)	1.37 (0.052)	−0.015 (−0.032, −0.003)	0.097
Angular gyrus, right	11.52 (0.49)	11.73 (0.49)	−0.20 (−0.30, −0.11)	**<0.001**
Angular gyrus, left	6.98 (0.20)	7.10 (0.23)	−0.12 (−0.19, −0.06)	**<0.001**
Supramarginal gyrus, right	12.3 (0.50)	12.6 (0.52)	−0.21 (−0.31, −0.10)	**<0.001**
Supramarginal gyrus, left	7.24 (0.23)	7.36(0.24)	−0.13 (−0.18, −0.07)	**<0.001**
Superior temporal gyrus, right	19.68 (0.71)	20.03 (0.79)	−0.35 (−0.52, −0.17)	**<0.001**
Superior temporal gyrus, left	13.65 (0.37)	13.87 (0.44)	−0.21 (−0.33, −0.09)	**0.001**
Middle temporal gyrus, right	27.56 (1.07)	28.00 (1.09)	−0.44 (−0.67, −0.21)	**<0.001**
Middle temporal gyrus, left	28.99 (0.86)	29.47 (0.97)	−0.49 (−0.72, −0.25)	**<0.001**
Inferior temporal gyrus, right	22.11 (0.78)	22.42 (0.85)	−0.32 (−0.51, −0.14)	**0.001**
Inferior temporal gyrus, left	18.44 (0.47)	18.74 (0.57)	−0.31 (−0.46, −0.15)	**<0.001**
Language network	244.85 (8.20)	248.96 (8.94)	−4.11 (−6.12, −2.09)	**<0.001**

**All analyses are corrected for sex, age at scan, intracranial volume and handedness. Bold values remained significant after correcting for multiple comparisons.

(ID 420) Brain volumetric differences between extremely preterm children and control children corrected for sex, age at scan, handedness and intracranial volume.

None declared.

ID 422. Parental perceptions on the value of neonatal brain MRI

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Background: Brain MRI is often part of routine screening in extremely preterm infants (EPI, <28 weeks of gestation) to provide neurodevelopmental prognoses. Little research has been done on parental perceptions on routine MRI, while parents have to handle the results. This study aims to describe parental perceptions on the value and communication of routine MRI.

Methods: Parents of 205 EPI who were admitted to the neonatal intensive care of the Wilhelmina Children's Hospital between 2016 and 2021 were invited to complete an online survey on neonatal MRI. Data were analysed by frequency distributions and Fisher–Freeman–Halton exact tests to determine associations between different prognoses and responses about MRI. Bivariate analyses excluded parents who answered "do not remember", causing different total numbers per analysis.

Results: The analysis included parental responses of 85 EPI (41.5% response rate). 81% recalled being informed about the advantages of MRI, whereas only 44% recalled hearing about possible risks. 34.5% felt they had no choice regarding MRI and 37.3% were not asked whether they wanted to know the results. While the results were useful for 94.8% of parents and 63.2% reported that they reassured them about their child's development, 74% did not think their child received adjusted treatment based on the results. However, all parents who received a worrisome prognosis ($n = 5$) reported that their child received adjusted treatment after MRI, opposing 7.7% ($n = 26$) of parents who received a good prognosis ($p \leq 0.001$). For 66.7% of parents who received a worrisome prognosis ($n = 6$), the results caused anxiety about their child's development, opposing no parents ($n = 29$) who received a good prognosis ($p \leq 0.001$). Moreover, the poorer the prognosis, the more often parents reported that their child developed better than predicted ($p \leq 0.001$).

Conclusion: In this sample, parental perceptions on MRI are mainly positive. However, worrisome prognoses can cause parental anxiety, while these parents also reported their child to develop better than expected based on the prognosis. This suggests that such prognoses should be carefully discussed with parents. Additionally, communication can be improved by explaining both advantages and risks, and by presenting MRI as shared decision-making, with an important voice of parents.

