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GABA and glutamate in the preterm neonatal brain: In-vivo measurement by magnetic resonance spectroscopy

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Abstract

Cognitive and behavioral disabilities in preterm infants, even without obvious brain injury on conventional neuroimaging, underscores a critical need to identify the subtle underlying microstructural and biochemical derangements. The gamma-aminobutyric acid (GABA) and glutamatergic neurotransmitter systems undergo rapid maturation during the crucial late gestation and early postnatal life, and are at-risk of disruption after preterm birth. Animal and human autopsy studies provide the bulk of current understanding since non-invasive specialized proton magnetic resonance spectroscopy (^{1}H-MRS) to measure GABA and glutamate are not routinely available for this vulnerable population due to logistical and technical challenges. We review the specialized ^{1}H-MRS techniques including MEscher-GArwood Point Resolved Spectroscopy (MEGA-PRESS), special challenges and considerations needed for interpretation of acquired data from the developing brain of preterm infants. We summarize the limited in-vivo preterm data, highlight the gaps in knowledge, and discuss future directions for optimal integration of available in-vivo approaches to understand the influence of GABA and glutamate on neurodevelopmental outcomes after preterm birth.

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Declaration of Competing Interest

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Supplementary materials

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1. Introduction

The lifelong burden of neurodevelopmental impairment in survivors of premature birth is a major public health challenge with more than 50% of very preterm infants [born before 32 weeks gestational age (GA)] dependent on complex healthcare, specialized education and social support; and thus limiting quality of life. (Laptook et al., 2005; Hintz et al., 2015; Gire et al., 2018) In fact, even the relatively mature moderate-to-late (32–36 weeks GA) preterm infants, who are usually free of obvious brain injury on conventional neuroimaging, have a two-fold increased risk for cognitive and behavioral impairments compared with term-born infants. (Eeles et al., 2017; van Baar et al., 2009; Cserjesi et al., 2012; Spittle et al., 2017; Johnson et al., 2015) This underscores a critical need to identify the subtle but pervasive microstructural and biochemical derangements in the developing brain prematurely exposed to the extra-uterine environment.

Mid-to-late gestation is a rapid phase of neuronal organization and maturation of the principal neurotransmitter systems involving gamma-amino butyric acid (GABA) and glutamate. (G Xu et al., 2011; Volpe, 2017) The balance between the inhibitory GABA and excitatory glutamate signals are central to pathophysiology of several pediatric and adult neurologic disorders, which occurs more often in individuals born prematurely. (Horder et al., 2018; G. Ende, 2015; Ream and Lehwald, 2018) GABA-ergic signaling undergoes developmentally regulated transitions, (Ben-Ari, 2018; Tyzio et al., 2006; Ben-Ari, 2014) which in conjunction with synchronized glutamatergic system maturation, is crucial for normal brain development. Animal and human autopsy studies have reported microstructural and functional disruption of GABA and glutamatergic system following preterm birth which may explain the neurodevelopmental impairments even in absence of major injury on conventional neuroimaging.

Non-invasive neuroimaging using head ultrasound or magnetic resonance imaging (MRI) allow for longitudinal assessment of structural brain growth, but advanced tools are needed to specifically assess the development of the GABA and glutamatergic system. Proton magnetic resonance spectroscopy (^{1}H-MRS) allows in-vivo measurement of neurometabolites; however, reliable measurement of GABA and glutamate concentrations in preterm infants is hindered by challenges as reviewed further. (G Xu et al., 2011; GE Dwyer et al., 2018; Mullins et al., 2014) First, we summarize the neurophysiologic significance of the GABA and glutamatergic system and its developmental timeline during late gestation and early postnatal period based largely on animal or ex-vivo human studies. Then, we will review the ^{1}H-MRS methods used in adult studies, the technical challenges in their application in preterm infants, and the limited data from studies investigating the developing preterm brain. Finally, we discuss special considerations for interpretation of the available data, gaps in current knowledge and future directions to integrate in-vivo tools for a comprehensive understanding of the GABA and glutamate systems in the preterm brain.

2. GABA and glutamate in the developing brain: ex-vivo data

2.1. The critical role of GABA and glutamate in brain development

GABA and glutamate, the principal inhibitory and excitatory neurotransmitters respectively in the mature human brain, act via ionotropic ($GABA_{A,}$ AMPA and NMDA) and metabotropic (G-coupled $GABA_B$ and glutamate) receptors. (Volpe, 2017; Wu and Sun, 2015; Petroff, 2002) In the developing brain, in addition to neurotransmission, they play a crucial role in neuronal migration, differentiation, dendrite and synapse formation and organization of neural circuits. (G Xu et al., 2011; Volpe, 2017; Huang et al., 2007; Cellot and Cherubini, 2013; Gaiarsa et al., 2011) GABA and glutamate system maturation involves developmentally synchronized neuronal migration and organization, perinatal ion-channel transitions, synapse and dendrite maturation, followed by pruning extending through the early childhood and adolescence. (G Xu et al., 2011; Ben-Ari, 2014; Wu and Sun, 2015; Huang et al., 2007; Ben-Ari et al., 2012; Kilb, 2012; Demarque et al., 2002; Smyser et al., 2010; Represa and Ben-Ari, 2005)

GABA and glutamate are located in cytoplasmic, vesicular (synaptic) and extracellular pools, each with distinct roles and pathophysiologic relevance. (Volpe, 2017; Petroff, 2002) The cytoplasmic pool primarily plays metabolic role; whereas vesicular neurotransmitters are found in high concentrations within the pre-synaptic boutons and mediates synaptic neurotransmission. (Wu and Sun, 2015; Petroff, 2002; Kilb, 2012; Martin and Rimvall, 1993; Belelli et al., 2009; Maddock et al., 2016; Ito, 2016) There is significant turnover and cycling among the pools with the extrasynaptic pool contributing upto 70% of the total brain tissue GABA. (Petroff, 2002) During early development, a substantial part of GABAergic transmission may occur via non-synaptic processes. (Demarque et al., 2002) In fact, the tonic $GABA_A$-mediated membrane depolarization provides the first excitatory drive necessary for promoting neurite outgrowth and synapse formation as well as chemotropic action on cell migration. (Cellot and Cherubini, 2013; Demarque et al., 2002; Demarque et al., 2002; Demarque et al., 2002) GABA plays tonic, neuromodulatory role in cortical inhibition, via free extracellular GABA acting on extra-synaptic $GABA_A$ receptors. (Belelli et al., 2009) Further, GABA exerts metabotropic action via $GABA_B$ receptors, contributing to cell survival, migration, differentiation, as well as to the maturation and plasticity of developing synaptic connections. (Gaiarsa et al., 2011) Interestingly, the presynaptic $GABA_B$ mediated control of transmitter release develops early and regulates neuronal growth before the conventional post synaptic $GABA_B$ mediated inhibition is established; (Gaiarsa et al., 2011) emphasizing the importance of developmental timeline and its potential disruption after preterm birth.

GABA and glutamate metabolic pathways are interconnected with glutamine being a common precursor involving cyclical turnover within synthetic, re-uptake and breakdown pathways and regulated transport across neurons and glial cells, suggesting multiple levels of intertwined neuromodulatory roles. (Petroff, 2002) Further, GABA-mediated large membrane depolarizations have been shown to facilitate glutamatergic signaling as well as regulate the formation of glutamatergic synapses in the developing cortex in vivo with contribution of NMDA receptors. (Ito, 2016; Mohajerani and Cherubini, 2006; Kasyanov

et al., 2004) This emphasizes the importance of their simultaneous investigation in the developing brain.

