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Adolescent escitalopram administration modifies neurochemical alterations in the hippocampus of maternally separated rats

Jiaojie Hui [a,b], Zhijun Zhang [a,b,*], Shanshan Liu [a,b], Guangjun Xi [a,b], Xiangrong Zhang [a,b], GaoJun Teng [a,c], Kevin C. Chan [d,e], Ed X. Wu [d,e], Binbin Nie [f,g], Baoci Shan [g], Lingjiang Li [h], Gavin P. Reynolds [i]

[a] School of Clinical Medicine, Southeast University, Nanjing, Jiangsu, China
[b] Department of Neurology, Affiliated ZhongDa Hospital of Southeast University, Nanjing, Jiangsu, China
[c] Institution of Molecular Imaging, Southeast University, Nanjing, Jiangsu, China
[d] Laboratory of Biomedical Imaging and Signal Processing, The University of Hong Kong, Pokfulam, Hong Kong, China
[e] Department of Electrical and Electronic Engineering, The University of Hong Kong, Pokfulam, Hong Kong, China
[f] Physical Science and Technology College, Zhengzhou University, Zhengzhou, Henan, China
[g] Key Laboratory of Nuclear Analytical Techniques, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing, China
[h] Mental Health Institute, The Second Xiangya Hospital of Central South University, Changsha, Hunan, China
[i] Department of Psychiatry, Queen's University Belfast, BT9 7BL, Belfast, UK

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Abstract

Early life stress is a potential precursor of eventual neuropsychiatric diseases and may result in altered neurodevelopment and function of the hippocampus, which thus provides a site at which potential interventions to modify the effects of early life stress may act. In this study, Sprague–Dawley rat pups comprising male and female animals underwent maternal separation (MS) for 180 min from postnatal days (PND) 2 to 14, or were left with their dams. They subsequently received daily administration of saline (0.9%), escitalopram (10 mg/kg), or no treatment during adolescence (PND 43–60). All adult animals underwent brain magnetic resonance imaging (MRI) and bilateral hippocampal proton magnetic resonance spectroscopy (^{1}H-MRS). Neither MS nor escitalopram treatment had a significant effect on hippocampal volume. Adult rats that experienced MS displayed significantly increased choline-containing compounds (Cho) and decreased *N*-acetylaspartate (NAA), glutamate (Glu) and Myo-inositol (MI) relative to the stable neurometabolite creatine (Cr) in hippocampus. Administration of escitalopram during adolescence could modify the alterations of NAA/Cr, Glu/Cr and MI/Cr. The effects of MS on

* Corresponding author. Department of Neurology, affiliated ZhongDa Hospital of Southeast University, No. 87 DingJiaQiao Road, Nanjing, Jiangsu, China. Tel.: +86 25 83272023; fax: +86 25 83285132.
E-mail address: zhijunzhang838@yahoo.com.cn (Z. Zhang).

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hippocampal neurochemistry were most significant in the right hippocampus. These results indicate that MS in rats has long-term consequences on hippocampal neurochemistry reflective of neural density/functional integrity, especially on the right hippocampus, and adolescent administration with escitalopram can at least partially ameliorate these neurochemical alterations. Furthermore, these metabolite changes seem to be more sensitive indicators of the results from early life stress than volume changes.

1. Introduction

Early life stress is associated with changes in behavioral, cognitive and neurobiological responses to stress, and increases risk of developing stress-related neuropsychiatric disorders in adulthood, but the mechanisms are unclear (Heim and Nemeroff, 2001). The hippocampus plays a critical role in learning, memory storage and retrieval (Clark et al., 2000; Hollup et al., 2001). It is not only vulnerable to stress but also implicated in emotional regulation and stress reactivity (Eren et al., 2007).

Previous structural magnetic resonance imaging (MRI) studies reported that people with stress-related diseases associated with a history of childhood abuse showed significant volume reduction (Bremner et al., 1997, 2003; Vythilingam et al., 2002; Irle et al., 2005) in the hippocampus compared with non-abused patients and healthy subjects, and the hippocampal volume reduction was correlated with the extent and duration of childhood abuse (Bremner et al., 1997; Driessen et al., 2000). These findings suggest that early life stress may be involved in the pathogenesis of hippocampal abnormalities, and thus is a risk factor for stress-related neuropsychiatric disorders later in life. However, laterality was inconsistent across these MRI studies, with volume decreases being reported in the right (Irle et al., 2005), left (Bremner et al., 1997; Vythilingam et al., 2002), and both hippocampi (Driessen et al., 2000; Bremner et al., 2003). In addition, some studies failed to observe a difference in hippocampal volume between people with a history of childhood abuse and those without the history (Cohen et al., 2006; Weniger et al., 2008).

Magnetic resonance spectroscopy (MRS) is a noninvasive technique which can determine in vivo, in localized brain regions, several cerebral metabolites considered relevant to neural density/functional integrity (*N*-acetylaspartate, NAA; choline-containing compounds, Cho; glutamate, Glu; Myo-inositol, MI) (Lyoo and Renshaw, 2002). Previous clinical studies have reported changes of hippocampal MRS measures in stress-related neuropsychiatric disorders, such as depression (Yildiz-yesiloglu and Ankerst, 2006; Capizzano et al., 2007), anxiety (Mathew et al., 2008), bipolar disorder (Capizzano et al., 2007), posttraumatic stress disorder (PTSD) (Freeman et al., 1998; Schuff et al., 2001, 2008; Villarreal et al., 2002; Mohanakrishnan et al., 2003; Mahmutyazicioglu et al., 2005) and schizophrenia (Deicken et al., 1998). To our knowledge, there has been only one published clinical MRS study addressing the brain neurochemical changes mainly associated with early life stress, suggesting reduced NAA of anterior cingulate cortex (ACC) in abused children and adolescences with PTSD, although this did not report measures in the hippocampus (De et al., 2000).

However, retrospective clinical studies are limited in that they do not provide direct evidence of a cause–effect relationship. Animal studies are indispensable to improve our understanding of the consequences of early life stress. Only a few animal studies of early life stress have employed a structural MRI (Spinelli et al., 2009) or an MRS approach (Mathew et al., 2003; Marco et al., 2007). The MRI study found an enlarged vermis, dorsomedial prefrontal cortex, and dorsal ACC associated with early life stress in monkeys with no apparent differences in the corpus callosum and hippocampus (Spinelli et al., 2009). One MRS study reported that the fatty-acid amide hydrolase inhibitor (URB597) could up-regulate NAA and down-regulate glutamate and glutamine in the hippocampus of adult rats exposed to early maternal deprivation (Marco et al., 2007). Another MRS study found significantly decreased NAA and increased glutamate-glutamine-GABA (Glx) resonance in the ACC and significantly increased Cho in the medial temporal lobe of adult bonnet macaques exposed to early life stress (Mathew et al., 2003). These previous studies indicate brain volume and neurochemical changes occur in response to early life stress, possibly increasing the risk of development of neuropsychiatric disorders in adulthood.

