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Glutamate homeostasis in the adult rat prefrontal cortex is altered by cortical docosahexaenoic acid accrual during adolescence: An *in vivo* ^{1}H MRS study

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ABSTRACT

Major psychiatric disorders are associated with dysregulated glutamate homeostasis and deficits in the omega-3 fatty acid docosahexaenoic acid (DHA). This study determined the effects of dietary-induced alterations in brain DHA accrual on cortical glutamate homeostasis in the adult rat brain. Adolescent rats were fed a control diet (n = 20), a n-3 fatty acid-deficient diet (DEF, n = 20), or a fish oil-fortified diet containing preformed DHA (FO, n = 20). In adulthood ^{1}H MRS scans were performed with voxels in the prefrontal cortex (PFC) and thalamus. Compared with controls, erythrocyte, PFC, and thalamus DHA levels were significantly lower in DEF rats and significantly higher in FO rats. In the PFC, but not the thalamus, glutamate was significantly elevated in DEF rats compared with controls and FO rats. Glutamine did not differ between groups and the glutamine/glutamate ratio was lower in DEF rats. No differences were observed for markers of excitotoxicity (NAA, GFAP), or astrocyte glutamate transporter (GLAST, GLT-1) or glutamine synthetase expression. Across diet groups, PFC DHA levels were inversely correlated with PFC glutamate levels and positively correlated with GLAST expression. Together these findings demonstrate that rat cortical DHA accrual during adolescence impacts glutamate homeostasis in the adult PFC.

1. Introduction

Converging evidence indicates that mood and psychotic disorders are associated with abnormalities in glutamate neurotransmission. Postmortem brain studies suggest that patients with psychiatric disorders exhibit altered glutamate transporter and *N*-methyl-D-aspartate (NMDA) receptor subunit expression (Beneyto et al., 2008; Feyissa et al., 2009; Matute et al., 2005; Rao et al., 2012; Uezato et al., 2009) and glutamate concentrations (Hashimoto et al., 2007). The non-competitive glutamate NMDA receptor antagonist ketamine has psychotogenic effects (Lahti et al., 1995; Malhotra et al., 1997), as well as rapid antidepressant effects (McGirr et al., 2015), and increases cortical glutamine in healthy human subjects (Rowland et al., 2005) and glutamate in the rat PFC (Kim et al., 2011). Meta-analyses of ^{1}H magnetic resonance spectroscopy (^{1}H MRS) studies indicate that schizophrenia (Merritt et al., 2016) and bipolar disorder (Gigante et al., 2012) are associated with elevated, and major depressive disorder (MDD)(Yildiz-Yesiloglu and Ankerst, 2006) with lower, glutamate and/or glutamine levels in cortical and subcortical brain regions. While these findings suggest that a dysregulation in glutamate homeostasis is associated with the pathophysiology of mood and psychotic disorders, little is known about pathogenic mechanisms and associated risk factors.

A separate body of translational evidence has identified a deficiency in omega-3 polyunsaturated fatty acids (n-3 PUFA), including docosahexaenoic acid (DHA), as a potential neurodevelopmental risk factor for psychiatric disorders (McNamara et al., 2015; Tesei et al., 2017). Meta-analyses indicate that bipolar I disorder (McNamara and Welge, 2016), MDD (Lin et al., 2010), and schizophrenia (van der Kemp et al., 2012) are associated with erythrocyte (red blood cell) membrane DHA deficits. DHA is the most abundant n-3 PUFA in the mammalian brain, and erythrocyte and frontal cortex DHA levels are positively correlated in adult humans (Carver et al., 2001). DHA increases rapidly in the human frontal cortex (Carver et al., 2001) and rat brain (Green et al., 1999) during active periods of synaptic maturation, and emerging evidence suggests that DHA is required for the structural and functional maturation of glutamatergic synapses (Cao et al., 2009; Moreira et al., 2010; Yoshida et al., 1997). Furthermore, astrocytes play a key role in maintaining glutamate homeostasis (Hertz and Zielke, 2004), and DHA is required for optimal astrocyte maturation (Champeil-Potokar et al., 2004, 2006; Joardar et al., 2006) and modulates astrocyte glutamate

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transporter activity and expression (Champeil-Potokar et al., 2015; Berry et al., 2005; Grintal et al., 2009; Harbeby et al., 2012). However, the effects of altering DHA levels on glutamate homeostasis in brain have not been systematically evaluated *in vivo* using ^{1}H MRS.