2.2. Late gestation and early postnatal timeline of GABA and glutamate system micro-structural development

Glutamatergic and a majority of GABAergic neurons originate in the fetal dorsal telencephalic zone and migrate along radial glia fibers early in gestation to reach the developing cortical subplate and eventually neocortex by third trimester. (G Xu et al., 2011; Rakic and Sidman, 1970; Rymar and Sadikot, 2007; Letinic et al., 2002) This synchronized neuronal migration declines by term age and is completed by 6 postnatal months; (G Xu et al., 2011) in parallel with increasing GABAergic neuronal density in the cortex during late gestation and reaching a peak at term age. Hence in the event of preterm birth during the 2nd trimester, some neurons may still be migrating through the white matter; as some GABA-ergic neurons have been reported in the cerebral white matter as late as 31 gestational weeks. (Rakic and Sidman, 1970) From midgestation to infancy, the $GABA_A$ receptor and glutamic acid decarboxylase enzyme (GAD65 and GAD67, synthesizing synaptic and cytoplasmic GABA respectively) measures increase in the cortical middle laminae; in line with the striking increase in thickness of layers I-III after 30 weeks gestation; likely reflecting expansion by migrated GABAergic interneurons. (Volpe, 2017; Rakic and Sidman, 1970) A similar increase in glutamate levels during late gestational period, followed by a 25% decline due to apoptosis and then a gradual increase into young adulthood has been described in mouse parietal cortex. (Benitez-Diaz et al., 2003) The GABA-ergic interneurons provide most of the early regulatory signals modulating neuronal growth esp glutamatergic pyramidal cell growth and organization in the developing cortex. (Chen and Kriegstein, 2015; Khazipov et al., 2001; Tyzio et al., 1999) The last trimester of human gestation and early postnatal life is also a period of rapid axonal development, oligodendrocyte proliferation, dendritogenesis, and synapse formation; (Volpe, 2017; Rymar and Sadikot, 2007) which remain is at risk of disruption after preterm birth.

2.3. Preterm birth and postnatal exposures alter GABA and glutamatergic pathway

McClendon et al. reported marked abnormalities in medium spiny projection neurons in the caudate nucleus of preterm sheep after *in-utero* global cerebral hypoxia ischemia. (McClendon et al., 2014) Shaw et al. demonstrated decreased GAD and lower mRNA levels of $\alpha 6$ and δ subunits of $GABA_A$ receptors in the cerebellum of preterm guinea pigs. (Shaw et al., 2018; JC Shaw et al., 2015) Malik et al. observed suppressed glutamatergic neurogenesis in preterm rabbits pups. (Malik et al., 2013) In an ex-vivo human autopsy study, Robinson et al. (Robinson et al., 2006) reported loss of caudate nucleus GABAergic neuron expression in preterm neonates' brains with white matter lesions. Several medications including opioids, sedatives and caffeine are administered uring postnatal intensive care and are known to influence GABA and glutamatergic systems as well as impact neurodevelopmental outcomes. (Steinhorn et al., 2015; Isokawa, 2016; Ruangkittisakul et al., 2015; Wikstrom et al., 2018; Duerden et al., 2016; Schuurmans et al., 2015)

2.4. Perinatal GABA-ergic signal transition: neuroprotective implications following premature birth

$GABA_A$ receptors are ligand-gated chloride ion (Cl^-) channels which on activation, allow the passage of Cl^- ions dependent on the neuronal transmembrane Cl^- gradient. In the mature brain, intraneuronal Cl^- levels are maintained at a low level by the KCC2 co-transporter channels (and other mechanisms) by extruding Cl^- into the extracellular space; (Farrant and Kaila, 2007; Rivera et al., 1999) and $GABA_A$ receptor activation allows intracellular Cl^- influx, resulting in hyperpolarization and its inhibitory effect. (Farrant and Kaila, 2007) Ex-vivo studies on the immature mice brain indicate that a reverse Cl^- ion gradient (intracellular Cl^- high) is maintained by the NKCC1 transporter and low expression of the KCC2 co-transporter; (Ben-Ari, 2014; Ben-Ari et al., 2012; Ben-Ari et al., 2007), allowing Cl^- efflux after $GABA_A$ receptors activation and resulting in a depolarizing response instead. In fact, this reversed GABA signal has also been observed during pathologic states like epilepsy and traumatic brain injury; (Ben-Ari et al., 2012) and may be relevant in the preterm brain, where the extra-uterine hypoxic-ischemic stress may alter neurosignalling and further disrupt brain development.

The perinatal transition of Cl^- channels is mediated by the oxytocin surge during labor, (Ben-Ari, 2018; Tyzio et al., 2006) and may underlie the neuroprotective anti-apoptotic effect of vaginal delivery, compared with Ceasarean delivery. (Castillo-Ruiz et al., 2018) Further, Shaw et al. reported increase in mRNA level of $GABA_A$ receptor subunits during the fetal-to-neonatal transition following term birth; but not following preterm birth. (JC Shaw et al., 2015) $GABA_A$ receptors have binding sites for the neuro-steroid allopregnanolone receptor, and may mediate its neuroprotective role in reducing anoxic injury and neuronal stress during labor. (Ben-Ari, 2018; Tyzio et al., 2006) Erythropoietin, a promising neuroprotective agent, upregulates KCC2 chloride channels after birth following prenatal hypoxia-ischemia. (Jantzie et al., 2014)

2.5. Glutamate receptor transitions, role in apoptosis, and excitotoxic neuronal injury following perinatal hypoxia-ischemia

Glutamate is expressed early during brain development, its receptors undergo developmentally regulated transitions and plays essential role in activity-dependent plasticity and synaptic refinement. (Demarque et al., 2002; McDonald and Johnston, 1990; Liguz-Lecznar et al., 2015; Desfeux et al., 2010; Lujan et al., 2005) Both glutamatergic receptor activation (NMDA) and antagonism have been implicated in augmenting excitotoxic and apoptotic neuronal injury respectively. (McDonald and Johnston, 1990; Kaindl and Ikonomidou, 2007) In fact, NMDA receptor mediated excitotoxic injury is known to play a central role in hypoxic-ischemic brain injury in term neonates and may also play a critical role before, during and after preterm birth which often is associated with intermittent hypoxia-ischemia. (Johnston et al., 2001; Low et al., 2003)

Thus, perinatal stress may alter these developmentally regulated transitions especially in absence of labor before preterm birth and/or may unleash potentially excitotoxic events following perinatal hypoxiaischemia which remains undetected by conventional neuroimaging, but predispose to cognitive deficits.