None declared.

ID 424. A comparison of four brain MRI scoring systems in neonatal hypoxic-ischemic encephalopathy: interrater reliability and prediction of outcome

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Background: Brain MRI scan results are main predictors for the outcome of neonates with hypoxic–ischemic encephalopathy (HIE). There is currently no uniform method to assess these brain MRI scans. The aim was to determine which MRI-score demonstrates the highest interrater reliability and most accurately predicts the outcome at 2 years of age.

Methods: Four MRI scoring systems for neonates with HIE were selected: (1) Weeke score; (2) NICHD score; (3) Rutherford score and (4) Trivedi score (the adapted Bednarek). All scores (except the Rutherford score) included assessments of diffusion weighted imaging. Two blinded raters retrospectively assessed and scored the brain MRI images of 161 neonates with HIE, born between 2010 and 2014, treated with hypothermia. Results of all four scoring systems were compared and interrater reliability was assessed. Outcome was assessed by standardized neurodevelopmental testing at the age of 2 years. Neurodevelopmental impairment (NDI) was defined as a motor or cognitive composite score of at least 1 standard deviation below the reference mean on the Bayley Scales of Infant and Toddler Development Third Edition (i.e. a score<85 points), diagnosis of cerebral palsy (Gross Motor Function Classification System of ≥2), hearing loss requiring hearing aids or severe visual loss. The combined outcome was defined as death or NDI.

Results: Interrater reliability analyses demonstrated good reliability of the Weeke score (intraclass correlation coefficient (ICC) 0.740) and the Trivedi score (ICC 0.759), a reasonable reliability of NICHD (weighted Kappa 0.646) and an insufficient reliability of the Rutherford score (ICC 0.526). The reliability improved when analyzing only high quality scans. All four different scoring systems performed well in distinguishing between a normal or NDI: Weeke score AUC 0.71 (0.60–0.82), NICHD score AUC 0.69 (0.59–0.79), Trivedi score AUC 0.67 (0.56–0.78) and Rutherford score AUC 0.66 (0.55–0.77). This was also true for predicting the combined outcome: Weeke score AUC 0.88 (0.82–0.94), NICHD score AUC 0.86 (0.79–0.93), Trivedi score AUC 0.85 (0.79–0.92) and Rutherford score AUC 0.79 (0.71–0.87).

Conclusion: Trivedi score and Weeke score performed best on interrater reliability which possibly can be attributed to the use of DWI. All four MRI brain-scores proved to be usable predictors for the outcome at 2 years of age.

None declared.

ID 432. Insulin growth factor 1 stimulated release of extracellular vesicles from the choroid plexus—novel mode of blood-brain-barrier signalling

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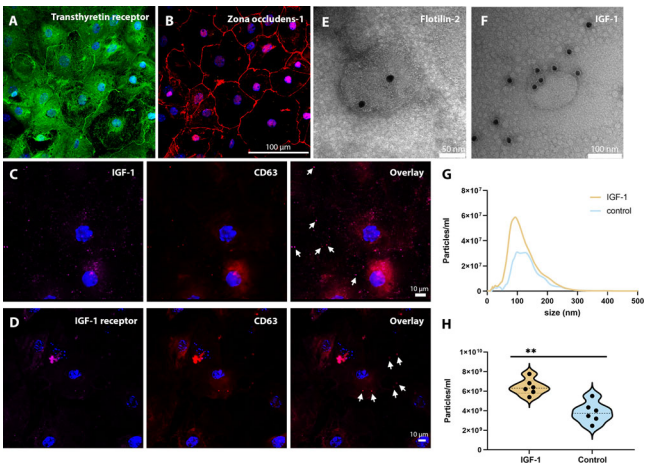
Background: It is well accepted that insulin-like growth factor 1 (IGF-1) plays a crucial role in brain development. However, previous studies have inferred that the transfer of systemically administered IGF-1 over the blood brain barrier is restricted, which would present a limitation for beneficial treatment effects within the brain. Extracellular vesicles (EVs) are small, cell-derived phospholipid membrane enclosed vesicles that constitute important cell-to-cell messengers, regulating diverse cellular functions of recipient cells. EVs released from the choroid plexus (CP) to intraventricular cerebrospinal fluid (CSF) have been shown to cross the ependyme and exert a regulatory function in periventricular brain tissue. We hypothesized that exposure of the CP to blood-born IGF-1 induces release of EVs into intraventricular CSF destined to regulate surrounding brain parenchyma. We evaluated this in a primary culture of CP epithelial (CPE) cells using a Transwell in vitro model.