The present study investigated the effects of alterations in rat brain DHA accrual during adolescent development on *in vivo* glutamate and glutamine levels in the young adult PFC and thalamus using ^{1}H MRS at 7 T. Three distinct groups of rats were generated with high, medium (control), and low blood and regional brain DHA levels. Following scanning, postmortem erythrocyte, PFC, and thalamus DHA levels, and astrocyte glutamate transporters GLAST (EAAT1) and GLT-1 (EAAT2), glutamine synthetase (GS), and glial fibrillary acidic protein (GFAP) expression were determined. Based on the translational evidence reviewed above, our specific prediction was that blood and brain DHA levels would be inversely correlated with glutamate and glutamine levels.

2. Methods

2.1. Animals and diets

Post-weaning (P20) male Long-Evans hooded rats from different nulliparous dams were purchased (Harlan Farms, Indianapolis, IN), and randomized to one of three diets (n = 20/diet group) from P21 until young adulthood (P90). Control (CON) rats were maintained on an α-linolenic acid (ALA, 18:3*n*-3)-fortified diet (TD.04285, Harlan-TEKLAD, Madison, WI). Deficient (DEF) rats were maintained on an ALA-free diet (TD.04286), and *n*-3 PUFA enriched rats were maintained on diet containing 1.1% fish oil in place of ALA (FO, TD.110837, Harlan-TEKLAD, Madison, WI). Diets were closely matched for all non-fat nutrients and fatty acid composition with the exception of ALA, which was absent from the DEF and FO diets, and DHA and eicosapentaenoic acid (EPA, 20:5*n*-3) which were present in the FO diet but not the CON and DEF diets (**Supplemental** Table 1). Rats were housed 2 per cage with food and water available *ad libitum*, and were maintained under standard vivarium conditions on a 12:12 h light:dark cycle. All experimental procedures were approved by the University of Cincinnati and Children's Hospital Institutional Animal Care and Use Committees, and adhere to the guidelines set by the National Institutes of Health.

2.2. ^{1}H MRS

Adult (P90) male rats were anesthetized with 2.5–3.5% isoflurane in air, positioned supine with their teeth in a bite bar, and scanned in a 7T Bruker Biospec system (Bruker BioSpin, Ettlingen, Germany)(Fig. 1**A**). Respiration was monitored and body temperature was maintained at 36–38 °C using an animal monitoring system (SAI Inc., Stony Brook, NY). The head was centered inside a 38 mm Litz coil (Doty Scientific, Inc., Columbia, SC), and a set of localizers from each orthogonal plane were collected. Following acquisition of these localizers, fast spin echo (RARE) images (effective TE 45 ms, TR 3300 ms, RARE factor 16, matrix 256 × 256, FOV 35 mm, 20 slices in the axial direction and 10 slices in the sagittal direction) were collected for voxel placement. Voxels were placed in the bilateral mPFC (3 mm × 3 mm × 3 mm, 256 averages, Fig. 1**C,D**) and thalamus (6 mm × 4 mm × 4 mm, 128 averages, Fig. 1**E,F**). The voxel was shimmed using FASTMAP to an average line width of 10.5 Hz. Water suppressed data were acquired using VAriable Pulse Power and Optimized Relaxation delays (VAPOR) water suppression with a 120 Hz bandwidth followed by Point RESolved Spectroscopy (PRESS) with Outer Volume Suppression (OVS) localization with a TE of 20 ms, TR of 2500 ms, 2048 points, and a spectral width of 3301 Hz. A ^{1}H MRS spectrum acquired from the rat mPFC is illustrated in Fig. 1**B**. Unsuppressed data were acquired by turning the VAPOR RF pulses off and acquiring 4 averages. The spectra were imported into LCModel for quantitation, using the unsuppressed acquisition for phasing, eddy-current correction, and an internal water reference. A provided simulated basis set was used to fit the spectra to obtain peak areas which were scaled to the water area (assumed concentration 45 mM) to provide concentration estimates. These estimates were not corrected for metabolite relaxation times and are reported in institutional units (IU). Estimates were retained if the Cramer-Rao lower bound (CRLB) reported by LCModel was less than 25%. Primary measures of interest were glutamate, glutamine, glutamate + glutamine (Glx), and the glutamine/glutamate ratio. A secondary measure of interest was *N*-acetyl aspartate (NAA) which served as an index of excitotoxicity (Roffman et al., 2000).