3. Advanced neuroimaging: in-vivo tools for investigating GABA and glutamate systems

MRI allows non-invasive assessment of structural brain growth and its alterations after premature birth. Longitudinal volumetric and morphometric MRIs of human fetal brain show a 4-fold increase in cortical volume and a dramatic increase in surface gyrification between 28 and 40 weeks of post-menstrual age (PMA), which parallels the GABA and glutamatergic neuronal migration and cortical organization observed in ex-vivo studies. Standard ^{1}H-MRS enables measurement of neurometabolites like N-acetyl-aspartate (NAA), choline (Cho) creatine (Cr), lactate (Lac) and inositol (Ins) in the preterm brain, and their alterations have been associated with neurodevelopmental outcomes. (Card et al., 2013) However, these dominant metabolite signals overlap the smaller signals from GABA and glutamate necessitating specialized techniques for reliable measurement. (Fig. 1, Mullins et al., 2014, GE Dwyer et al., 2018, Kreis et al., 2002)

3.1. Technical challenges with in-vivo quantification of GABA and glutamate by ^{1}H-MRS

Glutamate concentrations in the adult human brain range from 3 to 10 mM. Specialized techniques, including higher magnetic fields, echo-time (TE)-averaging and ultra-short Stimulated Echo Acquisition Mode (STEAM) sequence, have been used for optimal resolution of the glutamate signal. (GE Dwyer et al., 2018; Ramadan et al., 2013; Tkac et al., 2001) Nonetheless, on lower magnetic field strengths (1.5–3 T), the glutamate signal is overlapped by glutamine and therefore most publications report the composite glutamate + glutamine (Glx) signal at 2.2–2.5 ppm. (Ramadan et al., 2013) However, measuring in-vivo GABA has been more challenging due to lower concentrations (1–2 mM), which are overlapped by signals from creatine at 3 ppm and NAA at 2.2 and 1.9 ppm on the 1.5–3 T ^{1}H-MRS (Fig 1a). (Mullins et al., 2014; Mikkelsen et al., 2017; AD Harris et al., 2017; Chang et al., 2003) Ultrahigh magnetic field strength (7–9 T) scanners help increase the spectral resolution and measure GABA concentrations in animal studies. (I Tkac et al., 2003; J Ramu et al., 2016) For human studies using clinical (3 T) scanners, specialized ^{1}H-MRS acquisitions with shorter echo-time or spectral editing techniques have been used. (Mullins et al., 2014; GE Dwyer et al., 2018; Shungu et al., 2016; G. Ende, 2015) Among spectral editing techniques, MEscher-GArwood Point Resolved Spectroscopy (MEGA-PRESS) and MEGA-sLASER have been used in adult human studies, in which two sets of spectra are acquired with editing frequency pulses at 1.9 ppm (ON) and 7.5 ppm (OFF). The ON and OFF spectra are then subtracted to remove overlapping signals and unmask the J-coupled signals from GABA (Fig. 1b). (Mullins et al., 2014; GE Dwyer et al., 2018; Puts et al., 2018) Nonetheless, contamination of GABA signal at 3 ppm by the co-edited macromolecule (MM) signal remains a major challenge. Techniques to address MM contamination, include acquiring a metabolite-nulled MM spectrum and using it as prior knowledge during analysis and acquiring MM suppressed J-difference data; and have their own drawbacks like increasing acquisition time or reducing GABA signal. Hence, the convention is to report the GABA signal acquired using J-difference method as GABA+ indicating MM contribution. (Mullins et al., 2014)

We performed a literature search for English language publications in Pubmed, MEDLINE and Google Scholar using combinations of the key words 'GABA, glutamate, magnetic resonance spectroscopy, newborn, neonate and premature'. We reviewed in detail all manuscripts reporting GABA (and/or glutamate) measurements using j-edited ^{1}H-MRS on infants less than 6 months age including preterm infants. We have briefly reviewed several studies that acquired PRESS sequence on a 1.5 T scanner and reported glutamate measurements from preterm or term newborns suffering from cerebral hypoxia. Outside of the newborn/preterm studies, we have summarized some of the peer-reviewed articles describing age and maturational aspects of GABA and/or glutamate ^{1}H-MRS measurements from the animal, pediatric and adult human populations. More thorough review of ^{1}H-MRS studies in older children and adults have been published recently. (GE Dwyer et al., 2018; Porges et al., 2020; AD Harris et al., 2017)

4. In-vivo ^{1}H-MRS measurement of GABA and glutamate in the developing animal brain

Ultra-high magnetic field (7–9 T) ^{1}H-MRS under general anesthesia has been used to measure GABA and glutamate concentrations in the developing rat brain (Table 1). Tkac et al. (I Tkac et al., 2003) reported longitudinal metabolite profile of rat brain between 7 and 28 postnatal days (PND), corresponding to early human brain development from 34 weeks of gestation to 2–3 years after birth. (BD Semple et al., 2013) Ultrashort echo-time STEAM sequences from voxels placed in the hippocampus, the striatum, and the cerebral cortex demonstrated increasing glutamate and glutamine concentrations across all regions. The ratio of Glu/Gln peaked between PND 14 and 21, with regional variations. GABA concentrations increased from PND 7 to peak around PND 14, and then declining slightly by PND 28.

Using 7T PRESS sequences, Morgan et al. (JJ Morgan et al., 2013) reported increasing Glu+Gln- concentrations in the rat forebrain between the neonatal stage (P7) to young adulthood (P60) and Ramu et al. (J Ramu et al., 2016) reported slight increase in Glu- levels and stable GABA levels between PND 14 and 35 in the left dorsal hippocampus and anterior cingulate cortex of rat brain. With the caveat of anaesthetized scans and developmentally more complex human brain, animal data suggests a consistent increase in brain glutamate concentrations from late gestation, through the neonatal stage into adolescence and reaching a plateau in adulthood; whereas GABA concentrations remain either stable or increase slightly during the early stages of brain development. (BD Semple et al., 2013; Clancy et al., 2007)

5. In-vivo ^{1}H-MRS studies measuring GABA and glutamate in human brain

5.1. Studies in healthy children and adults

^{1}H-MRS studies involving healthy children and adults have reported age, gender and regional differences in the brain GABA and glutamate concentrations (Table 2). Holmes et al. (MJ Holmes et al., 2017) reported increase in glutamate concentrations with advancing age in the mid-frontal gray matter, peri-trigonal white matter and right basal ganglia of

children between 5 and 10 years of age. Saleh et al. (Saleh et al., 2020) recently reported a positive correlation between age and GABA+ concentrations in the frontal voxel of healthy children (5–14 years). However, Maes et al. (J Ramu et al., 2016) did not observe an age dependent change in macromolecule-suppressed GABA concentrations in 7–14 year old healthy children, and they speculated whether the early increase in GABA+ levels could be driven mostly by changes in co-edited macromolecules.

Shimizu et al. reported lower Glu/Cr levels in frontal and occipital cortices of young adults (18–33 years) compared to children (4–13 years). (Shimizu et al., 2017) O'Gorman et al. (O'Gorman et al., 2011) observed no age-dependent frontal and parietal GABA or Glu-concentration changes in healthy adults between 25 and 38 years age; with higher GABA and Glu-levels in males compared to females. Gao et al. (Gao et al., 2013) reported a declining GABA and Glx/Cr level in healthy adults between 20 and 76 years of age; with more rapid decline in GABA+ levels in the frontal region of women than men. A positive correlation of cognition with frontal GABA concentrations (EC Porges et al., 2017) and a decline in regional GABA concentrations with advancing age has been reported in healthy elderly population. (EC Porges et al., 2017; Maes et al., 2018) In a pre-print meta-analysis, Porges et al. have integrated data from 8 published reports that include subjects between the ages of 8 years to the 8th decade of life, to show a lifespan brain GABA trajectory in which an early period of rapid increase is followed by a period of stability during early adulthood, and a gradual decrease during adulthood and aging. (Porges et al., 2020)

Grewal et al. reported lower GABA concentrations in the frontal compared to the parietal and occipital cortices, but no interhemispheric variations. (Grewal et al., 2016) Consistently, van der Veen et al. (van der Veen and Shen, 2013) reported higher GABA/Cr ratio in the occipital cortex compared with medial prefrontal cortex. Choi et al. (IY Choi et al., 2006) reported 8-fold higher GABA concentrations and 5-fold higher GABA/Cr ratio in the gray matter compared with white matter in healthy adults.