Maternal separation (MS) is a well-characterized model of early life stress in rodents (Sanchez et al., 2001; Mirescu et al., 2004). Adult rats repeatedly separated from their mothers on postnatal days demonstrate depression-like behaviors and memory deficits (Cui et al., 2006; Leventopoulos et al., 2009) in adulthood, with decreased sucrose preference, longer immobility in the forced swimming test and shorter time spent in target quadrant during probe trial of the Morris water maze test, findings we have replicated. This animal model is potentially valuable in studying the mechanisms underlying the pathophysiology of affective disorders associated with early life stress.

An important challenge is the potential reversibility of the consequences of early life stress. It appears that selective serotonin reuptake inhibitors (SSRIs) may be beneficial in the treatment of children and adults exposed to early life stress (Heim and Nemeroff, 2001). In addition, administration of SSRIs (escitalopram and fluoxetine) reverses the behavioral or neurochemical changes of MS rats (El et al., 2006; Leventopoulos et al., 2009; Marais et al., 2009), although the mechanisms of their actions are not well established.

Several previous studies have shown that the consequences of early life stress in both humans and animal models may be lateralized, with changes of regional volumes and neuronal activity in right hemisphere (Lyons et al., 2002; Irle et al., 2005; Stevenson et al., 2008). In addition, other studies have shown sex differences in behavioral tests (Ito et al., 2006) and hypothalamic–pituitary–adrenal (HPA) axis

response to stress (Kalinichev et al., 2002) in animals experiencing early life stress.

In the present study, we employed MS to investigate whether early life stress in rats could induce changes in hippocampal volume and neurochemistry, and whether any such changes could be modified by adolescent administration with escitalopram. Furthermore, we also aimed to address the possible presence of laterality and sex differences in the effects of MS and escitalopram intervention.

2. Experimental procedures

2.1. Animals

Timed-pregnant Sprague–Dawley rats (Animal House Center, Southeast University, China) were provided on gestation days 16–18, individually housed in a temperature (21 ± 2 °C) and humidity (55% ± 5%) controlled room on a 12-h light/dark cycle (lights on at 07:00 am) with food and water provided ad libitum. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by Jiang Su Animal Care and Use Committee.

2.2. Early life experience

The day of delivery was designated as PND 0. As previously described (Huot et al., 2001; Mirescu et al., 2004), on PND 1, litter composition was standardized to 10 pups (5–6 males and 4–5 females). All pups were randomly assigned to MS or control (CON) group from PND 2 to14 inclusive. For MS pups, dams were first removed and placed in an adjacent cage. Pups were then transferred to a plastic container lined with bedding from the home cage and placed in an incubator maintained at roughly 34 °C, a temperature consistent with nest measurements. After the separation period, MS pups were returned to their home cages, where they were reunited with their dams. MS was carried out for a period of 180 min between 08:00 am and 11:00 am. CON pups remained with their dams over this period. On PND 21, all rats were weaned and housed with the same sex in regular cages (4–5/cage) until adulthood.

2.3. Pharmacological administration

Escitalopram oxalate tablets (H. Lundbeck A/S, Copenhagen, Denmark) were dissolved in saline (0.9%) and administered by gavage at a dose of 10 mg/kg body weight (Eren et al., 2007). From PND 43 to 60, MS and CON animals received daily administrations of saline, escitalopram, or no treatment. There were six experimental groups (n = 12–14, 6–7 animals of each sex): CON, CON + saline, CON + escitalopram, MS, MS + saline, and MS + escitalopram.

2.4. MRI and proton MRS (^{1}H-MRS) acquisition

MRI was undertaken on a 7.0 T animal MRI scanner (70/16 PharmaScan, Bruker Biospin GmbH, Germany) using a 38 mm birdcage rat brain quadrature resonator for radiofrequency transmission and reception. Rats were anesthetized using inhaled isoflurane/O_2 (3% for induction and 1.5–2% for maintenance). During MRI scan, the rat was prostrated on a custom-made holder to minimize head motion while respiration was monitored at a rate of 50 breaths/min. All animals were scanned in random order on six consecutive days (PND 70–75). The total anesthesia and scan time for one animal was about 60 min.

Scout T_2-weighted imaging (T_2WI) in three planes with a fast spin echo (FSE) pulse sequence was first acquired to control rat head positioning. Next, a coronal T_2WI scan was acquired using rapid-acquisition relaxation-enhancement (RARE) pulse sequence with the following parameters: field of view = 4.5 cm × 4.5 cm, matrix size = 256 × 256, repetition time (TR) = 3500 ms, echo time (TE) = 12 ms, slice thickness = 1.0 mm, slice gap = 1.5 mm, acquisition time = 1 min 24 s.

For single-voxel ^{1}H-MRS, the volume of interest (VOI, 3.5 mm × 3.5 mm × 3.5 mm) was placed over the hippocampal region in the coronal T_2W images. After first- and second-order localized voxel shimming with fast automatic shimming technique b mapping along projections (FASTMAP), a full-width half-maximum line width of water signal of ≤ 20 Hz would be achieved. The water signal was suppressed by variable power radiofrequency pulses with optimized relaxation delays (VAPOR). Point resolved spectroscopy (PRESS) sequence was used for signal acquisition, with TR = 2500 ms, TE = 20 ms, number of average = 512 and scan duration for each side = 21 min. In addition, the order of acquisition of the right and left hippocampal spectra alternated between subsequent animals scanned, to minimize the introduction of artifactual hemispheric differences.

2.5. Volume measurements of hippocampus

The coronal T_2WI data was analyzed using statistical parametric mapping (SPM5) (http://www.fil.ion.ucl.ac.uk/spm/software/spm5). The volume of hippocampus was extracted using our in-house developed atlas-based region of interest (ROI) extraction method based on T_2WI rat brain template and digital atlas (BB Nie). Boundaries of the hippocampus were determined following the guidelines of Watson et al. (Watson et al., 1992), including hippocampal proper, dentate gyrus, subiculum, fimbria and alveus. To account for variations in brain size, hippocampal volume was normalized to intracranial volume (ICV) which was extracted based on the PCNN (Pulse Coupled Neural Network) method (Murugavel and Sullivan, 2009).

2.6. ^{1}H-MRS spectral processing

The ^{1}H-MRS data were processed using jMRUI software (version 3.0, http://www.mrui.uab.es/mrui) (Gruetter, 1993) according to the methods described by Chan et al. (Chan et al., 2009). In short, metabolite peak areas were determined using the quantum estimation (QUEST) method combined with a subtraction approach for background modeling after preconditioning. A simulated basis set was used to estimate peak areas. Fig. 1 indicates the typical jMRUI quantitation results for ^{1}H-MRS. To reduce systematic variations between animals, we applied a relative quantification method using the creatine (Cr) peak as the internal spectral reference (Atwood et al., 2007). The numerical time-domain modal functions of 10 metabolites [alanine (Ala), aspartate (Asp), Cr, Cho, Glu, glycine (Gly), NAA, taurine (Tau), lactate (Lac) and MI] were used as prior knowledge in QUEST and NAA/Cr, Cho/Cr, Glu/Cr, and MI/Cr were statistically evaluated in the present study. These metabolite model signals were quantum-mechanically simulated in NMR spectra calculations using operators (NMRSCOPE) for the in vivo experimental protocol. The reliability of metabolite determination was assessed using the Cramer-Rao lower bounds (CRLB) (Cudalbu et al., 2008). The mean of the relevant estimates and the corresponding error values (±1 standard deviation (S.D.), 70% confidence interval) were computed from jMRUI. An estimate was considered as relevant when the corresponding bound was found below 15%.

