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Permalink
https://escholarship.org/uc/item/0m26f2kt

Journal
Metabolic Brain Disease, 31(5)

ISSN
0885-7490

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Publication Date
2016-10-01

DOI
10.1007/s11011-016-9850-x

Peer reviewed

Reduced glutamate in white matter of male neonates exposed to alcohol *in utero*: a [1]H-magnetic resonance spectroscopy study

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Abstract

In utero exposure to alcohol leads to a spectrum of fetal alcohol related disorders (FASD). However, few studies used have used proton magnetic resonance spectroscopy (^{1}H-MRS) to understand how neurochemical disturbances relate to the pathophysiology of FASD. Further, no studies to date have assessed brain metabolites in infants exposed to alcohol *in utero*. We hypothesize that neonates exposed to alcohol *in utero* will show decreased glutamatergic activity, pre-emptive of their clinical diagnosis or behavioural phenotype. Single voxel ^{1}H-MRS data, sampled in parietal white and gray matter, were acquired from 36 neonates exposed to alcohol *in utero*, and 31 control unexposed healthy neonates, in their 2nd-4th week of life. Metabolites relative to creatine with phosophocreatine and metabolites absolute concentrations using a water reference are reported. Male infants exposed to alcohol *in utero* were found to have reduced concentration of glutamate with glutamine (Glx) in their parietal white matter (PWM), compared to healthy male infants ($p = 0.02$). Further, male infants exposed to alcohol *in utero* had reduced concentration and ratio for glutamate (Glu) in their PWM ($p = 0.02$), compared to healthy male infants and female infants exposed to alcohol *in utero*. Female infants showed higher relative Glx and Glu ratios for parietal gray matter (PGM, $p < 0.01$), compared to male infants. We speculate that the decreased Glx and Glu concentrations in PWM are a result of delayed oligoden-drocyte maturation, which may be a result of dysfunctional thyroid hormone activity in males exposed to alcohol *in utero*. Further study is required to elucidate the relationship between Glx and Glu, thyroid hormone activity, and oligodendrocyte maturation in infants exposure to alcohol *in utero*.

Keywords

MRS; Alcohol exposure; Gray matter; Oligodendrocytes; Thyroid hormone

Introduction

Exposure to alcohol during gestation is known to lead to a spectrum of foetal alcohol related disorders (Asma Khalil 2010). Recent magnetic resonance imaging studies in fetal alcohol spectrum disorder (FASD) show reduced brain volumes and reduced diffusivity of white matter – these reductions are suggested to result from delayed maturation and reduced neuronal plasticity (Human studies - (Treit et al. 2013; Spottiswoode et al. 2011; Lebel et al. 2012; Wozniak et al. 2011,2009,2006); Animal studies - (Leigland et al. 2013a, b). A recent resting state fMRI study in FASD has established that functional connectivity is related to observed brain structure abnormalities (Wozniak et al. 2013).

Proton magnetic resonance spectroscopy (^{1}H-MRS) may be particularly useful insofar as it can be used to infer information about cellular microstructure in vivo and to point to abnormalities in neurotransmission and cellular metabolism in FASD. However, to date very few studies have investigated brain metabolite concentrations in FASD using ^{1}H-MRS in children and adolescents, and there are no studies in neonates exposed to alcohol *in utero* (Donald et al. 2015). One study found *increased* n-acetyl-aspartate (NAA) for caudate nucleus of FASD children ($n = 11$) compared to control children ($n = 4$), a marker of neuronal integrity, the NAA concentration in these children was associated with facial dysmorphometry measures (Cortese et al. 2006). In contrast, a second study in adolescents and young adults with FASD ($n = 10$) found *no change* in NAA within frontal cortex, anterior cingulate cortex, left parietal cortex, hippocampi, frontal white matter, temporal white matter, caudate nucleus, cerebellum cortex or vermis. However they did find *increased* NAA within the right parietal cortex and bilateral thalami, when compared with controls ($n = 10$) (Fagerlund et al. 2006). A third ^{1}H-MRS study reported *decreased* choline, a marker of phospholipid membrane turnover and myelination, in frontal/parietal white matter of preadolescent children with diagnosed FASD ($n = 20$) compared with non-impaired children ($n = 61$; Astley et al. 2009). A second study reported *decreased* choline in the left striatum of children with FASD ($n = 8$) when compared to controls (Goncalves Rde et al. 2009). Finally, a more recent study which assessed young children with heavy alcohol exposure ($n = 37$) and non- or minimally exposed controls ($n = 17$), found several ^{1}H-MRS relationships within the cerebellum; *decreased* NAA was related to increased maternal use of alcohol around time of conception, *decreased* choline was related to higher levels of alcohol consumption during pregnancy, and alcohol consumption around time of conception and during pregnancy were related to *increased* glutamate with glutamine (Glx) (du Plessis et al. 2014). These studies demonstrate the utility of ^{1}H-MRS for targeting neurochemical abnormalities associated with symptoms of FASD during childhood. However, differences in the age range and clinical profiles of the developmental samples studied, differences in the anatomic locations of ^{1}H-MRS voxel placement, variations in imaging acquisition and analysis approaches have made it difficult to compare results across studies. Thus further empirical investigation at different stages of development is required and supported.