Methods: CPE were collected from p3-p9 mice pups and plated on Transwell membranes. Cells were stimulated with 40 ng/ml IGF-1 at the basal (blood) side for 24 h, apical supernatant was collected, and cells were fixed. EVs were prepared from cell culture supernatant. The size and quantity of EVs were measured with nanoparticle tracking analysis. EV morphology and protein immunolabeling were evaluated with transmission electron microscopy (TEM). CP protein markers were analysed using immunocytochemistry.

Results: Functional characteristics of CPE cells were ascertained by positive staining for the transthyretin receptor and zona occludens-1 (Fig. 1a, b), absent staining for several fibroblast markers and an increasing transepithelial electric resistance during culture. The CPE cells also stained positively for IGF-1, the IGF-1 receptor and the exosomal marker CD63 (Fig. 1c, d). TEM of purified EVs from apical supernatant displayed typical exosome and microvesicle morphology, positive immunolabeling for IGF-1 and the exosomal marker Flotilin-2 (Fig.1e, f). Exposure of CPE cells to IGF-1 caused an increased release of EVs into the apical supernatant ($p = 0.004$) (Fig.1g, h).

Conclusion: Simulated blood-borne exposure of IGF-1 stimulates CP epithelial cells to secrete IGF-1 positive EVs in a Transwell in vitro system. We will further expand this study to investigate the miRNA secretome of CP derived EVs upon IGF-1 stimulation, both in vitro and in vivo.



(ID 432) a–d Immunocytochemical staining of the transthyretin receptor, zona occludens-1, IGF-1, the IGF-1 receptor and CD63. Arrows display colocalization. e, f TEM; immunogold labelling of Flotilin-2 and IGF-1. g, h Nanoparticle tracking analysis.

None declared.

ID 443. Metabolic and haemodynamic reactivity indices of cerebral autoregulation provide an early assessment of injury severity and predict neurological outcome following neonatal encephalopathy

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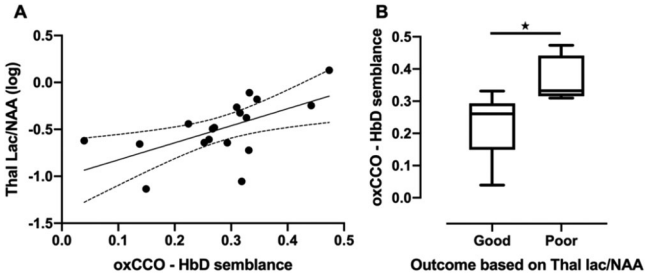
Background: Neonatal encephalopathy (NE) resulting from intrapartum events remains a significant global health problem. An urgent need exists for a cot-side biomarker for early stratification of injury severity and prediction of neurological outcome. Broadband near-infrared spectroscopy (BNIRS) monitors real-time changes in mitochondrial metabolism (oxCCO) and cerebral oxygenation (HbD). Wavelet semblance (reactivity index of phase difference) of BNIRS variables can measure cerebral autoregulatory disturbance at 48h of life during therapeutic hypothermia and predict neurodevelopmental outcome. A novel dual monitoring platform with BNIRS and diffuse correlation spectroscopy (DCS) directly monitors microvascular cerebral blood flow (BFI) along with mitochondrial metabolism and oxygenation. We hypothesised that optical wavelet reactivity indices early after a hypoxic ischaemic (HI) injury in a preclinical model of NE will relate to outcome.