2.7. Statistical analysis

Data were analyzed using SPSS software, version 13.0 (SPSS Inc., Chicago) and values were presented as mean ± S.D. The results were considered statistically significant if $p < 0.05$.

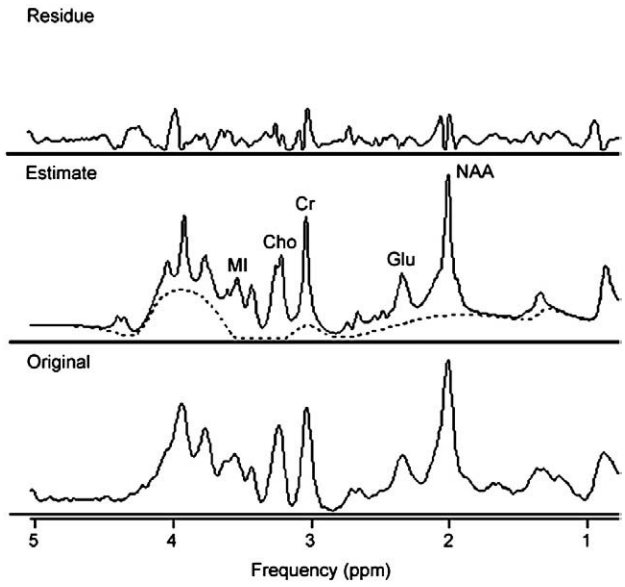

Figure 1 jMRUI QUEST quantitative results of the volume of interest selected in the right hippocampus of one rat. From top to bottom: residue of QUEST quantitation, estimated spectrum and background signals (dashed line) using QUEST and simulated metabolite basis set signals, and original spectrum. Spectral peaks identified are Myo-inositol (MI), choline-containing compounds (Cho), creatine (Cr), glutamate (Glu) and *N*-acetylaspartate (NAA). QUEST, quantum estimation.

The results were analyzed in two stages. Initial analysis was to determine the effect of maternal separation on hippocampal volume and each metabolite ratio, and to determine whether this was affected by the presence of the daily saline gavage, acting as a control for the drug administration. The second stage was to determine the effect of escitalopram administration on any change in hippocampal volume and metabolite ratio induced by MS.

In both analyses, hippocampal volume and metabolite ratios were measured in the bilateral hippocampi. Repeated-measures analysis of variance with hemisphere as the within-subject variable and sex, rearing (MS or control for the first stage), intervention (none and saline for the first stage; saline and escitalopram for the second stage) as between-subjects factors was initially determined. If no significant laterality effect or no interaction of laterality with other factors emerged, the metabolite ratio of both hemispheres was combined. In the case of missing ^{1}H-MRS data on one side for some animals, the mean measure reflected the contribution of the existing side. Given that there was some lateralized missing ^{1}H-MRS value reducing the sample number of repeated-measures analysis, we also undertook a univariate ANOVA for each metabolite ratio with sex, rearing and intervention as fixed factors. This was followed by the Bonferroni testing as post hoc analysis to assess the differences within factor-specific groups when necessary. In addition, if there was no significant effect of sex, further separate analyses for each sex were not conducted.

3. Results

Of the 79 animals undergoing neuroimaging studies, the coronal T_2WI scan was obtained in each animal, while the ^{1}H-MRS data sets were obtained in 75. In addition, the numbers of samples taken into statistical analysis of ^{1}H-MRS data for right and left hippocampus were reduced because of poor quality spectra that were precluded from analysis (see Table 1 for detail).

3.1. Effects of maternal separation and escitalopram administration on hippocampus volume measurements

As expected, male rats had larger mean ICV (1172.5 ± 82.3 mm^3) compared with female rats (1112.5 ± 63.3 mm^3) [$F(1, 78) = 13.150$, $p = 0.001$]. Table 2 showed the ICV and normalized hippocampal volume. Repeated-measures analysis of variance showed no significant effect of rearing [$F(1, 51) = 0.382$, $p = 0.540$], saline [$F(1, 51) = 0.197$, $p = 0.659$] or drug [$F(1, 52) = 0.286$, $p = 0.596$] on normalized hippocampal volume. However, there was a significant effect of laterality on this area both in the analysis for the effect of rearing [$F(1, 51) = 6.456$, $p = 0.015$] and drug [$F(1, 52) = 10.702$, $p = 0.002$]. Across all the subjects, the left normalized hippocampus was significantly larger than the right normalized hippocampus [$F(1, 78) = 11.426$, $p = 0.001$], although this difference did not remain significant when the CON group was examine alone [$F(1, 12) = 0.453$, $p = 0.507$].

3.2. Effects of maternal separation on hippocampus ^{1}H-MRS measurements

Repeated-measures analysis of variance showed a significant effect of rearing for NAA/Cr [$F(1, 43) = 4.871$, $p = 0.034$] but not for Cho/Cr [$F(1, 43) = 0.498$, $p = 0.485$], Glu/Cr [$F(1, 43) = 0.557$, $p = 0.460$] or MI/Cr [$F(1, 43) = 2.063$, $p = 0.160$], and a significant effect of sex for Cho/Cr [$F(1, 43) = 7.137$, $p = 0.011$] but not for NAA/Cr [$F(1, 43) = 3.193$, $p = 0.082$], Glu/Cr [$F(1, 43) = 0.027$, $p = 0.871$] or MI/Cr [$F(1, 43) = 0.121$, $p = 0.730$]. It also showed a significant effect of laterality for NAA/Cr [$F(1, 43) = 7.195$, $p = 0.011$] and Glu/Cr [$F(1, 43) = 5.087$, $p = 0.030$], laterality × rearing interaction for Cho/Cr [$F(2, 42) = 5.074$, $p = 0.030$] and laterality × sex interaction for MI/Cr [$F(2, 42) = 4.820$, $p = 0.035$].

Therefore, separate univariate ANOVAs in each hemisphere for each metabolite ratio were undertaken, showing that MS had significant effects on NAA/Cr [$F(1, 48) = 7.759$, $p = 0.008$], Glu/Cr [$F(1, 48) = 6.744$, $p = 0.013$] and MI/Cr [$F(1, 48) = 5.800$,

Table 1 Number of samples taken into statistical analysis for each group in the ^{1}H-MRS study. Abbreviations: CON, control; MS, maternal separation; ESC, escitalopram; HIP, hippocampus; M, male; F, female.