It is important to characterize the permanent effects or outcome of *in utero* exposure to alcohol in children and adolescents, however is it feasible to intervene and improve the clinical and behavioural outcome of these children at a younger age – as neonates. The

teratogenic effects of alcohol have not been fully elucidated due to the complex cellular influences it exerts (Goodlett et al. 2005). The severity of FASD has been associated with the ability of the mother to metabolize alcohol, and the production of acetaldehyde – a highly toxic metabolite (Gemma et al. 2007). Alcohol has been shown to decrease DNA synthesis, inhibit growth factors and protein synthesis, and reduce available glucose – all of which are necessary for healthy development (Shibley and Pennington 1997). Studies in neonates exposed to alcohol *in utero* primarily assess the severity of FASD, investigating clinical phenotype through dysmorphometry and biomarkers obtained from meconium (Memo et al. 2013). As yet no studies have been able to suggest strategies of intervention to improve the neurobiological effects of *in utero* alcohol exposure.

Glutamate, the brain's major excitatory neurotransmitter is required to signal processes of myelination; achieved by glutamate's activation of AMPA/kainate and NMDA receptors on immature oligodendrocytes (Karadottir and Attwell 2006). The expression of these glutamate receptors on oligodendrocytes is achieved by the release of neuregulin and brain derived neurotrophic factor (BDNF) required for natural brain maturation (Lundgaard et al. 2013), which is reportedly attenuated in FASD (Balaszczuk et al. 2013). Oligodendrocytes sheath axons to ensure fast and efficient communication between brain regions, which is again attenuated in children and adolescents with FASD (Treit et al. 2013; Spottiswoode et al. 2011; Wozniak et al. 2013). In healthy neonates glutamate concentration increases exponentially in white matter, and gray matter, during the first 3 months of life, then tapers to a steady level in the second year of life (Bluml et al. 2013). Could this be a window of opportunity to develop an intervention, instead ofsimply characterizing metabolite profiles in childhood and adolescence?

We hypothesize that neonates exposed to alcohol *in utero* will show decreased glutamatergic activity, pre-emptive of their clinical diagnosis or behavioural phenotype. To address these important gaps in the literature, the current investigation used single voxel ^{1}H-MRS to compare metabolite concentrations in infants exposed to alcohol *in utero* (Table 2), who have not yet been diagnosed with FASD, during their second to fourth week of life, hypothesizing that neonates exposed to alcohol *in utero* would show decreased glutamate concentrations.

Materials and methods

Participants and recruitment

We report data from 36 infants (19 female, 17 male) who were exposed to alcohol *in utero* and 31 infants (10 female, 21 male) who were not exposed to alcohol *in utero,* i.e. no history or biological evidence of substance abuse by the mother. Ethical approval for this study was obtained from the Faculty of Health Sciences Research Ethics Committee at the University of Cape Town (HREC REF 525/2012). The study was conducted in accordance with the Declaration of Helsinki (WMA General Assembly 2000).

Infants and their mothers were recruited from a larger population-based birth cohort study, the Drakenstein Child Lung Health Study, located in a peri-urban area of the Western Cape, South Africa. This population represents a low to middle-income community of

approximately 200,000 people, for a review of the population recruited please see Stein et al. (2015). The mothers were recruited at 20–24 weeks gestation and written informed consent was obtained. Inclusion of mothers and infants to the alcohol exposed group was determined on the basis of a minimum score of 9 for alcohol use on the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST; WHO ASSIST Working Group 2002; Gryczynski et al. 2015). The mothers were also required to confirm their use of alcohol during pregnancy for each trimester at levels consistent with WHO moderate–severe alcohol use (either drinking 2 or more times a week or 2 or more drinks per occasion; Table 2). Infants and their mothers from either group – alcohol exposed or control, were excluded if the mother's urine tested positive for other drugs of abuse or mother reported use of drugs other than alcohol or cigarette smoking (a cotinine score of >500 indicated active smoking; Table 1), if infants were premature <36 weeks old, exhibited low Apgar scores – less than 7 at 5 min, had a history of neonatal complications, or had an identified genetic syndrome from clinical observation.