Functional role of the measured GABA and glutamate levels have been investigated by several studies. (van der Veen and Shen, 2013; IY Choi et al., 2006; Hermans et al., 2018; Harada et al., 2011) Hermans et al. (Hermans et al., 2018) employed edited ^{1}H-MRS and transcranial magnetic stimulation (TMS) in the left sensorimotor cortex and observed that older adults (63–74 years) had reduced GABA mediated short and long-interval intracortical inhibition. Acute increase in GABA concentrations (Coxon et al., 2018) and glutamate/Cr ratios following exercise, (Maddock et al., 2016) association with default mode network deactivation during working memory task (Chen et al., 2018) and correlation of GABA and glutamate concentrations with cognitive function (EC Porges et al., 2017; Huang et al., 2017) emphasizes their relevance in executive task performance. The importance of GABA and glutamate in cognitive impairment with normal aging is further highlighted by the findings in adults with neurologic disorders.

5.2. Studies in subjects with neurologic disorders

Altered GABA and glutamate concentrations have been reported in pediatric and adult disorders, including epilepsy, autism, attention-deficit disorder and schizophrenia. (Horder et al., 2018; MJ Holmes et al., 2017; Bluml et al., 2013; G. Ende, 2015; Blum and

Mann, 2002) A meta-analysis of 40 ^{1}H-MRS studies reported lower brain GABA levels in subjects with depression and autism spectrum disorders (Schur et al., 2016) Ende et al. (G. Ende, 2015; Ende et al., 2016) have reviewed the relationship between in-vivo GABA and glutamate and adult neuropsychological conditions. Impulsivity scores were positively correlated with Glu, and negatively correlated with GABA concentrations in adolescents. (Murphy et al., 2012; Hayes et al., 2014) Improved selective motor inhibition was associated with increased glutamate levels in the premotor cortex of children with Tourette Syndrome, (Mahone et al., 2018) emphasizing the importance of their role in behavior regulation. Infact, in a randomized trial of bumetanide treatment in children with autism spectrum disorder, treatment was associated with a reduction in GABA/Glx ratio and symptom improvement. (Zhang et al., 2020) Interestingly, neuro-behavior disorders including autism are more common in survivors of prematurity; which emphasizes the importance of in vivo investigation of GABA and glutamate changes during early postnatal life of preterm infants.

5.3. Challenges in GABA and glutamate quantification by ^{1}H-MRS in neonatal/reterm populations

The standard 1.5 T clinical neonatal MRIs have lower signal-to-noise ratios (SNR) and spectral resolution from a limited voxel volume due to relatively small brain volume in the newborn (even smaller in preterm infants). (Mullins et al., 2014; Kreis et al., 2002; Grewal et al., 2016; Tanifuji et al., 2017; Kwon et al., 2014) The J-difference acquisitions for MEGA-PRESS to resolve GABA and glutamate peaks are time consuming and very susceptible to motion artifacts, a major limitation of non-sedated scans in neonates. Using sedation for research MRI studies is not only ethically questionable, but may also alter brain GABA and glutamate metabolism and spectroscopic signals. Motion correction strategies employed in adult studies include retrospective correction using navigators sequences to correct for frequency and phase variations due to motion. (Andronesi et al., 2020) However, prospective motion correction using navigator sequences require the use of additional interleaved pulse sequence, and are not widely used in neonatal acquisitions with additional disadvantage of increased specific absorption rate and longer acquisition time in these vulnerable preterm babies. Smaller preterm infants need an MRI-compatible transport incubator for thermoregulation, a ventilator for respiratory support, cardiorespiratory monitoring and intravenous infusions during the MRI scan, which emphasizes the importance of nursing expertise for close monitoring as well as feed and bundle approach to minimize motion artifacts.

Adult studies use tissue correction strategies usually assuming negligible metabolite contribution by CSF and a relatively higher concentration of GABA and glutamate in gray (2-fold) compared to white matter. (EC Porges et al., 2017; Harris et al., 2015) However in the preterm brain, these assumptions may not be universally applicable given the temporal changes in the sub-cortical plate, neuronal migration through the white matter, ill-defined gray-white matter delineation and metabolic and functional maturation of GABA and glutamatergic neurons. Additionally, evolving sulcation and gyration patterns, increasing cortical layer thickness, larger CSF to parenchymal volume proportions and relatively small regional brain volumes of the neonatal brain add challenges in application of adult (or even pediatric) anatomical segmentation atlases. Ideally, age specific brain atlases

(Makropoulos et al., 2018) should be used along with implementation of tissue correction and motion correction strategies. (Andronesi et al., 2020; Quadrelli et al., 2016) Despite these challenges affecting the spectral quality, investigations including preterm infants are of utmost importance for understanding this crucial phase of brain development.

5.4. Studies in preterm and term infants

Till date, only a few studies have reported either GABA and/or glutamate in-vivo concentrations in the preterm brain during their early postnatal life. Kreis et al. (Kreis et al., 2002) in 2002, reported in-vivo glutamate and GABA concentrations in nine preterm infants (mean GA of 32.7 ± 1.6 weeks) who were longitudinally scanned at PMA 32–35 weeks and at 39–43 weeks (TEA); and 12 full term infants who were scanned once at 38–42 weeks PMA. Non-sedated short-TE ^{1}H-MRS were acquired on a 1.5 T scanner (TE=20 ms, TR=3000 ms, 128 acquisitions, bandwidth=1953 Hz) from voxels placed over the thalamus, paramedian occipital gray matter and centrum semiovale white matter. They observed significant increases in glutamate concentrations with advancing GA in preterm infants from 3.8 ± 2.0 mM/kg at <35 weeks PMA to 4.4 ± 1.2 mM/kg at TEA (p<0.05). However, glutamate concentrations were similar between full-term infants and preterm infants at TEA (4.6 ± 1.3 vs 4.4 ± 1.2 mM/kg, p = NS). The term infants had higher concentrations of glutamate in the thalamus compared with gray and white matter voxels (p<0.0001). The authors acknowledged limited resolution for the GABA measurements, which did not show significant age trends (0.1 ± 0.5 vs 0.1 ± 0.2 mM/kg, p = NS) or regional variations. Several reports from term infants with cerebral hypoxia-ischemia have reported Glx/Cho or Glx/NAA ratios using 1.5 T ^{1}H-MRS PRESS (TE=30 ms) but did not find significant association with outcomes or diseased state. (Holshouser et al., 1997; Roelants-Van Rijn et al., 2001) Bluml et al. reported trends in Glx levels in 5 brain regions of 309 children between 0 and 18 years age based on 1.5 T ^{1}H-MRS PRESS (TE = 35 ms) and reported that Glx levels peaked at approximately 4–6 months and then declined or reached a plateau by 1–2 years in cortical gray and subcortical white matter, with continued increase throughout the first 2 years in the deep nuclear gray matter. (Bluml et al., 2013)

Koob et al. reported in-vivo Glx/H_2O measures from 16 preterm (GA 29.1 ± 2 weeks) and 16 term infants scanned at PMA 39.2 ± 1 and 39.8 ± 1 weeks respectively. (Koob et al., 2016) They used a 1.5T MR scanner to acquire PRESS sequence (TE=30 ms, TR=1500 ms, 256 averages and TE=135 ms, TR=1500 ms, 278 averages) from a 4.5 cm^3 voxel centered at the level of the white matter underlying the central sulcus within the centrum semiovale. 1H-MRS spectra were acquired with water saturation with the chemical shift-selective module at both TEs. An additional spectrum was acquired at a TE of 30 ms without water saturation. At a TE of 30 ms, Glx/H2O tended to be lower in preterm cohort (−20 %), but relationship with GA or PMA at scan was not found to be statistically significant.