	CON	CON + saline	CON + ESC	MS	MS + saline	MS + ESC
Total number (M/F)	13(7/6)	13(7/6)	12(6/6)	12(6/6)	12(6/6)	13(6/7)
Left HIP (M/F)	12(7/5)	11(6/5)	11(5/6)	12(6/6)	10(4/6)	11(6/5)
Right HIP (M/F)	13(7/6)	12(6/6)	12(6/6)	12(6/6)	12(6/6)	13(6/7)

Table 2 Intracranial volume and normalized hippocampal volume. Data are presented as mean ± standard deviation (S.D.). Abbreviations: CON, control; MS, maternal separation; ESC, escitalopram; ICV, intracranial volume; HIP, hippocampus.

	CON	CON + saline	CON + ESC	MS	MS + saline	MS + ESC
ICV (mm^3)						
	1130.6 ± 83.4	1139.9 ± 84.8	1163.4 ± 73.8	1155.6 ± 93.3	1114.6 ± 75.2	1151.6 ± 66.8
Normalized HIP volume (absolute HIP volume/ICV × 1000)						
Left HIP	29.4 ± 3.5	30.2 ± 3.4	29.6 ± 2.8	30.6 ± 1.9	30.5 ± 2.1	29.9 ± 3.5
Right HIP	28.4 ± 4.1	28.8 ± 4.0	26.9 ± 3.2	28.7 ± 3.7	28.6 ± 3.5	27.8 ± 3.4

$p = 0.021$] and Cho/Cr [$F(1, 48) = 4.895$, $p = 0.033$] in the right but not left hippocampus.

Further Bonferroni testing demonstrated decreases of NAA/Cr, Glu/Cr and MI/Cr and increase of Cho/Cr in the right hippocampus of MS group compared with CON group, although without significant differences (Table 3).

There were also significant effects of sex on NAA/Cr [$F(1, 44) = 7.355$, $p = 0.010$] and Cho/Cr [$F(1, 44) = 5.672$, $p = 0.022$] in the left hippocampus, and separate univariate ANOVA for left NAA/Cr and Cho/Cr showed no significant effect of rearing in both male and female rats. However, there were significant decreased left NAA/Cr [$F(1, 44) = 7.698$, $p = 0.008$] and increased left Cho/Cr [$F(1, 44) = 6.302$, $p = 0.016$] in male rats.

Neither administration of saline by gavage nor its interaction with laterality, rearing or sex showed significant effects on any metabolite ratio.

3.3. Effects of escitalopram administration on hippocampus ^{1}H-MRS measurements

Repeated-measures analysis of variance showed a significant effect of drug for NAA/Cr [$F(1, 41) = 17.275$, $p = 0.000$] but not for Cho/Cr [$F(1, 41) = 0.809$, $p = 0.375$], Glu/Cr [$F(1, 41) = 0.936$, $p = 0.340$] or MI/Cr [$F(1, 41) = 2.148$, $p = 0.152$], and a significant effect of sex for Cho/Cr [$F(1, 41) = 8.072$, $p = 0.008$] but not for NAA/Cr [$F(1, 41) = 0.279$, $p = 0.601$], Glu/Cr [$F(1, 41) = 1.146$, $p = 0.292$] or MI/Cr [$F(1, 41) = 0.010$, $p = 0.920$]. It also showed a significant effect of laterality for Cho/Cr [$F(1, 41) = 9.270$, $p = 0.004$] and Glu/Cr [$F(1, 41) = 4.458$, $p = 0.042$], a laterality × drug interaction for NAA/Cr [$F(2, 40) = 4.940$, $p = 0.033$] and no significant effect of laterality or its interaction terms for MI/Cr.

Separate univariate ANOVAs in each hemisphere for NAA/Cr, Cho/Cr and Glu/Cr showed that escitalopram administration had significant effects on NAA/Cr in bilateral hippocampi [left: $F(1, 42) = 4.751$, $p = 0.036$; right: $F(1, 48) = 25.422$, $p = 0.000$] and on Glu/Cr in the right hippocampus [$F(1, 48) = 4.649$, $p = 0.037$].

Bonferroni testing demonstrated significant increases of NAA/Cr in MS + escitalopram ($p = 0.000$, compared with MS + saline group) and CON + escitalopram ($p = 0.046$, compared with CON + saline group) groups for the right hippocampus. It also showed a significant increase of Glu/Cr in MS + escitalopram group compared with MS + saline group ($p = 0.048$) (Table 3). However, there were no significant differences of MRS measurements in the left hippocampus.

There was no effect of laterality on MI/Cr, so the bilateral MI/Cr ratios were combined in further analysis. Univariate ANOVA for combined MI/Cr showed that escitalopram administration had a significant effect on combined MI/Cr [$F(1, 48) = 13.218$, $p = 0.001$]. Bonferroni testing showed a significant increase of combined MI/Cr in MS + escitalopram ($p = 0.003$) and CON + escitalopram ($p = 0.001$) groups compared with MS + saline group.

There was also significant effects of sex on Cho/Cr in bilateral hippocampi [left: $F(1, 42) = 6.325$, $p = 0.017$; right: $F(1, 48) = 4.744$, $p = 0.035$], and separate univariate ANOVA for left and right hippocampus showed this to be due to higher values in male rats [left: $F(1, 42) = 6.735$, $p = 0.013$; right: $F(1, 48) = 4.203$, $p = 0.046$].

4. Discussion

The major findings of this report were as follows: firstly, neither MS nor adolescent escitalopram treatment had a significant effect on hippocampal volume. However, adult rats that had undergone MS displayed significantly increased Cho/Cr and decreased NAA/Cr, Glu/Cr and MI/Cr in the hippocampus and administration of escitalopram during

Table 3 Effects of maternal separation and adolescent escitalopram administration on hippocampal ^{1}H-MRS measurements of adult rats. #$p < 0.05$, in comparison to the CON + saline group. *$p < 0.05$, ***$p < 0.001$ in comparison to the MS + saline group. Data are presented as mean ± standard deviation (S.D.). Abbreviations: CON, control; MS, maternal separation; ESC, escitalopram; MI, Myo-inositol; Cho, choline-containing compounds; Cr, creatine; Glu, glutamate; NAA, *N*-acetylaspartate.

Laterality	Metabolites	CON	CON + saline	CON + ESC	MS	MS + saline	MS + ESC
Left	NAA/Cr	1.19 ± 0.07	1.20 ± 0.08	1.24 ± 0.11	1.18 ± 0.07	1.18 ± 0.04	1.24 ± 0.08
	Cho/Cr	0.18 ± 0.03	0.17 ± 0.04	0.16 ± 0.03	0.16 ± 0.02	0.16 ± 0.03	0.16 ± 0.03
	Glu/Cr	1.12 ± 0.08	1.03 ± 0.19	1.09 ± 0.15	1.07 ± 0.09	1.15 ± 0.12	1.09 ± 0.10
	MI/Cr	0.64 ± 0.05	0.71 ± 0.08	0.74 ± 0.08	0.69 ± 0.05	0.67 ± 0.06	0.70 ± 0.12
Right	NAA/Cr	1.19 ± 0.08	1.17 ± 0.07	1.28 ± 0.13 #	1.13 ± 0.04	1.13 ± 0.05	1.30 ± 0.10 ***
	Cho/Cr	0.16 ± 0.03	0.17 ± 0.02	0.19 ± 0.04	0.18 ± 0.03	0.19 ± 0.04	0.20 ± 0.04
	Glu/Cr	1.24 ± 0.10	1.15 ± 0.17	1.18 ± 0.12	1.13 ± 0.13	1.06 ± 0.11	1.21 ± 0.13 *
	MI/Cr	0.68 ± 0.12	0.71 ± 0.09	0.76 ± 0.10	0.62 ± 0.06	0.64 ± 0.05	0.71 ± 0.12

adolescence could modify these deficits in NAA/Cr, Glu/Cr and MI/Cr. Additionally, the effects of MS on hippocampal neurochemistry were significant in right hippocampus. Finally, there were no significant sex differences in the neurochemical changes induced by MS or escitalopram administration.