Methods: Combined BNIRS-DCS monitoring was performed in 18 newborn piglets during and after induced HI injury. Insult severity was graded to simulate moderate HI in seven piglets and severe HI in six piglets. Five piglets did not have HI insult (control). Reactivity indices were calculated as mean oxCCO-HbD and BFI-HbD semblance over one-hour of monitoring at one-hour post-insult using wavelet analysis. All animals had MR imaging and proton MR spectroscopy in a 3T scanner 6 h post-insult. Thalamic Lac/NAA 0.39 was used as cut-off threshold for neurological outcome along with TUNEL+ cell count in thalamic region on brain histology.

Results: Both oxCCO-HbD (metabolic reactivity) and BFI-HbD semblance (vascular reactivity) correlated with thalamic Lac/NAA ($p = 0.009$, $r^2 = 0.353$ and $p = 0.057$, $r^2 = 0.234$ respectively). Both oxCCO-HbD and BFI-HbD semblance correlated with thalamic TUNEL+ histology ($p = 0.056$ and 0.020). Both oxCCO-HbD and BFI-HbD semblance were significantly different between groups based on insult severity ($p = 0.046$ and 0.002) and between groups of animals with good and poor neurological outcome based on thalamic Lac/NAA ($p = 0.002$ and 0.025) (Fig. 1).

Conclusions: Early optical markers of metabolic and vascular reactivity (indicating

mitochondrial injury and cerebrovascular autoregulatory impairment) following HI insult can assess injury severity and predict neurological outcome in an animal model of NE. These translational findings can be important for clinical decisions and needs to be investigated further in a clinical study.



(ID 443) - Fig. 1. Linear regression analysis between oxCCO-HbD semblance and thalamic lac/NM, $p = 0.009$ (A), oxCCO-HbD semblance was significantly different between outcome groups based on thalamic lac/NM cut-off threshold 0.39 (B).

None declared.

ID 472. Preliminary results of the PASSIoN trial (Perinatal Arterial Stroke treated with Stromal cells IntraNasally)

Lisanne M Baak[1], Nienke Wagenaar[1], Niek E van der Aa[1], Floris Groenendaal[1], Jeroen Dudink[1], Maria-Luisa Tataranno[1], Liesbeth S Smit[2], Reint K Jellema[3], Timo R de Haan[4], Henk J ter Horst[5], Willem P de Boode[6], Sylke J Steggerda[7], Colin G de Haar[8], Linda S de Vries[1], Frank van Bel[1], Cora HA Nijboer[9], Manon JNL Benders[1]*

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Background: Perinatal arterial ischemic stroke (PAIS) is an important cause of perinatal brain damage in the term-born neonate with lifelong neurodevelopmental disorders in 50–75% of patients. Currently, there is no treatment available to alleviate neurological damage after PAIS. Mesenchymal stromal cells (MSCs) have shown promising results in animal studies of PAIS. In this study, we assessed the safety and feasibility of intranasal delivery of bone marrow-derived allogeneic MSCs in neonates with PAIS.

Methods: We conducted a phase I/II, open-label, single-arm, nationwide intervention study in the NICU at the University Medical Center Utrecht, the Netherlands (ClinicalTrials.gov/show/NCT03356821). Ten (near-)term (≥36 weeks of gestation) neonates with MRI-confirmed PAIS in the middle cerebral artery (MCA) region, with presenting symptoms within the first week after birth and parental consent, were included. Neonates received one dose of $\pm 50 \times 10^6$ MSCs via intranasal droplets as soon as possible after confirmation of the MCA stroke, but within the first week after presenting symptoms. We monitored (sub)acute safety by measuring vital parameters, blood markers and occurrence of adverse events, and we repeated the MRI at 3 months of age.