Kwon et al. (Kwon et al., 2014) performed ^{1}H-MRS at term corrected age in 20 preterm (birth weight <1500 gs) and 25 healthy term control newborns (born between 37 and 41 weeks' PMA). Using a 3T MRI scanner, GABA-edited MEGA-PRESS spectra were acquired from a 2 × 3 × 3 cm^3 voxel (TR=1500 ms, TE=68 ms, flip angle=90°, bandwidth=1200 Hz) localized over the right frontal region of the brain. They reported

that the preterm infants had lower regional GABA (0.013 ± 0.004 vs 0.016 ± 0.004 units/mm^3, $p = 0.049$) and glutamate (0.031 ± 0.0008 vs 0.042 ± 0.01 units/mm^3, $p = 0.005$) concentrations compared with term infants. Interestingly, preterm infants showed a positive correlation ($r = 0.735$, $p = 0.01$) between GABA concentrations and functional resting state intrinsic connectivity distribution in the right Broadman Areas BA9, BA44 and BA6, while term controls demonstrated a negative correlation (r=−0.564, $p = 0.02$). These frontal regions are involved in language and executive function and its uncertain whether the observed differences are related to the cognitive and behavioral impairments in surviving preterm infants.

Tomiyasu et al. (Tomiyasu et al., 2017) measured in-vivo GABA levels using 3T MEGA-PRESS (TE=69 ms, TR=1500 ms, bandwidth=1200 Hz, 128 signal averages) in the basal ganglia and cerebellum of 38 neonates of which 28 were born between 23 and 36 weeks GA (scanned between 35 and 41 weeks PMA); and the remaining 10 were term infants (scanned between 38 and 43 weeks PMA). The neonatal scans were performed under thiopental sedation and the measured basal ganglia and cerebellar GABA+/water ratios were significantly lower ($p < 0.001$) than 12 control children (range 6–16 years). Similarly, GABA+/Cr ratios were significantly lower in the basal ganglia ($p = 0.001$), but were higher in the cerebellum ($p = 0.006$) of neonates compared to the control children; which was speculated by authors to be due to lower Cr concentrations in the neonatal cerebellum. The normalized GABA+ levels were significantly lower in neonates than in children in both regions, regardless of the approach employed ($p < 0.01$). Comparisons between preterm and term neonates were not reported and therefore the effect of prematurity remained unclear.

Tanifuji et al. (Tanifuji et al., 2017) performed ^{1}H-MRS at 37–46 week PMA (period A) and 64–73 week PMA (period B) in 20 preterm infants (GA range 24–34 weeks). GABA was measured using the edited MEGA-PRESS sequence (TE=68 ms, TR=1500 ms, 256 signal averages) while Glx was measured by the unedited PRESS sequence. They reported that right basal ganglia GABA concentrations remained unchanged between 1.06 (1.0–1.4) at period A to 1.13 (0.93–1.55) at period B (p=NS); whereas Glx demonstrated significant increase from 3.7 (3.54–4.7) to 6.7 (5.9–7.3), (p<0.01) during this period. GABA/Cr ratio decreased significantly at period B ($p = 0.03$), but there was no significant difference in GABA/Cho ratios ($p = 0.58$). Both Glx/Cr and Glx/Cho ratios were significantly increased (p<0.01) at period B. The authors speculated that the stable GABA and GABA/Cho levels indicate unchanged levels between period A and B; and the observed decrease in the GABA/Cr ratio may be driven by more rapidly increasing Cr levels with brain growth.

We recently reported early postnatal profiles of in vivo GABA and glutamate concentrations in the developing right frontal brain of 38 very preterm infants without structural brain injury [median GA of 28.0 (IQR 26.0, 28.9) weeks; 19 males (50%)]. (SK Basu et al., 2020) Non-sedated ^{1}H-MRS MEGA-PRESS sequences (TE=68 ms, TR=2000 ms, 256 signal averages) were acquired at a median postmenstrual age of 38.4 (range 33.4–46.4) weeks on a 3T scanner from a 20 mm × 15 mm × 15 mm (2.7 cm^3 average volume) voxel placed in the right frontal lobe mostly encompassing sub-cortical white matter. GABA+ concentrations were measured from the J-difference spectra whereas glutamate and Glx measured from the unedited PRESS (OFF) spectra. We observed that with advancing post-

menstrual age, the concentrations of glutamate increased significantly (β=0.22, p = 0.02) whereas those of GABA+ did not. Advancing postnatal age at the time of imaging positively correlated with GABA+ (β=0.06, p = 0.02) and glutamate OFF (β=0.11, p = 0.02) and Glx OFF (β= 0.12, p = 0.04), perhaps indicating postnatal activation. Male infants had higher GABA+ (1.66±0.07 vs. 1.33±0.11, p = 0.01) concentrations compared with female infants. Glutamate concentrations measured from the J-difference spectra moderately correlated with measurements from OFF spectra (Spearman correlation r = 0.54, p = 0.02); whereas Glx measurements had low correlation.

Maria et al., recently used HERMES (Hadamard Encoding and Reconstruction of MEGA-Edited Spec- troscopy) sequence on a 3T scanner to simultaneously measure concentrations of GABA, Glx and glutathione (GSH) in the brain of 18 'healthy' neonates born between 29 – 41 weeks GA and scanned at a median 41 (39–47) weeks PMA. (Maria et al., 2021) They placed relatively larger ~ 15 cm^3 voxels (anterior cingulate 31.25 × 25 × 20 mm^3 with ~70% gray matter and left thalamus 25 × 25 × 25 mm^3 with ~ 40% gray matter) and acquired sequences at TE = 80 ms, TR=2000 ms, 2 kHz receiver bandwidth, 2048 data points, 320 averages, 90°excitation/180°refocusing pulses and 20 ms editing pulses at 1.9 ppm for GABA and 4.56 ppm for GSH, with VAPOR water suppression, with excitation frequency set at 3 ppm. Prior to performing tissue correction, they reported higher GABA+ and Glx concentrations in the thalamus, which was not observed after performing tissue correction (assuming equal metabolite concentrations in gray and white matter).

6. Gaps in knowledge and future directions

Inferences drawn from above information need to be considered with the understanding of the heterogeneity between imaging techniques, animal and more mature human studies, regional differences in neuronal activity as well as level of maturation at diiferent ages.

6.1. Special considerations with ^{1}H-MRS GABA and glutamate measurements

6.1.1. What are we measuring?—In vivo ^{1}H-MRS measures the total concentration of GABA or glutamate within a localized brain tissue voxel; and cannot distinguish amongst the cytoplasmic, vesicular and extracellular pools. While it is presumed that the vesicular GABA or glutamate is mostly invisible to the ^{1}H-MRS, recent reports have debated this. (Wang et al., 2013) Additionally, GABA from some of these pools may be more tightly bound to macromolecules than others, making variable co-edited contribution to the "total measured " concentrations. Studies reporting association of behavior and MRS-derived GABA, (Hermans et al., 2018; CJ Stagg et al., 2011; Kolasinski et al., 2019; CJ Stagg et al., 2011; Floyer-Lea et al., 2006) indicate correlation with the neurotransmitter and neuromodulator GABA pool. However, in the developing brain the extra-synaptic GABA mediates the early neurosignalling and developmental regulation; and may substantially contribute to the ^{1}H-MRS GABA signal. It remains unclear if the observed differences in ^{1}H-MRS measured GABA and glutamate concentrations reflect a global change or a selective change in a specific pool and/or changes in co-edited macromolecules. (Bell et al., 2021) This emphasizes the need of interpreting ^{1}H-MRS data in conjunction with other modes of in vivo investigation like connectivity, electrophysiological studies as well as

ex-vivo data using immunehistochemical staining to correlate functional and anatomical identification.