The infants were scanned in the second to fourth week after birth. On the day of scanning physical attributes were recorded, including: length (cm), weight (kg), and head circumference (cm). The Dubowitz neurological exam, a validated measure of infant neuromotor and neurobehavioural status (Dubowitz et al. 1998) was performed by a qualified neonatal nurse (Table 1). Drug use history was also taken.

MRI scanning of infants and ^{1}H-MRS data collection

All infants were scanned without anaesthesia during natural sleep after they had been fed. During scanning, infants were wrapped in cotton swaddling with earplugs and mini-muffs for ear protection. A pulse oximeter was used to monitor respiration, and a qualified neonate clinician was present with the infant in the scanner room for the duration of the imaging session. Imaging data were acquired on a 3 T Allegra Siemens head-only system using a transmit-receive head coil. To overcome limitations with scanning smaller volumes of tissue, voltage was reduced to optimize signal, and the head coil was loaded with a wet clay inlay (40×40 cm with a thickness of 2 cm, standard sculpting clay commercially bought – white stoneware clay with grog). The wet clay was developed in house and served to absorb extraneous radio signal that was not absorbed by the infant, which served to improve the signal to noise.

Anatomical images were obtained for ^{1}H-MRS voxel placement using a T2-weighted sequence acquired in a sagittal direction (TR = 3500 msec, TE 354 msec, FOV = 160×160 mm, slice thickness =1.0 mm, 128 slices, inplane resolution =1.3×1.3 mm, scan time 6:29 min). The T2-weighted images were reconstructed in 3D to facilitate accurate placement of voxels in left parietal white matter, which was positioned dorsolateral to trigone of the lateral ventricle (PWM; standard placement Fig. 1) and bilateral parietal gray matter. The center of this voxel was the divide of the two hemispheres and included bilateral precuneus and posterior cingulate (PGM; standard placement Fig. 2). ^{1}H-MRS acquisition included a standard PRESS sequence (TE = 30 msec, TR = 2000 msec, 128 averages, delta = −2.6 ppm delta frequency, volume of interest (VOI) $25 \times 25 \times 25$ mm, scan time 4:24 min) and water reference was obtained from the same tissue (2 averages, scan time 0:12 min)).

¹H-MRS data processing

Processing of ¹H-MRS data were performed using the widely validated LCModel software package (Provencher 1993) with basis set for PRESS sequences with a TE = 30 msec. Default parameters (ppmend =0.2 and ppmst =3.85) were used and a Cramér-Rao lower bound of <20 % was determined as the criterion for filtering out low quality spectra. From the 36 infants exposed to alcohol *in utero* – 31 PGM and 17 PWM spectra survived visual inspection and filtering. From 31 control infants – 26 PGM and 20 PWM spectra survived visual inspection and filtering. Relative metabolite concentrations are reported in relation to creatine with phosphocreatine (Cr + PCr) - glutamate with glutamine (Glx), glutamate (Glu), n-acetyl-aspartate with n-acetyl-aspartyl-glutamate (NAA + NAAG), n-acetyl-aspartate (NAA), choline containing metabolites (glycerophosphocholine + phosphocholine (GPc + PCh)), and *myo*-inositol (mI). Absolute concentrations, obtained with use of a water reference, include glutamate with glutamine (Glx), glutamate (Glu), n-acetyl-aspartate with n-acetyl-aspartyl-glutamate (NAA + NAAG), n-acetyl-aspartate (NAA), choline containing metabolites (glycerophosphocholine + phosphocholine (GPc + PCh)), *myo*-inositol (mI), and creatine containing metabolites (phosphocreatine + creatine (PCr + Cr)). Absolute ¹H-MRS metabolites concentrations are reported in mmol/l (Table 3).

Statistical analysis

Statistical analyses were performed using the Statistica version 12 software package (Dell Statistica 2015). Factorial univariate analysis (ANOVA) was used to model group differences (group, sex, group*sex) for each brain area, PGM or PWM, and gender. We were unable to include brain area as a within variable due to the fall-out of either PWM or PGM creating a mismatch of data and the model was compromised. Significant findings were followed by Tukey's post-hoc tests, *p*-values <0.05. We report mean and standard deviation.

Correlation analyses were performed on metabolite data (relative or absolute) that revealed significant difference(s) from ANOVA with clinical measures. We report rho and *p*-values <0.01 as significant.

Results

Physical properties, Dubowitz scores, and maternal smoking

No differences in physical properties were found by group at this young age. No differences were found in outcome from the Dubowitz neurological exam or its subscales by group. No differences were found for the number of active smokers (Table 1). Use of alcohol by the mothers during pregnancy are reported in Table 2.