4.1. Effects of maternal separation and escitalopram administration on hippocampus volume measurements

Numerous studies in human beings have reported diminished hippocampus volume in psychiatric patients experiencing early life stress (Bremner et al., 1997, 2003; Driessen et al., 2000; Vythilingam et al., 2002; Irle et al., 2005) and antidepressant treatments increase adult hippocampal neurogenesis (Banasr and Duman, 2007). In the present study, we found no hippocampal volume differences as a function of MS and escitalopram treatment, which was consistent with a recent animal study in the monkey (Spinelli et al., 2009). This apparent inconsistency with human findings may in part reflect the alcohol dependence and abuse common in psychiatric patients and which are associated with hippocampus atrophy (Laakso et al., 2000).

One meta-analysis found larger left than right hippocampus in the adult healthy people (Woon and Hedges, 2008). In the current study, we found that the left hippocampus was larger than the right hippocampus across all the subjects, although this difference did not remain significant when CON group was examined alone. It still needs more studies to authenticate this finding in rats.

4.2. Effects of maternal separation on hippocampus ^{1}H-MRS measurements

The NAA signal in ^{1}H-MRS, mainly comprising NAA and a small proportion of *N*-acetylaspartylglutamate (NAAG), is predominantly a marker of neuron density and function (Baslow, 2003). Maltreated children and adolescents with PTSD (De et al., 2000) and adult bonnet macaques exposed to early life stressors demonstrated decreased NAA/Cr in ACC (Mathew et al., 2003), which might suggest neuronal loss or dysfunction resulting from early life stress. We found a significant effect of MS on hippocampal NAA in the present study, providing further support for the loss of neuronal integrity and indicating the involvement of hippocampus in the developmental deficit induced by MS.

Our finding of increased hippocampal Cho/Cr by MS was consistent with the finding that Cho/Cr ratios both in medial temporal lobe and ACC were significantly elevated following early life stress in bonnet macaques (Mathew et al., 2003), although abnormalities of Cho/Cr ratio in ACC were not found in maltreated children and adolescents with PTSD (De et al., 2000). Various hypotheses have been invoked to explain the elevated Cho resonance in the brain, including increased membrane phospholipid turnover (Miller, 1991), increased myelin turnover (Jung et al., 1999), alteration in endocrine status and local metabolic rates (Gupta et al., 1995). The stress associated with early weaning in mice has been shown to over-stimulate subsequent expression of myelin basic proteins (Chan et al., 1998). Thus our results may reflect the augmentation of myelin breakdown products and rapid cell membrane synthesis resulting from early life stress.

The MI signal includes contributions from free MI, glycine, and a small component from inositol monophosphates (Brand et al., 1993). MI, considered to be a marker for astrocytes (Brand et al., 1993), makes the greatest contribution to the MI peak (Coupland et al., 2005). Astrocytes have important functions in maintaining ionic, neurotransmitter and metabolic homeostasis in brain and play vital roles in maintaining neuroplasticity via multiple mechanisms including support of synaptogenesis, synapse maintenance and secretion of neurotrophins (Newman, 2003). Leventopoulos et al. reported the reduction of astroglia density in hippocampus, prefrontal cortex, cingulate cortex, and amygdala following MS in rats (Leventopoulos et al., 2007). In the present study, there was a significant decrease in hippocampal MI after MS, which might suggest that astrocytic loss or dysfunction in this region contributed to the impact of early adverse life events.

Increasing evidence suggests that stress-related neuropsychiatric disorders are associated with perturbation in the metabolism of Glu. Early studies reported that the concentration of Glu was abnormally decreased in brain regions including the hippocampus, frontal and anterior cingulate cortices of major depressive disorder (MDD) and schizophrenic patients (Tsai et al., 1995; Caetano et al., 2005; Rosenberg et al., 2005). Our finding that adult rats undergone MS displayed significantly decreased Glu/Cr in hippocampus suggested reduced metabolism of Glu after early life stress.

Several previous studies have shown that the consequences of early life stress in both humans and animal models may be lateralized, with changes of regional volumes and neuronal activity in right hemisphere (Lyons et al., 2002; Irle et al., 2005; Stevenson et al., 2008). The right-sided abnormalities of hippocampal Cho/Cr and MI/Cr resulting from MS in this study are consistent with these studies and may indicate early life stress primarily interferes with the development of the right hemisphere.

We found no significant sex differences in the neurochemical changes induced by MS, contrary to some other studies which showed sex differences in behavioral tests (Ito et al., 2006) and HPA stress responses (Kalinichev et al., 2002) in early-stress-experienced animals. However, we did find a difference between males and females in left hemisphere NAA and Cho, indicating some underlying neurochemical sex differences, which could confound, or contribute to, MS-induced differences in other studies.

4.3. Effects of escitalopram administration on hippocampus ^{1}H-MRS measurements

How escitalopram influences NAA/Cr in MS rats is unclear. However, chronic treatment with this and other SSRIs reversed stress-induced inhibition of neurogenesis in the dentate gyrus (DG) (Malberg et al., 2000; Jayatissa et al., 2006) and re-established granule cell density (Jayatissa et al., 2008). SSRIs could also reverse the delay of the differentiation of precursors into mature neurons (Jaakomovits et al., 2006; Wang et al., 2008) and the death of

neuronal cell of hippocampus (Jin et al., 2009). Consequently, neuronal number and function may be increased.

Human and rat glial cells express the gene encoding serotonin transporter (Kubota et al., 2001). Moreover, a functional fluoxetine-sensitive high-affinity uptake system for serotonin has been found in astrocytes (Dave and Kimelberg, 1994; Inazu et al., 2001). Boldizsar et al. reported fluoxetine could prevent the stress-induced numerical decrease of astrocytes in hippocampus (Czeh et al., 2006). In the present study, we found a significant increase of MI/Cr in escitalopram treated animals. Our findings, in the light of these previous studies, suggest that SSRIs exert a direct effect on astrocytes that cannot be ignored when attempting to elucidate their mechanism(s) of action.