6.1.2. Can we simultaneously measure GABA and glutamate concentrations?—

Given the strong metabolic, functional and developmental relationship between GABA and glutamate, their simultaneous measurement is essential in understanding both healthy and pathophysiologic processes in the developing brain. MEGA-PRESS yields most reliable measurements for GABA; (GE Dwyer et al., 2018) whereas TE-averaged PRESS (J resolved PRESS), unedited short TE PRESS or STEAM sequences are used more conventionally for glutamate and glutamine measurements. (Ramadan et al., 2013; Yang et al., 2008; C Choi et al., 2006; Hancu, 2009; Maddock et al., 2018) Recent studies have reported simultaneous measurements from MEGA-PRESS sequences, reporting GABA from the J-edited and glutamate (and/or Glx) from both the J-edited as well as the more conventional unedited PRESS with fair correlation. (O'Gorman et al., 2011; Gao et al., 2013; Maddock et al., 2018; AD Harris et al., 2017; SK Basu et al., 2020; Maria et al., 2021)

6.1.3. Are measured GABA and glutamate concentrations related to functional imaging parameters?—

Functional connectivity measured by blood-oxygen-level-dependent contrast amplitudes provides a non-invasive assessment of the development and activation status of the neural circuits, which is functionally dependent on neurotransmitter balance predominantly GABA and glutamate. In adults, studies report correlations between GABA and glutamate concentrations and connectivity parameters that vary with age, paired brain regions being investigated for connectivity, and state of health or disease. (Donahue et al., 2010; Duncan et al., 2014; Duncan et al., 2019; Gonen et al., 2020; Gao et al., 2018) For example, seed-based functional connectivity measures of anterior cingulate cortex negatively correlated with GABA concentrations and a positive correlation with glutamate concentrations in healthy controls but not in individuals with neurologic disease. (Shukla et al., 2019) In a report in preterm infants, Kwon et al. reported a positive correlation between frontal GABA concentrations and intrinsic functional connectivity at term corrected age; whereas term-born infants demonstrated an inverse correlation. (Kwon et al., 2014) Prospective longitudinal studies with simultaneous fMRI and ^{1}H-MRS measurements in this population are needed to investigate whether this divergent correlation is related to their postnatal age, stage of maturation and most importantly, neurocognitive outcomes.

6.2. Inferences based on existing data from the developing brain

The trajectory of GABA concentrations from early childhood to late aging has been described by Porges et al. (Porges et al., 2020) Keeping the above considerations in mind, we combine the neonatal data from animal and human studies and draw the following inferences:

- GABA concentrations demonstrate a stable to slight upward trajectory during the early developmental phase (late gestation-early postnatal life) followed by a declining trend after 3–6 postnatal months. While this may indicate synaptogenesis and functional maturation followed by neuronal pruning after the first few months of life, different pools contributing to the overall measured

GABA+ may have distinct trajectories. We can speculate that the overall GABA+ trajectory includes increasing intra-cellular and synaptic GABA with a decreasing extra-cellular GABA with maturation; further superimposed by the age-dependent increase in co-edited macromolecules. The observed decline in GABA/Cr ratio during infancy could be driven by more rapidly increasing Cr concentrations with brain growth.

- Glutamate concentrations increase with advancing age during late gestation to early infancy and beyond into adolescence. This finding is consistent across animal as well as neonatal and adult human studies and likely reflects an increase in both the intra-synaptic neurotransmitter component as well as the more ubiquitous extra-synaptic glutamate along with glutamine (measured as Glx) in cellular metabolic cycles. Since the absolute number of glutamatergic neurons decrease after birth due to maturational pruning, this continuing uptrend indicates higher levels of intraneuronal glutamate (and glutamine) in mature neurons.
- Regional variations in GABA and glutamate concentrations have been reported in adult literature, with lower GABA concentrations in the frontal cortex compared to parietal and occipital cortex. This is more pertinent for neonates since phylogenetically ancient caudal and midline structures mature earlier than the frontal-prefrontal regions, while premature exposure to ‘unnatural’ extra-uterine stimulus (e.g. pain, noise, light) after preterm birth may alter development as demonstrated by resting-state connectivity studies. (De Asis-Cruz et al., 2020; De Asis-Cruz et al., 2015) GABA and glutamate concentrations seem to be symmetric with little interhemispheric differences in adult brain (no neonatal data).
- Gray matter GABA and glutamate concentrations are higher than white matter concentrations in the adult brain and accordingly a correction factor of 0.5 is used for tissue fraction adjustment. (Harris et al., 2015) In absence of specific data from the developing neonatal brain, we speculate that cortical gray matter GABA concentrations increase through late gestation and early postnatal life as GABA-ergic neurons reach the sub-plate and undergo further organizational and metabolic maturation after birth. Due to this ongoing transition of neurons from white matter to sub-cortical plate to the layered neocortex, tissue correction strategies need to include age-specific correction factors for neonatal data especially when acquired before term-equivalent age.
- Preterm infants have lower GABA and glutamate concentrations compared with age-corrected term infants. Further investigation is needed to explore whether this can serve as an early bio-marker of delayed/impaired overall development even in absence of structural brain injury.
- The divergent relationship of GABA with resting state connectivity in preterm versus term infants reported in one study (Kwon et al., 2014) is intriguing. It remains uncertain whether it is related to GABA signaling shifts, migration

and maturational changes as well as altered connectivity in preterm infants and further, its value as an early biomarker to predict developmental outcomes.

These speculative inferences need prospective longitudinal in vivo investigation and confirmation in the preterm developing brain with long term follow-up for neurodevelopmental outcomes.

6.3. Future directions for ^{1}H-MRS GABA and glutamate measurements in neonatal population

6.3.1. Consensus recommendations for neonatal GABA-editing acquisitions

—Recent consensus expert recommendations have been published regarding ^{1}H-MRS acquisition parameters, J-difference editing, ^{1}H-MRS reporting standards, motion correction and partial tissue correction based on investigations in the adult human brain. (AD Harris et al., 2017; Andronesi et al., 2020; Choi et al., 2020; Lin et al., 2021; Wilson et al., 2019) While it seems logical to use the same parameters for neonatal studies, they need to be rationalized for feasibility in neonatal populations due to the special circumstances discussed earlier. For example, since the basal ganglia or thalamus volume is only 4–8 cm^3 in term neonates (smaller in the preterm infant), (Loh et al., 2017) a larger voxel acquisition might yield better quality spectrum, but at the expense of regional specificity. This is specifically pertinent to the neonatal brain where there is no consensus on the ratio of gray vs white matter levels of GABA or glutamate, with tissue correction strategies assuming equal gray and white matter contributions, effectively correcting only for the CSF. Similarly, longer sequences acquiring higher averages would improve SNR and spectral fit, but at the expense of longer scan duration, which in turn increases potential for motion artifact and adverse events in a high-risk critically ill population dependent on intensive care. Perhaps, the goal should be to acquire spectra from the largest possible voxel that fits in the specific region of interest and for the shortest duration that would yield sufficient signal averages for acceptable spectral quality. Additional considerations include developing age specific brain atlases, (Makropoulos et al., 2018; Glasser et al., 2016) alongwith consensus recommendations for tissue and motion correction strategies specific to newborns, adapted from existing adult literature. (Andronesi et al., 2020; Quadrelli et al., 2016)