Parietal white matter ¹H-magnetic resonance spectroscopy and clinical correlates

Parietal white matter metabolite (PWM) concentration for group by gender was found to be different for relative Glu and absolute Glu, which revealed similar differences ($F_{1,33} = 4.64$, $p = 0.04$), where control male infant Glu was higher than alcohol exposed males ($p = 0.02$). In addition absolute Glx was found to differ by group ($F_{1,33} = 4.875$, $p = 0.03$), where alcohol exposed infants reported lower Glx than control infants ($p = 0.02$). No other metabolite differences were found (Table 3). Those metabolites (relative or absolute) that

showed significant differences were assessed for relationships with clinical variables, the following relationships were found. For PWM relative Glu was related to age in days for female infants exposed to alcohol (rho =0.87, $p < 0.01$) and total Dubowitz score for control males (rho =0. 88, $p < 0.01$) No other significant relationships were found.

Parietal gray matter [1]H-magnetic resonance spectroscopy and clinical correlates

Parietal gray matter (PGM) metabolite concentration by gender revealed that relative Glx ($F_{1,53} = 11.05$, $p < 0.01$) and relative Glu ($F_{1,53} = 10.39$, $p < 0.01$) were higher in female infants compared to male infants ($p < 0.01$ for both). (Table 3). No significant relationships were found for relative Glx or Glu with clinical variables.

Discussion

This is the first study to examine variations in glutamate (Glu) and glutamate with glutamine (Glx) in neonates with and without prenatal exposure to alcohol. Our main findings indicate that parietal white matter (PWM) - Glx (absolute concentration) and Glu (ratio and absolute concentration) were decreased in male infants exposed to alcohol. While parietal gray matter (PGM) Glx (ratio) and Glu (ratio) were higher in females when compared to males (Table 3). This study provides support to our hypothesis: decreased glutamate concentrations may serve as a marker of the maturational lag in neonates exposed to alcohol *in utero* – i.e. decreased oligodendrocyte maturation in white matter, a result of poor thyroid hormone signalling. We would suggest following this line of investigation as it may serve as a potential route for early intervention, at least in male neonates that have been exposed to alcohol *in utero*.

As previously mentioned, Glu, is required to signal processes of myelination; achieved by Glu's activation of AMPA/kainate and NMDA receptors on immature oligodendrocytes (Karadottir and Attwell 2006), so too is effective thyroid hormone signalling required for the development and maturation of oligodendrocytes (Valcana et al. 1975; Emery 2010; Fernandez et al. 2004; Franco et al. 2008; Mohacsik et al. 2011). Oligodendrocytes sheath axons to ensure fast and efficient communication between brain regions, which is known to be attenuated in children and adolescents with FASD (Treit et al. 2013; Spottiswoode et al. 2011; Wozniak et al. 2013). These studies therefore implicate that the density of oligodendrocytes in white matter are attenuated in male neonates exposed to alcohol *in utero*.

Thyroid hormone signalling is required for the development and maturation of myelin (Valcana et al. 1975; Emery 2010; Fernandez et al. 2004; Franco et al. 2008; Mohacsik et al. 2011). It has been shown, in basic studies, that *in utero* alcohol affects thyroid hormone signalling – increased thyroid stimulating hormone and decreased thyroxine 4 (Herbstman et al. 2008). Which may be related to decreased feedback from activated α-1 thyroid hormone receptors; as α-1 thyroid hormone receptor mRNA encoding is reduced in rodents exposed to alcohol *in utero* (Scott et al. 1998). A recent human and rabbit study has comprehensively shown that treatment with thyroxine restores myelination and clinical recovery after neonatal intraventricular haemorrhage, by induction of necessary oligodendrocyte transcription

factors (Vose et al. 2013). These data suggest that the reduced Glu found in the current study associated with delayed maturation of oligodendrocytes.

Why did we find this decrease in Glu and Glx only in the male infants exposed to alcohol *in utero*? As mentioned earlier, thyroid hormone signalling is affected in neonates exposed to alcohol *in utero* – increased thyroid stimulating hormone and decreased thyroxine 4, these levels are *further affected in male infants exposed to alcohol in utero* (Herbstman et al. 2008). This supports the proposed relationship between decreased glutamate, decreased thyroxine hormone signalling, and immature oligodendrocytes. Future studies should aim to determine the differential effects of alcohol *in utero* in males and females. Specifically we should address the potential mechanism(s) by which female neonates Glu and Glx concentration are not changed in PWM and why they are greater in PGM. By understanding these mechanism(s) we may be able to provide early intervention therapies to correct this delayed myelination in male neonates exposed to alcohol *in utero*.