Only very few studies address the effects of SSRIs on Glu. It was reported that both acute and chronic administration of citalopram could significantly inhibited the release of Glu in rat prefrontal cortex induced by sodium channel activator, veratridine (Golembiowska and Dziubina, 2000). However, the MRS measurement of Glu in hippocampus is related to the ubiquitous metabolism of Glu. Therefore, the finding in our study that administration of escitalopram during adolescence could increase hippocampal Glu/Cr in rats exposed to early life stress still requires detailed investigation to verify.

Escitalopram, like other SSRIs, has been reported to demonstrate greater efficacy in female than male subjects with depression (Young et al., 2009). The underlying biology of this effect remains unclear, but may relate to differences in aetiological factors. However, in the present study we did not identify differences between the male and female rats in their neurochemical responses to escitalopram.

4.4. Limitations of the study

The study reported here must be considered preliminary because of the following limitations: first, we measured metabolite ratios but not absolute concentrations. The ratio approach is potentially vulnerable if there is a change in the denominator, which in this case is Cr. Furthermore, even though the position of the VOI was carefully selected, to obtain higher signal-to-noise ratios upon limited scan time, the spatial resolution of ^{1}H-MRS inevitably included some tissue outside the hippocampus that may have introduced spurious metabolites variations.

4.5. Conclusions

In conclusion, the present study suggests that hippocampal neurochemistry changes seem to be more sensitive indicators of the results from early life stress than volume changes. Additionally, the altered hippocampal neurochemistry, in particular that of right hippocampus, may be a neurochemical phenotype during childhood for increased risk of developing stress-related neuropsychiatric disorders. Finally, this neurochemical phenotype may provide a potential target for treatments to ameliorate the effects of early life stress.

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Contributors

Jiaojie Hui was involved in the design of the research proposal and performance of the study, collected the data, undertook the statistical analyses and wrote the first draft of the manuscript. Zhijun Zhang supervised the work, providing instruction, funding and instrumentation for this study. Shanshan Liu and Guangjun Xi assisted with animal care and MRI study. Xiangrong Zhang contributed to supervising the study. GaoJun Teng provided MRI scanner. Kevin C. Chan and Ed X. Wu contributed to analysis of the MRS data. Binbin Nie and Baoci Shan contributed to analysis of the structural MRI data. Lingjiang Li supervised the research and provided some fund support. Gavin P. Reynolds contributed substantially to the statistical analysis and final version of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

None of the authors have a conflict of interest, financial or otherwise, directly or indirectly related to this work.

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References

Atwood, T., Payne, V.S., Zhao, W., Brown, W.R., Wheeler, K.T., Zhu, J.M., Robbins, M.E., 2007. Quantitative magnetic resonance spectroscopy reveals a potential relationship between radiation-induced changes in rat brain metabolites and cognitive impairment. Radiat. Res. 168 (5), 574–581.

Banasr, M., Duman, R.S., 2007. Regulation of neurogenesis and gliogenesis by stress and antidepressant treatment. CNS Neurol. Disord. Drug Targets 6 (5), 311–320.

Baslow, M.H., 2003. N-acetylaspartate in the vertebrate brain: metabolism and function. Neurochem. Res. 28 (6), 941–953.

Brand, A., Richter-Landsberg, C., Leibfritz, D., 1993. Multinuclear NMR studies on the energy metabolism of glial and neuronal cells. Dev. Neurosci. 15 (3–5), 289–298.

Bremner, J.D., Randall, P., Vermetten, E., Staib, L., Bronen, R.A., Mazure, C., Capelli, S., McCarthy, G., Innis, R.B., Charney, D.S., 1997. Magnetic resonance imaging-based measurement of hippocampal volume in posttraumatic stress disorder related to childhood physical and sexual abuse — a preliminary report. Biol. Psychiatry 41 (1), 23–32.

Bremner, J.D., Vythilingam, M., Vermetten, E., Southwick, S.M., McGlashan, T., Nazeer, A., Khan, S., Vaccarino, L.V., Soufer, R., Garg, P.K., Ng, C.K., Staib, L.H., Duncan, J.S., Charney, D.S., 2003. MRI and PET study of deficits in hippocampal structure and