Results: In all ten neonates, intranasal administrations of MSCs were feasible. We did not observe any adverse events, except for one patient that developed a mild transient fever shortly after MSC treatment without further clinical implications. Blood infection parameters (CRP, procalcitonin and leukocyte levels) remained stable pre- versus post-administration. MRI scans at 3 months of age ($n = 8$, 2 pending) did not show signs of infection or cerebral tumorigenicity and 63% ($n = 5/8$) of infants had minimal to no posterior limb of the internal capsule (PLIC) involvement while the corticospinal tract initially showed diffusion restriction on DWI. Visual inspection of the amount of tissue loss on MRI following MSC therapy looks promising; however, quantitative analysis still needs to be performed. Currently, most neonates are too young to report on their functional outcome.

Conclusion: Intranasal MSC application of ten neonates with PAIS was safe and feasible. Most infants showed symmetrical myelination of the PLIC 3 months after MCA stroke. Future placebo-controlled studies with larger patient populations are needed to determine the therapeutic effect of MSCs.

This study was funded by ZonMw, the Netherlands. The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the abstract.

ID 478. Morphine exposure and neurodevelopmental outcome in extremely preterm infants

Michele Luzzati[1], Caterina Coviello[1], Henriette Swarenburg-Veye[2], Jeroen Dudink[2], Carlo Dani[1], Corine Koopmans[2], Linda S deVries[2], Floris Groenendaal[2], Manon Benders[2], Maria Luisa Tataranno[2]*

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Background: Opioids are the most common drugs used to treat pain and stress in infants receiving mechanical ventilation in the NICU. However, controversial data regarding their effects on long-term neurological outcome have been reported.

Methods: We conducted a retrospective study in extremely preterm infants (gestational age (GA) <28 weeks), admitted to the Wilhelmina Children's Hospital NICU, Utrecht, between 2008 and 2011 with the aim to investigate the association between morphine exposure up to term age and neurodevelopmental outcome at 2 and 5 years. Morphine administration was expressed as cumulative dose (mg/kg) until term-equivalent age (TEA). Neurodevelopmental outcome was assessed at 2 years with the Bayley Scales of Infant and Toddler Development (BSID-III-NL) and at 5 years with the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III-NL). Multivariable linear regression analysis was used to assess the association between morphine exposure and outcome. Analyses were adjusted for confounders: GA, patent ductus arteriosus, long-term mechanical ventilation (>7 days), postnatal corticosteroids, number of painful procedures, intraventricular hemorrhage (IVH), white matter injury (WMI), cerebellar hemorrhage and maternal education.

Results: 106 extremely preterm infants were included in the study, 64 received morphine (60.4%) at a mean dose 2.03 ± 2.09 mg/kg during their NICU admission. Infants exposed to morphine were more frequently male, had a lower GA and birth weight, longer mechanical ventilation, a higher incidence of IVH, bronchopulmonary dysplasia and WMI at TEA compared to not-exposed infants. Moreover, exposed subjects revealed a significantly worse motor performance at 2 years ($p < 0.005$), whereas no differences were observed in cognitive and language of Bayley-III-NL and in WPSSI-III-NL score, respectively. At regression analysis morphine exposure did not represent a risk factor for a worse Bayley-III-NL scores at 2 years. Nevertheless, morphine-exposure resulted a risk factor for a lower Fullscale-IQ scores ($p = 0.008$, $B = -9.3$, CI -15.6 -3.1) and Performance-IQ scores ($p = 0.005$, $B = -17.5$, CI -27.9 -7) at 5 years of age.

Conclusion: Morphine exposure in extremely preterm infants is not associated with neurological outcome at 2 years. However, an association is found with poorer Fullscale -IQ and Performance IQ at 5 years. Future, prospective studies with larger sample sizes are needed to confirm these findings.

None declared.