Several limitations are apparent in the current study. First there was a loss of data due to movement artefact from infants waking during the scan. Second, the differentiation of brain tissue in neonates is different to that of adults – it is less differentiated, due to the rapid development and brain structures undergoing differentiation, Figs. 1 & 2. Third, large ^{1}H-MRS single voxels were used to improve the signal-to-noise ratio, which affects the homogeneity of tissue within the voxels. Fourth, we did not apply partial voluming correction which accounts for the inhomogeneity of tissue within the voxel. Fifth, we did not address potential chemical shift artefact related to the overlap of metabolite and water scans for each voxel. Sixth, although also a strength of this study, neonates were examined prior to FASD diagnosis and thus were compared on the basis of known alcohol exposure only. While this suggests that Glx and Glu concentrations are an independent marker of prenatal alcohol exposure, we cannot rule out the possibility that some reports of maternal history of alcohol exposure may be less accurate. Lastly, we did not collect thyroid hormone measurements in the current study and much of our discussion as a result is speculative, however the discussion does provide direction to future studies.

In conclusion, we found concentrations of Glx and Glu in parietal white matter to be decreased in male neonates exposed to alcohol *in utero*. We speculate that the decreased Glx and Glu is a result of delayed oligodendrocyte maturation, a result of dysfunctional thyroid hormone activity. Further study is required to elucidate the relationship between Glx and Glu, thyroid hormone activity, oligodendrocyte maturation, particularly in male infants who have been exposed to alcohol in utero, as this may be a suitable window where we can effect positive change in the neurodevelopment of these neonates.

Acknowledgments

Abbreviations

^{1}H-MRS	Proton magnetic resonance spectroscopy
FASD	fetal alcohol spectrum disorders
Glx	glutamate with glutamine concentration
Glu	glutamate concentration
PWM	parietal white matter
PGM	parietal gray matter