6.3.2. Are GABA and glutamate concentrations related to neurodevelopmental outcomes in premature infants?—

To date, there are no reports linking neurodevelopmental outcomes in preterm infants with in vivo GABA and glutamate concentrations. Ex-vivo infant autopsies indicate lower GABA and glutamate concentrations in the gray matter of preterm infants compared with term infants. Drawing parallels from the observation that lower GABA and glutamate concentrations are associated normal adult aging, lower cognition and neuropsychiatric conditions, it supports the hypothesis of similar association with neurodevelopmental impairments in preterm infants that needs verification by prospective studies. (EC Porges et al., 2017; Huang et al., 2017)

6.3.3. Can we integrate in vivo modalities like ^{1}H-MRS, resting state connectivity and electroencephalography for comprehensive assessment of the GABAergic system function?—

Electrical brain activity detected by electroencephalography (EEG), allows in vivo assessment of neurosignalling and has been

reported to qualitatively and quantitatively evolve with advancing age and maturation in the preterm infants and is associated with neurodevelopmental outcomes. (Pavlidis et al., 2017; Arora et al., 2018; Huning et al., 2018) Slow endogenous activity transients are mostly recorded in prenatal and preterm developing brain and typically diminish with advancing age, in parallel with functional maturation of GABAergic neurotransmission. (Vanhatalo et al., 2005) Since electrocortical signals recorded by EEG are dependent on GABA and glutamate (and other) neurotransmitter function, it may help understand their bidirectional relationship with clinical events (e.g., hypoxia, hypotension) and administered medications (sedatives, opioids, caffeine) known to influence these pathways. (Malk et al., 2014) Simultaneous EEG with ^{1}H-MRS and MR resting state connectivity may further elaborate the influence of changing GABA and glutamate concentrations on the electrocortical maturation of the developing brain. Investigation of neonatal population as part of the Human Connectome Project will facilitate understanding the timeline of normative development of connectivity and thus may facilitate detecting disruptions following injury after premature birth. (Glasser et al., 2016; Smith et al., 2013)

Transcranial magnetic stimulation (TMS) is used to selectively stimulate the cortical GABA-ergic interneurons and to monitor its effect on function of postsynaptic fast acting ionotropic ($GABA_A$) and slower acting metabotropic ($GABA_B$) receptors by measuring the GABA induced short and long interval intracortical inhibition, respectively on EEG. (Reis et al., 2008; Hermans et al., 2018) TMS studies are currently limited to adult populations and its applications have been reviewed by Reis et al. (Reis et al., 2008) An alternative to single-voxel ^{1}H-MRS is the chemical shift imaging (CSI or MRSI) approach which provides a visual representation of the metabolic milieu of a brain slice. However, this approach has not, to our knowledge, been used in the neonatal population, and may need to overcome challenges such as spatial coverage around excitation edges, scalp lipid contamination and increased motion artifact contamination due to longer scan times. Integrated use of available in vivo modalities for future studies in preterm neonates may fill the existing gaps in knowledge.

Summary

GABA and glutamate play a vital role in fetal and neonatal brain development. Their developmental timeline becomes more pertinent in preterm infants whose developing brains are prematurely exposed to the extra uterine stress and predisposed to neurological disorders, perhaps in part as sequelae of early derangements in GABA and glutamate systems. Despite the technical challenges, neonatal ^{1}H-MRS measurements of GABA and glutamate provide crucial information that needs integrated interpretation with MRI-based functional connectivity and EEG for comprehensive in-vivo assessment of postnatal neurophysiology and mechanisms of injury after preterm birth. Prospective investigation of the relationship of in vivo GABA and glutamate measurements with neurodevelopmental outcomes and clinical correlates may serve as an early neuroimaging outcome or biomarker for future neuroprotective trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability statement

This is a literature review article and only refers to previously published data and articles.

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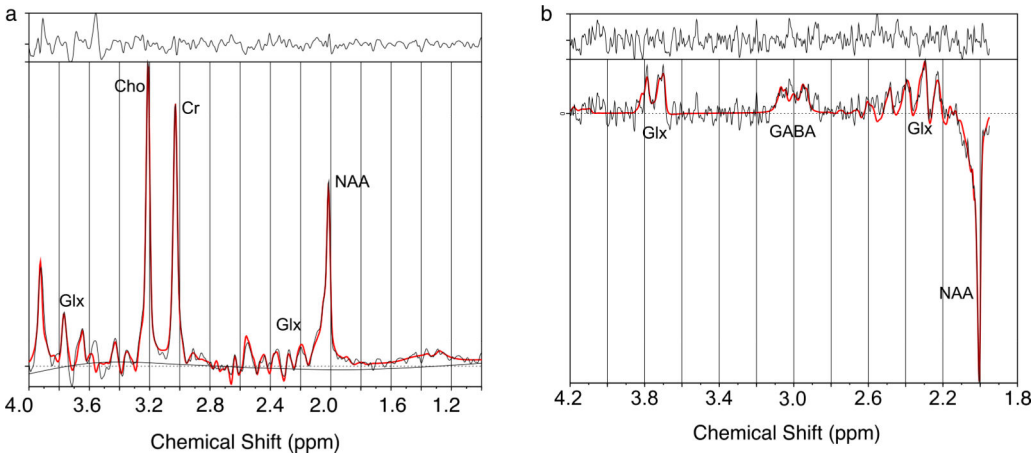

Fig. 1.

Representative ^{1}H-MRS spectrum on (a) standard PRESS and (b) MEGA-PRESS on 3T showing distinct signal peaks for GABA and Glx.[1]

[1]Representative spectra acquired from a preterm infant at term corrected age from a voxel centered over the right basal ganglia (17 × 27 × 10 mm^3). The spectrum was acquired on a 3T scanner with TE=68 ms, TR=2000 ms, NSA = 256 and the editing pulses at 1.9 ppm and 7.5 ppm.

Table 1

Summary of ^{1}H-MRS studies measuring GABA and glutamate in the developing animal brain.