ID 480. Systemic IGF-1 treatment affects gene expression in the brain in the preterm rabbit

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Background: Endogenous serum levels of IGF-1 are low in preterm infants following birth, and low levels are linked to morbidities and unfavorable neurodevelopmental outcome. IGF-1 affects brain cell proliferation, neurogenesis, maturation, and differentiation, however, the mechanistic effects of systemic exogenous IGF-1 on the brain, across the blood barrier still remain unclear. Using the preterm rabbit pup as a model for preterm infants, we investigated the impact of systemic peripheral IGF-1/IGFBP3 treatment on gene expression in the periventricular brain area.

Methods: Preterm rabbits ($n = 24$) were delivered by C-section on E29 (=3 days prior to term) and received 4 mg/kg IGF-1/IGFBP3 or NaCl subcutaneously every 12th hour, the first 3 days of life (6 doses). Total RNA transcriptome sequencing was performed on periventricular brain matter collected after the end of treatment (P0, 3 days after birth) and 10 days after the end of treatment (P10).

Results: IGF-1/IGFBP3 treatment resulted in significant changes in 1624 genes ($q \leq 0.05$, corrected for multiple testing) at P0 compared to the NaCl group. No differently expressed genes were found at P10. Over time, from P0 to P10, 1813 genes were affected exclusively by IGF-1/IGFBP3 treatment, 1583 genes were regulated exclusively in the NaCl group, and 1001 were affected in both groups. Top regulated KEGG pathways at P0 in the IGF-1/IGFBP3 treatment included the spliceosome, the AMPK and the Hedgehog signalling pathways, all involved in regulating brain cell proliferation, metabolism, stem cell maintenance, and development. Investigating the dynamics in gene expression from P0 to P10, top regulated pathways affected in the IGF-1/IGFBP3 group included the synaptic vesicle cycle, the cell cycle, and the GABAergic synapse pathways among others. The top 20 genes upregulated in the IGF-1/IGFBP3 group from P0 to P10 (not affected at P0) included SCN1B, HPCA, CNTNAP2 and ITPKA gene, critical for neuronal migration and proliferation, myelination and synaptic plasticity.

Conclusions: Systemic peripheral IGF-1/IGFBP3 treatment postnatally following preterm birth induced changes in genes and important pathways involved in neurodevelopment, such as the formation of neurons, neuronal survival, and synaptic plasticity. Further, IGF-1/IGFBP3 treatment triggered a different developmental cue affecting the dynamics of the gene expression during an important developmental period.

D.L. has received consulting fees from Shire PLC.

ID 518. Alterations in circadian genes BMAL1, CRY, CLOCK, and REV-ERBα are associated with inflammatory cytokine profile in neonatal encephalopathy

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Background: Circadian genes (CG) Brain and muscle Arnt-like protein-1 (BMAL1), REV-ERBα, and Circadian locomotor output cycles kaput (CLOCK) are present in all immune cells and promote an anti-inflammatory state. Cryptochrome (CRY) negatively regulates the expression of BMAL1. Dysregulated inflammation and delayed sleep-wake cycling onset are both associated with adverse outcome in neonatal encephalopathy (NE). Early randomised trials of melatonin treatment, a chronobiotic agent, as

an adjunct to therapeutic hypothermia (HT) has demonstrated improved outcomes. We explored circadian influences on inflammation in NE by investigating the association between CG expression and serum cytokines, and the effect of melatonin treatment on CG expression ex vivo.

Methods: Blood samples were collected from infants with NE requiring HT during the first week of life and divided into four treatment groups—vehicle, lipopolysaccharide (LPS), melatonin, and LPS +melatonin. Serum cytokine concentration was analysed by multiplex ELISA. Cytokines examined were GM-CSF, IFNγ, IL-1α, IL-1ra, IL-2, IL-1β, IL6, IL-8, IL-10, IL-18, TNFα, TNFβ, Epo, and VEGF. Whole blood RNA was isolated, cDNA was synthesized and analysed by quantitative PCR for the expression of BMAL1, REV-ERBα, CLOCK and CRY. Associations between CG expression and serum cytokine concentrations were examined by Pearson correlation following data log transformation, and differences between treatment groups in CG expression was examined by paired t-tests.