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Fig. 1.
Positioning of parietal white matter proton magnetic resonance spectroscopy (^{1}H-MRS) voxel and example spectra, left hemisphere. Peaks that contribute to glutamate (Glu), glutamine (Gln), and glutamate with glutamine (Glx) peaks are indicated in spectra
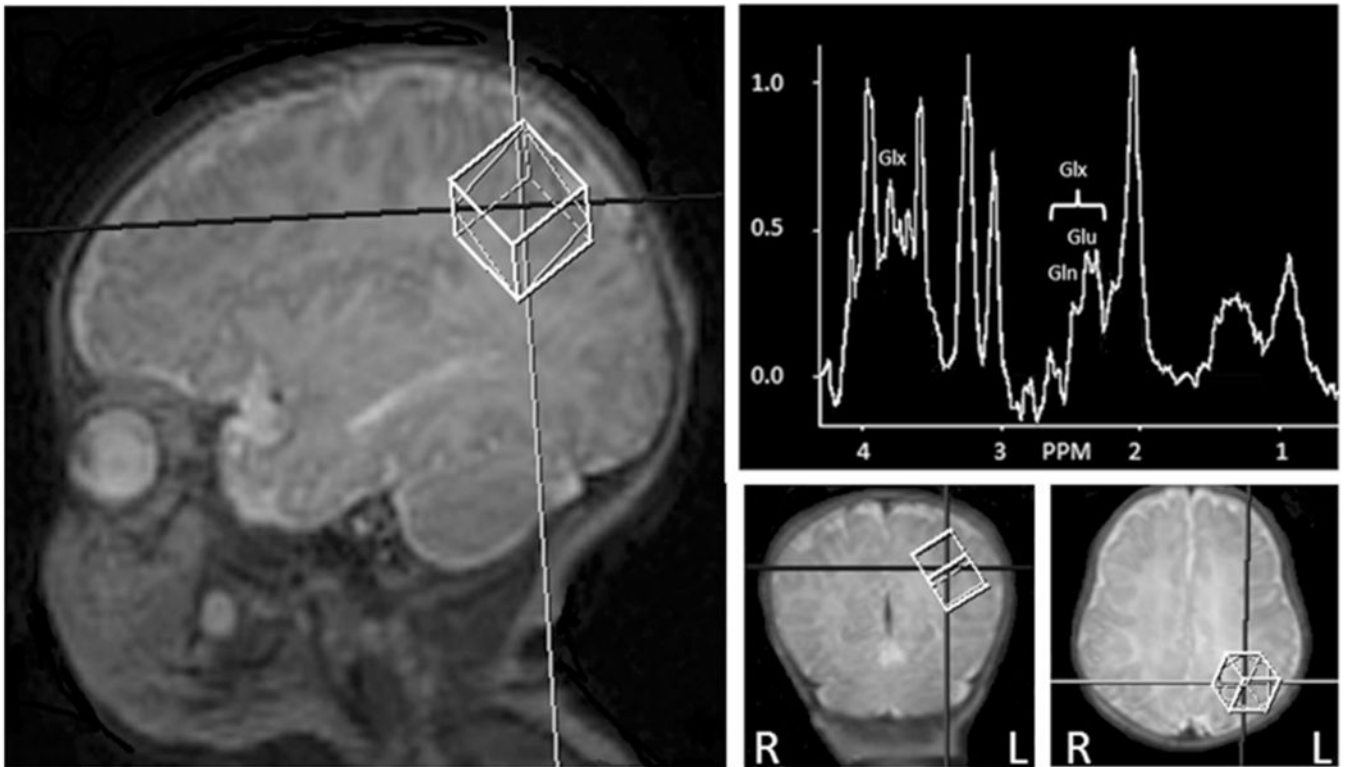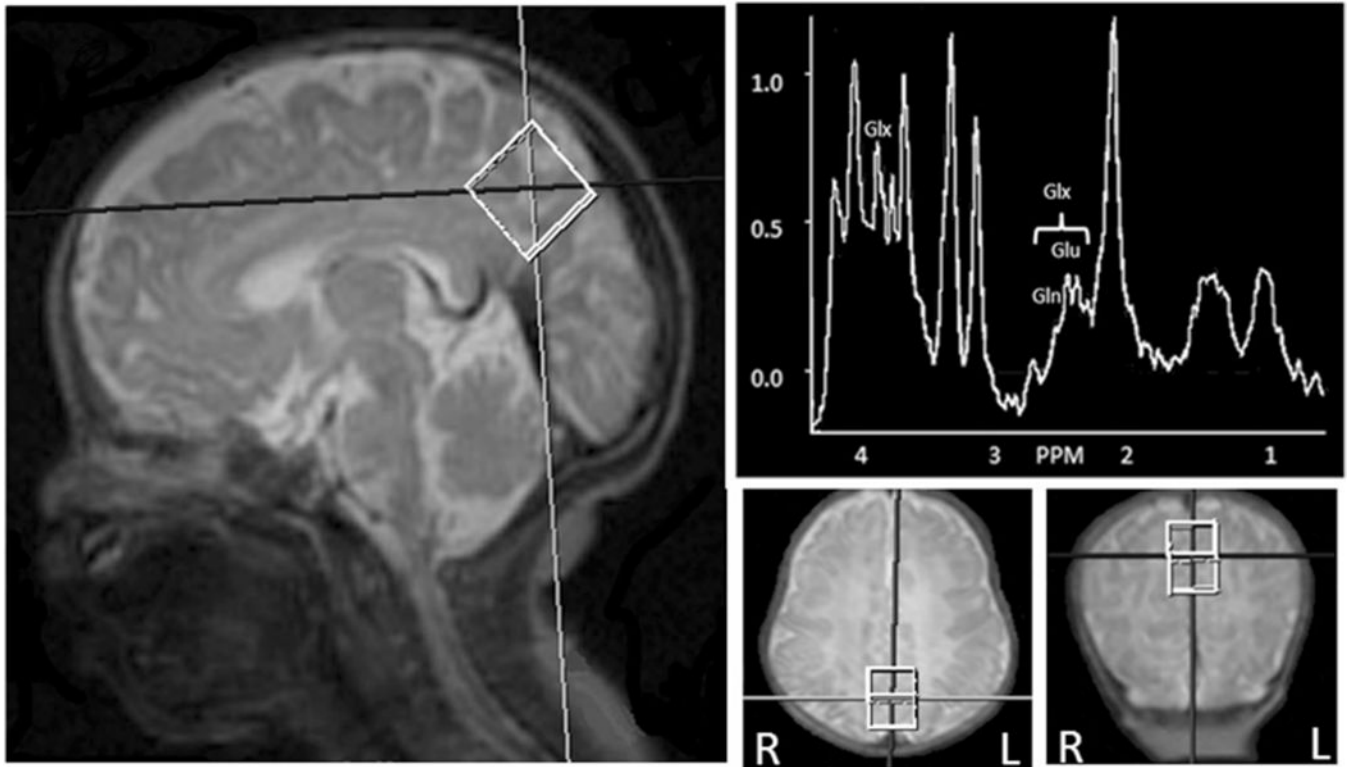
Fig. 2.
Positioning of parietal gray matter proton magnetic resonance spectroscopy (^{1}H-MRS) voxel and example spectra, midline. Peaks that contribute to glutamate (Glu), glutamine (Gln), and glutamate with glutamine (Glx) peaks are indicated in spectra

Table 1

Physical attributes, Dubowitz neurological exam scores, and maternal smoking status