2.3. Tissue collection

Immediately following scanning, isoflurane-anesthetized rats were sacrificed by decapitation. Whole venous trunk blood was collected into EDTA-coated tubes, and centrifuged at 4 °C for 20 min (1500 × *g*). Plasma and buffy coat were then removed and erythrocytes washed 3 times with 0.9% NaCl and stored at −80 °C. The brain was dissected on ice to isolate the left PFC and thalamus for fatty acid analyses, and the right PFC isolated for gene expression assays.

2.4. Gas chromatography

The gas chromatography procedure has been described in detail previously (McNamara et al., 2009). Briefly, erythrocyte, PFC, and thalamus total fatty acid composition were determined with a Shimadzu GC-2014 equipped with an auto-injector (Shimadzu Scientific Instruments Inc., Columbia MD). The column was a DB-23 (123–2332): 30 m (length), I.D. 0.32 mm wide bore, film thickness of 0.25 μM (J & W Scientific, Folsom CA). Fatty acid identification was determined using retention times of authenticated fatty acid methyl ester standards (Matreya LLC Inc., Pleasant Gap PA). Analysis of fatty acid methyl esters is based on areas calculated with EZstart 7.4 software. Fatty acid composition is expressed as weight percent of total fatty acids (mg fatty acid/100 mg fatty acids). All samples were processed by a technician blinded to treatment. The primary measure of interest was DHA.

2.5. qRT-PCR

Total RNA was isolated using the RNeasy Mini Kit and potential DNA contamination removed using RNase-free DNase (Qiagen, Valencia, CA). cDNA was prepared using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). Targets were amplified in quadruplicate wells of a 384 well TaqMan low density microfluidic card (Thermo Fisher, Waltham, MA) on an ABI 7900HT500 Real Time PCR System (Applied Biosystems, Foster City, CA). Primary genes of interest were the astrocyte glutamate-aspartate transporter (GLAST, *Slc1a3*, NM_001289941), glial-specific glutamate transporter-1 (GLT-1, *Slc1a2*, NM_001035233), glutamine synthetase (GS, *Glul,* NM_017073), and glial fibrillary acidic protein (GFAP, NM_017009). Data were analyzed as normalized cycle threshold values (ΔC_T) using PPIA (NM_017101) as the housekeeping gene.

2.6. Statistical analyses

Diet group differences in neurochemical, fatty acid, and qRT-PCR data were evaluated in each brain region separately with a one-way ANOVA. Post hoc comparisons were made with unpaired *t*-tests (2-tailed, $\alpha = 0.05$). Pearson (linear) correlation analyses were performed to determine relationships between brain fatty acid, gene expression, and neurochemical concentration data. Statistical analyses were performed with GB-STAT software (Dynamic Microsystems, Inc., Silver Springs MD).