Study	^{1}H-MRS field strength and sequence	Subjects and Study design	Brain voxel region	GABA findings	Glutamate findings
Tkac et al., 2003. (I Tkac et al., 2003)	9.4T, Ultrashort echo-time STEAM (TE = 2 ms)	8 rat pups, ^{1}H-MRS on PND 7, 10, 14, 21, and 28	Hippocampus, the striatum, and the cerebral cortex	GABA (range 0.7–1.1 μmol/g); ↑ from PND 7 to peak at PND 14 and then slightly ↓ by PND 28	Glutamate ↑ (> 0.10 μmol g^{-1} $day^{-1,}$ from 3.2 to 3.5 to 8.7–11.2 μmol/g) between PND 14 to 28. Glu/Gln-ratio ↑ from 2.0 to 4.5 by PND 21
Morgan et al., 2014. (JJ Morgan et al., 2013)	7T, PRESS (TE = 20 ms, TR = 1250–8000 ms with T1 relaxation correction)	9 male rats, ^{1}H-MRS on PND 7, 35, and 60	Forebrain, centered on the striatum	NA	Glx ↑ between P7 (5.38 ± 0.77 μmol/g) to P35 (8.96 ± 0.79 μmol/g) and further at P60 (10.4 ± 0.86 μmol/g)
Ramu et al., 2016. (J Ramu et al., 2016)	7T, PRESS (TE = 8 ms)	10 male rats, ^{1}H-MRS on PND 14–70	Anterior cingulate cortex and the left dorsal hippocampus	GABA levels remained stable in hippocampus (2.06 μmol/g, + 0.002 μmol/g/day) and the anterior cingulate cortex (2.27 μmol/g, − 0.005 μmol/g/day).	Glutamate levels ↑ (8.4–8.8 μmol/g, + 0.031 μmol/g/day) in both regions. Gln-levels marginally ↑ (2.3–3.1 μmol/g, + 0.01 μmol/g/day)
Raman et al., 2005. (Raman et al., 2005)	9.4T, ultra-short echo-time STEAM (TE = 2 ms, TM = 20 ms, TR = 5 s)	23 rat pups exposed to chronic hypoxia (10% O2 from PND 3 to P28), 14 normoxic controls	Left hippocampus	GABA levels remained stable longitudinally, ↑ in hypoxic rat pups	Gln-levels ↑ longitudinally, whereas Glu/Gln-showed slight ↓ between P 21 and P28.Glu/Gln-ratio ↑, whereas Gln-levels were ↓ in hypoxic rats
Traudt et al., 2012. (Traudt et al., 2012)	9.4 T, ultrashort echo-time single-voxel STEAM sequence (TE = 2 ms, TR = 5 s)	Twice daily 2 mg/kg morphine (n = 25) or normal saline (n = 20) from PND 3 to 7; ^{1}H-MRS on PND 8	Left hippocampus	↓GABA (–28%) and GAD enzyme levels in morphine group	Glu, Gln-levels were unchanged

Table 2

Summary of ^{1}H-MRS studies measuring GABA and glutamate in the mature human brain.

Study	^{1}H-MRS field strength and sequence	Subjects and Study design	Brain voxel region	GABA findings	Glutamate findings
Holmes et al., 2017. (MJ Holmes et al., 2017)	3T, PRESS (TR = 2000 ms, TE = 30 ms, 64 averages, 2:16 min duration)	64 children 5–10 years (29 were exposed to HIV, uninfected)	Mid-frontal gray matter, peri-trigonal white matter and right basal ganglia (1.5 × 1.5 × 1.5 cm^3 voxel)	NA	Glutamate ↑ (0.27 ± 0.06) with age in all regions. No sex-age or HIV-age interactions noted
Maes et al., 2017. (Maes et al., 2018)	3T, MEGA-PRESS (TE = 68 ms; TR = 2000 ms; 320 averages; 2048 points, bandwidth 2000 Hz,11 min duration)	85 young (18–35 years) and 85 older (60–75 years) adults	Left sensorimotor cortex and the midline occipital cortex (3 × 3 × 3 cm3). Voxel tissue correction performed	Unadjusted GABA ↓ in older adults. Adjusted for voxel CSF and white/ gray matter, age differences were not significant	NA
Gao et al., 2013. (Gao et al., 2013)	3T, MEGA-PRESS (TR = 2000 ms, TE = 68 ms, bandwidth 1000 Hz, 320 signal averages)	49 men and 51 women (20–76 years)	Frontal and parietal cortex. Voxel size: 3 × 3 × 3 cm3	GABA+/Cr and GABA+ /NAA were ↑ in frontal region. Concentrations ↓ with age ($r = -0.6$ with 4–5% decline per decade). ↓ in GABA levels with age was more rapid in women	Glx/Cr ratios demonstrated significant ↓ with age ($r = -0.4$). No gender effect in regional or age-related changes
O'Gorman et al., 2011. (O'Gorman et al., 2011)	3T, MEGA-PRESS (TR = 1800 ms, TE = 68 ms, 320 signal averages, 10 min duration)	14 healthy adults (7 female), age range 25–38 years	Dorsolateral prefrontal cortex. 30 ml voxel (25 × 40 × 30 mm3)	GABA concentrations were ↑ in males. No age effect noted	Glu-and Glx concentrations were ↑ in males; no age effect and no gender effect on Gln
Grewal et al., 2016. (Grewal et al., 2016)	3T, MEGA-PRESS (TR = 2000 ms; TE = 68 ms; bandwidth 2000 Hz; 2048 samples; 320 signal averages)	21 young adults (20–29 years)	Frontal, parietal and occipital cortex; both hemispheres. 15.6 cc voxel (2.5 × 2.5 × 2.5 cm3)	Frontal cortex levels ↓ (2.09 ± 0.5 mM) than others (2.8 ± 0.7 mM), but no interhemispheric differences. GABA with Cr as internal reference	NA
Huang et al., 2016. (Huang et al., 2017)	3T, MEGA-PRESS (TR = 2000 ms, TE = 68 ms, 64 averages, bandwidth 1000 Hz, duration 4:24 min)	Normal adults: 17 young and 15 elderly; 17 adults with Alzheimer's ds and 21 with mild cognitive impairment	Anterior cingulate cortex (ACC; 40 × 40 × 25 mm3) and right hippocampus (rHP; 40 × 20 × 20 mm3)	GABA+/Cr ratios were ↓ in elderly adults compared with young adults	Glx/Cr ratios were ↓ in elderly adults; with further ↓ in ACC in Alzheimers' disease and hippocampus in adults with cognitive impairment.
Van der Veen et al., 2013. (van der Veen and Shen, 2013)	3T, MEGA-PRESS (TR = 1500 ms, TE = 68 ms, 1024 acquisitions, 256 averages). Editing pulse bandwidth range 2.0–0.6 ppm	18 adult volunteers (22–57 years old, 13 male)	Occipital cortex and medial prefrontal cortex. Voxel 3 × 3 × 2 cm 3	GABA/Cr ratio was 16% ↑ in the occipital cortex (0.1103 ± 0.005) compared with medial prefrontal cortex (0.095 ± 0.004)	No regional difference in Glx.
Choi et al. (IY Choi et al., 2006)	3T MR GABA CSI with STEMS	13 healthy adult subjects	Gray vs White matter using CSI	GABA concentration in gray (1.30 ± 0.36 μmol/g) and white matter (0.16 ± 0.16 μmol/g). GABA/Cr ratios were ↑ in gray matter (0.16 ± 0.04) compared with white matter (0.03 ± 0.03).	
Hermans et al. (Hermans et al., 2018)	3T MEGA-PRESS	28 older (63–74 years) and 28 young adults (19–34		GABA levels did not differ between age groups.	

Study	^{1}H-MRS field strength and sequence	Subjects and Study design	Brain voxel region	GABA findings	Glutamate findings
		years); transcranial magnetic stimulation			
Harada et al., 2011. (Harada et al., 2011)	3T, MEGA-PRESS (TR = 1500 ms, TE = 68 ms, 256 signal averages	15 healthy young adults (avg 22 years). 8 men were scanned 1 week apart to test reproducibility; 7 women were scanned in follicular and luteal phase of menstrual cycle	Lentiform Nuclei (LN), left frontal lobe, or anterior cingulate Cortex (AC). Voxel size 3 × 3 × 3 cm^3	GABA level in LN (1.37 ± 0.34 mM) was the highest, and AC (0.79 ± 0.21 mM) was the lowest. Good reproducibility (0.72 correlation) in men; GABA levels were ↑ during follicular phase in women	NA

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