function in women with childhood sexual abuse and posttraumatic stress disorder. Am. J. Psychiatry 160 (5), 924–932.
Caetano, S.C., Fonseca, M., Olvera, R.L., Nicoletti, M., Hatch, J.P., Stanley, J.A., Hunter, K., Lafer, B., Pliszka, S.R., Soares, J.C., 2005. Proton spectroscopy study of the left dorsolateral prefrontal cortex in pediatric depressed patients. Neurosci. Lett. 384 (3), 321–326.
Capizzano, A.A., Jorge, R.E., Acion, L.C., Robinson, R.G., 2007. In vivo proton magnetic resonance spectroscopy in patients with mood disorders: a technically oriented review. J. Magn. Reson. Imaging 26 (6), 1378–1389.
Chan, J.R., Phillips, L.J., Glaser, M., 1998. Glucocorticoids and progestins signal the initiation and enhance the rate of myelin formation. Proc. Natl. Acad. Sci. USA 95 (18), 10459–10464.
Chan, K.C., Khong, P.L., Cheung, M.M., Wang, S., Cai, K.X., Wu, E.X., 2009. MRI of late microstructural and metabolic alterations in radiation-induced brain injuries. J. Magn. Reson. Imaging 29 (5), 1013–1020.
Clark, R.E., Zola, S.M., Squire, L.R., 2000. Impaired recognition memory in rats after damage to the hippocampus. J. Neurosci. 20 (23), 8853–8860.
Cohen, R.A., Grieve, S., Hoth, K.F., Paul, R.H., Sweet, L., Tate, D., Gunstad, J., Stroud, L., McCaffery, J., Hitsman, B., Niaura, R., Clark, C.R., McFarlane, A., Bryant, R., Gordon, E., Williams, L.M., 2006. Early life stress and morphometry of the adult anterior cingulate cortex and caudate nuclei. Biol. Psychiatry 59 (10), 975–982.
Coupland, N.J., Ogilvie, C.J., Hegadoren, K.M., Seres, P., Hanstock, C.C., Allen, P.S., 2005. Decreased prefrontal Myo-inositol in major depressive disorder. Biol. Psychiatry 57 (12), 1526–1534.
Cudalbu, C., Cavassila, S., Rabeson, H., van, O.D., Graveron-Demilly, D., 2008. Influence of measured and simulated basis sets on metabolite concentration estimates. NMR Biomed. 21 (6), 627–636.
Cui, M., Yang, Y., Yang, J., Zhang, J., Han, H., Ma, W., Li, H., Mao, R., Xu, L., Hao, W., Cao, J., 2006. Enriched environment experience overcomes the memory deficits and depressive-like behavior induced by early life stress. Neurosci. Lett. 404 (1–2), 208–212.
Czeh, B., Simon, M., Schmelting, B., Hiemke, C., Fuchs, E., 2006. Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. Neuropsychopharmacology 31 (8), 1616–1626.
Dave, V., Kimelberg, H.K., 1994. Na(+)-dependent, fluoxetine-sensitive serotonin uptake by astrocytes tissue-printed from rat cerebral cortex. J. Neurosci. 14 (8), 4972–4986.
De, B.M., Keshavan, M.S., Spencer, S., Hall, J., 2000. N-Acetylaspartate concentration in the anterior cingulate of maltreated children and adolescents with PTSD. Am. J. Psychiatry 157 (7), 1175–1177.
Deicken, R.F., Zhou, L., Schuff, N., Fein, G., Weiner, M.W., 1998. Hippocampal neuronal dysfunction in schizophrenia as measured by proton magnetic resonance spectroscopy. Biol. Psychiatry 43 (7), 483–488.
Driessen, M., Herrmann, J., Stahl, K., Zwaan, M., Meier, S., Hill, A., Osterheider, M., Petersen, D., 2000. Magnetic resonance imaging volumes of the hippocampus and the amygdala in women with borderline personality disorder and early traumatization. Arch. Gen. Psychiatry 57 (12), 1115–1122.
El, K.A., Gruber, S.H., Mork, A., Mathe, A.A., 2006. Adult life behavioral consequences of early maternal separation are alleviated by escitalopram treatment in a rat model of depression. Prog. Neuropsychopharmacol. Biol. Psychiatry 30 (3), 535–540.
Eren, I., Naziroglu, M., Demirdas, A., 2007. Protective effects of lamotrigine, aripiprazole and escitalopram on depression-induced oxidative stress in rat brain. Neurochem. Res. 32 (7), 1188–1195.
Freeman, T.W., Cardwell, D., Karson, C.N., Komoroski, R.A., 1998. In vivo proton magnetic resonance spectroscopy of the medial temporal lobes of subjects with combat-related posttraumatic stress disorder. Magn. Reson. Med. 40 (1), 66–71.
Golembiowska, K., Dziubina, A., 2000. Effect of acute and chronic administration of citalopram on glutamate and aspartate release in the rat prefrontal cortex. Pol. J. Pharmacol. 52 (6), 441–448.
Gruetter, R., 1993. Automatic, localized in vivo adjustment of all first- and second-order shim coils. Magn. Reson. Med. 29 (6), 804–811.
Gupta, R.K., Bhatia, V., Poptani, H., Gujral, R.B., 1995. Brain metabolite changes on in vivo proton magnetic resonance spectroscopy in children with congenital hypothyroidism. J. Pediatr. 126 (3), 389–392.
Heim, C., Nemeroff, C.B., 2001. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. Biol. Psychiatry 49 (12), 1023–1039.
Hollup, S.A., Kjelstrup, K.G., Hoff, J., Moser, M.B., Moser, E.I., 2001. Impaired recognition of the goal location during spatial navigation in rats with hippocampal lesions. J. Neurosci. 21 (12), 4505–4513.
Huot, R.L., Thrivikraman, K.V., Meaney, M.J., Plotsky, P.M., 2001. Development of adult ethanol preference and anxiety as a consequence of neonatal maternal separation in Long Evans rats and reversal with antidepressant treatment. Psychopharmacology (Berl) 158 (4), 366–373.
Inazu, M., Takeda, H., Ikoshi, H., Sugisawa, M., Uchida, Y., Matsumiya, T., 2001. Pharmacological characterization and visualization of the glial serotonin transporter. Neurochem. Int. 39 (1), 39–49.
Irle, E., Lange, C., Sachsse, U., 2005. Reduced size and abnormal asymmetry of parietal cortex in women with borderline personality disorder. Biol. Psychiatry 57 (2), 173–182.
Ito, A., Kikusui, T., Takeuchi, Y., Mori, Y., 2006. Effects of early weaning on anxiety and autonomic responses to stress in rats. Behav. Brain Res. 171 (1), 87–93.
Jaako-Movits, K., Zharkovsky, T., Pedersen, M., Zharkovsky, A., 2006. Decreased hippocampal neurogenesis following olfactory bulbectomy is reversed by repeated citalopram administration. Cell. Mol. Neurobiol. 26 (7–8), 1559–1570.
Jayatissa, M.N., Bisgaard, C., Tingstrom, A., Papp, M., Wiborg, O., 2006. Hippocampal cytogenesis correlates to escitalopram-mediated recovery in a chronic mild stress rat model of depression. Neuropsychopharmacology 31 (11), 2395–2404.
Jayatissa, M.N., Bisgaard, C.F., West, M.J., Wiborg, O., 2008. The number of granule cells in rat hippocampus is reduced after chronic mild stress and re-established after chronic escitalopram treatment. Neuropharmacology 54 (3), 530–541.
Jin, Y., Lim, C.M., Kim, S.W., Park, J.Y., Seo, J.S., Han, P.L., Yoon, S.H., Lee, J.K., 2009. Fluoxetine attenuates kainic acid-induced neuronal cell death in the mouse hippocampus. Brain Res. 1281, 108–116.
Jung, R.E., Brooks, W.M., Yeo, R.A., Chiulli, S.J., Weers, D.C., Sibbitt, W.L., 1999. Biochemical markers of intelligence: a proton MR spectroscopy study of normal human brain. Proc. Biol. Sci. 266 (1426), 1375–1379.
Kalinichev, M., Easterling, K.W., Plotsky, P.M., Holtzman, S.G., 2002. Long-lasting changes in stress-induced corticosterone response and anxiety-like behaviors as a consequence of neonatal maternal separation in Long-Evans rats. Pharmacol. Biochem. Behav. 73 (1), 131–140.
Kubota, N., Kiuchi, Y., Nemoto, M., Oyamada, H., Ohno, M., Funahashi, H., Shioda, S., Oguchi, K., 2001. Regulation of serotonin transporter gene expression in human glial cells by growth factors. Eur. J. Pharmacol. 417 (1–2), 69–76.
Laakso, M.P., Vaurio, O., Savolainen, L., Repo, E., Soininen, H., Aronen, H.J., Tiihonen, J., 2000. A volumetric MRI study of the hippocampus in type 1 and 2 alcoholism. Behav. Brain Res. 109 (2), 177–186.

Leventopoulos, M., Ruedi-Bettschen, D., Knuesel, I., Feldon, J., Pryce, C.R., Opacka-Juffry, J., 2007. Long-term effects of early life deprivation on brain glia in Fischer rats. Brain Res. 1142, 119–126.

Leventopoulos, M., Russig, H., Feldon, J., Pryce, C.R., Opacka-Juffry, J., 2009. Early deprivation leads to long-term reductions in motivation for reward and 5-HT1A binding and both effects are reversed by fluoxetine. Neuropharmacology 56 (3), 692–701.

Lyons, D.M., Afarian, H., Schatzberg, A.F., Sawyer-Glover, A., Moseley, M.E., 2002. Experience-dependent asymmetric variation in primate prefrontal morphology. Behav. Brain Res. 136 (1), 51–59.

Lyoo, I.K., Renshaw, P.F., 2002. Magnetic resonance spectroscopy: current and future applications in psychiatric research. Biol. Psychiatry 51 (3), 195–207.