Results: Decreased expression of BMAL1 and CRY (n = 36), and increased expression of CLOCK were significantly associated with higher serum concentration of the pro-inflammatory cytokine IFNγ. Decreased expression of REV-ERBα was significantly associated with increased serum concentration of the pro-inflammatory cytokine IL-2. There were no significant differences in CG expression between treatment groups when vehicle vs melatonin, and LPS-stimulated vs LPS +melatonin treatment groups were compared.

Discussion: Factors associated with the entrainment of CG expression in neonates are not fully understood. However, several factors likely to be associated with this entrainment including light exposure and regular feeding are disrupted in the care of patients with NE. While we have demonstrated an association between CG expression and inflammatory cytokines, CG expression does not appear to be influenced by melatonin treatment. Further research into circadian entrainment and the influence on inflammation and outcomes in NE are required.

None declared.

ID 534. Development of an automated real-time sleep-state prediction algorithm in preterm infants: the Sleep Well Baby project

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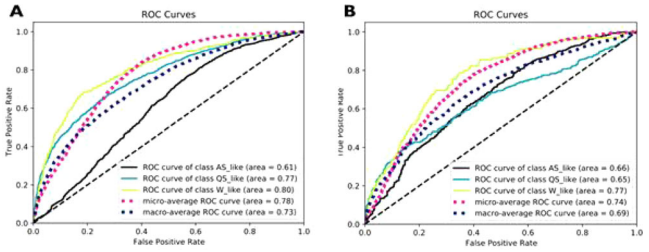
Introduction: In the womb, sleep is believed to be the major driver of neural activity, a process that is critical for neuronal survival, axonal guidance, and synapse maturation. However, in the NICU, preterm infants are exposed to a myriad of extrinsic stimuli that radically alter their sleep-wake states. To improve sleep in preterm infants, it is essential to co-align elective treatments on sleep and wake states. This is especially challenging because the active sleep states are hard to be distinguished from wake states. We aimed to develop an automated real-time sleep-state prediction algorithm in preterm infants based on vital signs that are routinely collected in neonatal intensive care unit (NICU).

Methods: We designed a novel and robust supervised machine-learning algorithm called SleepWellBaby (SWB) for real-time neonatal sleep-wake classification in the NICU. The SWB algorithm used the vital physiological parameters: heart rate, respiratory rate, and oxygen saturation as input features. The target variable consisted of minute-by-minute sleep states for which ground-truth labels were derived using our in-house behavioral sleep annotation system. The sleep–wake states were annotated as active sleep (AS), quiet sleep (QS), and wake. The random forest model was used as the underlying classifier. The model was trained on 23 unique preterm infants (28–32wk PMA; 90% of train data) via k-fold grouped cross-validation and randomized grid search. It was then tested on three independent infants (10% of train data). Bootstrapping was used to estimate 95% confidence intervals (CIs) of the area under the ROC curve (AUC). Model performance was later validated on nine newly observed infants.

Results: The macro-averaged area under curve (AUC) was 0.73 [0.71–0.74], with an AUC of 0.80 [0.78–0.83] for wake (Fig. 1). Similar performance was observed in the nine validation patients (Fig. 2), with an averaged AUC of 0.69 [0.67–0.72] and an AUC for wake of 0.77 [0.72–0.81] indicating good generalization performance of the model (Fig. 1).

Conclusion: We developed a well performing automated sleep-state prediction algorithm based on bedside vital parameters that generalized well to an independent sample of patients. Currently, we are preparing the next step of validating the model in real-world clinical practice settings.



(ID 534) - Fig. 1. Showing the receiver operating characteristics curves (ROC), illustrating the predictive ability of the SWB algorithm to distinguish between different sleep/wake states. (A) Cross-validation train results. (B) Pilot results.

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the abstract.