	Alcohol exposed infants (n = 36)			Alcohol exposed female infants (n = 17)		Alcohol exposed male infants (n = 19)		Control infants (n = 31)			Control female infants (n = 10)		Control male infants (n = 21)	
	ave.	std.dev	range	ave.	std.dev	ave.	std.dev	ave.	std.dev	range	ave.	std.dev	ave.	std.dev
Physical attributes														
Age (days old)	20.8	4.8	(12–35)	21.4	5.0	20.2	4.6	20.5	4.8	(11–31)	20.6	5.3	20.5	4.6
Gestational age (weeks)	38.7	1.5	(36–42)	39.0	1.2	38.5	1.7	38.6	1.7	(36–41)	38.2	1.5	38.2	1.8
Length (cm)	50.3	4.2	(45–65)	51.8	4.7	49.0	3.2	50.2	4.6	(38–62)	48.5	4.6	50.9	4.5
Weight (kg)	3.79	0.56	(2.63–5.30)	3.73	0.57	3.84	0.56	3.95	0.8	(2.07–5.66)	3.65	0.49	4.10	0.87
Head circumference (cm)	35.5	1.2	(33.0–37.5)	35.6	1.2	35.6	1.4	36.1	1.7	(31.8–39.0)	35.4	0.9	36.5	1.9
Dubowitz neurological exam														
Tone	8.4	1.8	(3.5–11.5)	8.5	1.7	8.4	1.9	8.7	1.3	(5.0–11.0)	8.0	1.5	9.0	1.1
Reflexes	4.8	0.6	(3.5–6.5)	4.9	0.4	4.7	0.7	4.8	0.6	(3.5–6.5)	4.7	0.7	4.8	0.6
Spontaneous movement	2.0	0.5	(0.5–2.0)	2.0	0.4	2.0	0.0	2.0	0.1	(1.5–2.0)	2.0	0.0	2.0	0.1
Behaviour	4.4	1.2	(1.0–7.0)	4.3	1.6	4.5	0.9	4.1	1.4	(2.0–7.0)	3.8	1.3	4.3	1.4
Abnormal signs	2.8	0.4	(2.0–3.0)	2.8	0.4	2.8	0.4	2.6	0.9	(0.0–3.0)	2.5	0.8	2.6	1.0
Optimality score/outcome	22.4	2.5	(15.0–26.5)	22.2	1.8	22.0	2.6	22.1	2.7	(15.5–26.0)	20.4	2.8	21.9	1.8
Smoking of nicotine cigarettes (continine score > 500)														
non-smoker / active-smoker	18/18							12/19						

No significant group differences were found for infants' physical attributes, Dubowitz neurological exam scores, or maternal smoking behaviour

Table 2

Maternal alcohol drinking during pregnancy

	Trimester 1	Trimester 2	Trimester 3
Alcohol usage (n,%)	30(83)	15(42)	10(28)
Once per week or less	19	11	4
2 to 3 times per week	5	4	6
4 to 5 times per week	1	0	0
Daily	0	0	0
Number of drinks per occasion			
< 2	3	1	0
2 to 3	10	5	2
4 or more	16	9	8

Use of alcohol during the three trimesters for alcohol exposed infants, consistent with WHO moderate–severe alcohol use (either drinking 2 or more times a week or 2 or more drinks per occasion)