Mahmutyazicioglu, K., Konuk, N., Ozdemir, H., Atasoy, N., Atik, L., Gundogdu, S., 2005. Evaluation of the hippocampus and the anterior cingulate gyrus by proton MR spectroscopy in patients with post-traumatic stress disorder. Diagn. Interv. Radiol. 11 (3), 125–129.

Malberg, J.E., Eisch, A.J., Nestler, E.J., Duman, R.S., 2000. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J. Neurosci. 20 (24), 9104–9110.

Marais, L., Hattingh, S.M., Stein, D.J., Daniels, W.M., 2009. A proteomic analysis of the ventral hippocampus of rats subjected to maternal separation and escitalopram treatment. Metab. Brain Dis. 24 (4), 569–586.

Marco, E.M., Adriani, W., Canese, R., Podo, F., Viveros, M.P., Laviola, G., 2007. Enhancement of endocannabinoid signalling during adolescence: modulation of impulsivity and long-term consequences on metabolic brain parameters in early maternally deprived rats. Pharmacol. Biochem. Behav. 86 (2), 334–345.

Mathew, S.J., Shungu, D.C., Mao, X., Smith, E.L., Perera, G.M., Kegeles, L.S., Perera, T., Lisanby, S.H., Rosenblum, L.A., Gorman, J.M., Coplan, J.D., 2003. A magnetic resonance spectroscopic imaging study of adult nonhuman primates exposed to early-life stressors. Biol. Psychiatry 54 (7), 727–735.

Mathew, S.J., Price, R.B., Mao, X., Smith, E.L., Coplan, J.D., Charney, D.S., Shungu, D.C., 2008. Hippocampal N-acetylaspartate concentration and response to riluzole in generalized anxiety disorder. Biol. Psychiatry 63 (9), 891–898.

Miller, B.L., 1991. A review of chemical issues in 1H NMR spectroscopy: N-acetyl-L-aspartate, creatine and choline. NMR Biomed. 4 (2), 47–52.

Mirescu, C., Peters, J.D., Gould, E., 2004. Early life experience alters response of adult neurogenesis to stress. Nat. Neurosci. 7 (8), 841–846.

Mohanakrishnan, M.P., Nasrallah, H.A., Lyons, J.A., Scott, M.F., Liberto, V., 2003. Single-voxel proton MR spectroscopy of right versus left hippocampi in PTSD. Psychiatry Res. 123 (2), 101–108.

Murugavel, M., Sullivan, J.M., 2009. Automatic cropping of MRI rat brain volumes using pulse coupled neural networks. Neuroimage 45 (3), 845–854.

Newman, E.A., 2003. New roles for astrocytes: regulation of synaptic transmission. Trends Neurosci. 26 (10), 536–542.

Rosenberg, D.R., Macmaster, F.P., Mirza, Y., Smith, J.M., Easter, P.C., Banerjee, S.P., Bhandari, R., Boyd, C., Lynch, M., Rose, M., Ivey, J., Villafuerte, R.A., Moore, G.J., Renshaw, P., 2005. Reduced anterior cingulate glutamate in pediatric major depression: a magnetic resonance spectroscopy study. Biol. Psychiatry 58 (9), 700–704.

Sanchez, M.M., Ladd, C.O., Plotsky, P.M., 2001. Early adverse experience as a developmental risk factor for later psychopathology: evidence from rodent and primate models. Dev. Psychopathol. 13 (3), 419–449.

Schuff, N., Neylan, T.C., Lenoci, M.A., Du, A.T., Weiss, D.S., Marmar, C.R., Weiner, M.W., 2001. Decreased hippocampal N-acetylaspartate in the absence of atrophy in posttraumatic stress disorder. Biol. Psychiatry 50 (12), 952–959.

Schuff, N., Neylan, T.C., Fox-Bosetti, S., Lenoci, M., Samuelson, K.W., Studholme, C., Kornak, J., Marmar, C.R., Weiner, M.W., 2008. Abnormal N-acetylaspartate in hippocampus and anterior cingulate in posttraumatic stress disorder. Psychiatry Res. 162 (2), 147–157.

Spinelli, S., Chefer, S., Suomi, S.J., Higley, J.D., Barr, C.S., Stein, E., 2009. Early-life stress induces long-term morphologic changes in primate brain. Arch. Gen. Psychiatry 66 (6), 658–665.

Stevenson, C.W., Halliday, D.M., Marsden, C.A., Mason, R., 2008. Early life programming of hemispheric lateralization and synchronization in the adult medial prefrontal cortex. Neuroscience 155 (3), 852–863.

Tsai, G., Passani, L.A., Slusher, B.S., Carter, R., Baer, L., Kleinman, J.E., Coyle, J.T., 1995. Abnormal excitatory neurotransmitter metabolism in schizophrenic brains. Arch. Gen. Psychiatry 52 (10), 829–836.

Villarreal, G., Petropoulos, H., Hamilton, D.A., Rowland, L.M., Horan, W.P., Griego, J.A., Moreshead, M., Hart, B.L., Brooks, W.M., 2002. Proton magnetic resonance spectroscopy of the hippocampus and occipital white matter in PTSD: preliminary results. Can. J. Psychiatry 47 (7), 666–670.

Vythilingam, M., Heim, C., Newport, J., Miller, A.H., Anderson, E., Bronen, R., Brummer, M., Staib, L., Vermetten, E., Charney, D.S., Nemeroff, C.B., Bremner, J.D., 2002. Childhood trauma associated with smaller hippocampal volume in women with major depression. Am. J. Psychiatry 159 (12), 2072–2080.

Wang, J.W., David, D.J., Monckton, J.E., Battaglia, F., Hen, R., 2008. Chronic fluoxetine stimulates maturation and synaptic plasticity of adult-born hippocampal granule cells. J. Neurosci. 28 (6), 1374–1384.

Watson, C., Andermann, F., Gloor, P., Jones-Gotman, M., Peters, T., Evans, A., Olivier, A., Melanson, D., Leroux, G., 1992. Anatomic basis of amygdaloid and hippocampal volume measurement by magnetic resonance imaging. Neurology 42 (9), 1743–1750.

Weniger, G., Lange, C., Sachsse, U., Irle, E., 2008. Amygdala and hippocampal volumes and cognition in adult survivors of childhood abuse with dissociative disorders. Acta Psychiatr. Scand. 118 (4), 281–290.

Woon, F.L., Hedges, D.W., 2008. Hippocampal and amygdala volumes in children and adults with childhood maltreatment-related posttraumatic stress disorder: a meta-analysis. Hippocampus 18 (8), 729–736.

Yildiz-Yesiloglu, A., Ankerst, D.P., 2006. Review of 1H magnetic resonance spectroscopy findings in major depressive disorder: a meta-analysis. Psychiatry Res. 147 (1), 1–25.

Young, E.A., Kornstein, S.G., Marcus, S.M., Harvey, A.T., Warden, D., Wisniewski, S.R., Balasubramani, G.K., Fava, M., Trivedi, M.H., John, R.A., 2009. Sex differences in response to citalopram: a STAR*D report. J. Psychiatr. Res. 43 (5), 503–511.