Table 3

¹H-Magnetic resonance spectroscopy relative and absolute metabolites

	Alcohol exposed infants		Alcohol exposed female infants		Alcohol exposed male infants		Control infants		Control female infants		Control male infants	
Parietal white matter	(n = 17)		(n = 9)		(n = 8)		(n = 20)		(n = 8)		(n = 12)	
	Mean	Std.Dev.	Mean	Std.Dev.	Mean	Std.Dev.	Mean	Std.Dev.	Mean	Std.Dev.	Mean	Std.Dev.
Relative concentration (relative to creatine with phosphocreatine)												
glutamate + glutamine (Glx)	1.90	0.57	2.02	0.62	1.78	0.52	2.00	0.63	2.09	0.79	1.87	0.21
glutamate (Glu)#	1.36	0.34	1.49	0.39##	1.23	0.23	1.42	0.32	1.49	0.36	1.32	0.21
n-acetyl-aspartate + n-acetyl-aspartyl-glutamate (NAA+NAAG)	1.00	0.17	1.00	0.21	1.01	0.13	1.01	0.14	1.00	0.14	1.04	0.15
n-acetyl-aspartate (NAA)	0.80	0.25	0.81	0.28	0.80	0.24	0.79	0.16	0.79	0.16	0.80	0.16
choline containing metabolites (GPC+PCh)	0.48	0.07	0.49	0.09	0.47	0.05	0.45	0.06	0.45	0.06	0.46	0.06
myo-inositol	1.78	0.41	1.65	0.45	1.91	0.34	1.90	0.31	1.93	0.33	1.87	0.29
Absolute concentration (water reference)												
glutamate + glutamine (Glx) *@	5.03	0.93	5.38	0.89##	4.64	0.85	5.68	0.85	5.77	0.97	5.53	0.66
glutamate (Glu)#	3.79	0.61	4.10	0.67##	3.45	0.30	4.10	0.81	4.16	0.92	4.00	0.66
n-acetyl-aspartate + n-acetyl-aspartyl-glutamate (NAA+NAAG)	2.76	1.10	2.61	1.23	2.93	1.00	3.09	0.76	2.99	0.75	3.24	0.81
n-acetyl-aspartate (NAA)	2.15	1.06	2.09	1.13	2.21	1.04	2.37	0.70	2.43	0.67	2.28	0.78
choline containing metabolites (GPC+PCh)	1.28	0.37	1.23	0.40	1.33	0.36	1.39	0.27	1.35	0.27	1.45	0.28
myo-inositol	5.00	1.71	4.68	1.91	5.37	1.50	5.80	1.31	5.65	1.45	6.03	1.08
creatine containing metabolites (PCr+Cr)	2.81	0.93	2.72	1.06	2.91	0.82	3.10	0.54	2.99	0.55	3.27	0.50
Parietal gray matter	(n = 31)		(n = 15)		(n = 16)		(n = 26)		(n = 8)		(n = 18)	
	Mean	Std.Dev.	Mean	Std.Dev.	Mean	Std.Dev.	Mean	Std.Dev.	Mean	Std.Dev.	Mean	Std.Dev.
Relative concentration (relative to creatine with phosphocreatine)												
glutamate + glutamine (Glx)%	1.89	0.39	1.99	0.37	1.81	0.40	1.84	0.43	2.17	0.48	1.70	0.32
glutamate (Glu)%	1.51	0.25	1.58	0.27	1.45	0.21	1.53	0.27	1.74	0.19	1.43	0.25

	Alcohol exposed infants		Alcohol exposed female infants		Alcohol exposed male infants		Control infants		Control female infants		Control male infants	
Parietal white matter	(n = 17)		(n = 9)		(n = 8)		(n = 20)		(n = 8)		(n = 12)	
	Mean	**Std.Dev.**	**Mean**	**Std.Dev.**	**Mean**	**Std.Dev.**	**Mean**	**Std.Dev.**	**Mean**	**Std.Dev.**	**Mean**	**Std.Dev.**
n-acetyl-aspartate + n-acetyl-aspartyl-glutamate (NAA+NAAG)	1.01	0.13	1.05	0.12	0.98	0.13	0.99	0.12	1.01	0.08	0.97	0.13
n-acetyl-aspartate (NAA)	0.70	0.18	0.75	0.17	0.66	0.18	0.72	0.17	0.69	0.16	0.73	0.17
choline containing metabolites (GPC+PCh)	0.43	0.06	0.44	0.07	0.43	0.05	0.43	0.04	0.45	0.05	0.42	0.03
myo-inositol	2.12	0.32	2.10	0.36	2.14	0.30	2.15	0.40	2.43	0.36	2.03	0.36
Absolute concentration (water reference)												
glutamate + glutamine (Glx)	6.49	1.16	6.55	1.20	6.45	1.17	6.23	1.02	6.16	0.99	6.26	1.05
glutamate (Glu)	5.12	0.69	5.12	0.67	5.12	0.73	5.14	0.71	5.04	0.88	5.19	0.65
n-acetyl-aspartate + n-acetyl-aspartyl-glutamate (NAA+NAAG)	3.45	0.36	3.43	0.23	3.47	0.45	3.40	0.67	2.99	0.75	3.58	0.57
n-acetyl-aspartate (NAA)	2.42	0.66	2.41	0.74	2.44	0.60	2.37	0.87	1.97	0.69	2.55	0.91
choline containing metabolites (GPC+PCh)	1.51	0.23	1.49	0.22	1.52	0.25	1.47	0.26	1.34	0.35	1.53	0.19
myo-inositol	7.28	0.85	7.11	0.90	7.44	0.79	7.24	0.94	7.09	1.16	7.30	0.86
creatine containing metabolites (PCr+Cr)	3.49	0.49	3.45	0.57	3.52	0.42	3.45	0.63	3.01	0.79	3.64	0.44

*
group difference (alcohol exposed infants vs. control infants)

%
sex difference (males vs. females)

#
group with sex difference

@
alcohol exposed infants significantly lower concentrations than control infants

##
alcohol exposed male infants significantly lower concentrations than male control infants

p > 0.05