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

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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 (1H-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
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; Capiziano et al., 2007), anxiety (Mathew et al., 2008), bipolar disorder (Capiziano 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 adolescents 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 (Kalnichev 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 to 14 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 temperature. 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 each): CON, CON + saline, CON + escitalopram, MS, MS + saline, and MS + escitalopram.

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

MRI was undertaken on a 7.0 T animal MRI scanner (7/16 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/O2 (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 T2-weighted imaging (T2WI) in three planes with a fast spin echo (FSE) pulse sequence was first acquired to control rat head positioning. Next, a coronal T2WI 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 1H-MRS, the volume of interest (VOI, 3.5 mm × 3.5 mm × 3.5 mm) was placed over the hippocampal region in the coronal T2WI 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 artificial hemispheric differences.

2.5. Volume measurements of hippocampus

The coronal T2WI 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 T2WI 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, fimbrria and alveus. To account for variations in brain size, hippocampal volume was normalized to intracranial volume (ICV) which was extracted based on the PCNII (Pulse Coupled Neural Network) method (Murugavel and Sullivan, 2009).

2.6. 1H-MRS spectral processing

The 1H-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 1H-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 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.
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 1H-MRS data on one side for some animals, the mean measure reflected the contribution of the other hemisphere.

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 1H-MRS data on one side for some animals, the mean measure reflected the contribution of the other hemisphere. Spectral peaks identified are Myo-inositol (MI), choline-containing compounds (Cho), creatine (Cr), glutamate (Glu) and N-acetylaspartate (NAA). QUEST, quantum estimation.

The results for 1H-MRS measurements were precluded from analysis (see Table 1 for detail).

### 3. Results

Of the 79 animals undergoing neuroimaging studies, the coronal T2WI scan was obtained in each animal, while the 1H-MRS data sets were obtained in 75. In addition, the numbers of samples taken into statistical analysis of 1H-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 mm³) compared with female rats (1112.5±63.3 mm³) [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 examined alone [F(1, 12)=0.453, p=0.507].

#### 3.2. Effects of maternal separation on hippocampus 1H-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)=0.207, 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, p=0.019].
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3.3. Effects of escitalopram administration on hippocampal 1H-MRS measurements

Repeated-measures analysis of variance demonstrated a significant effect of sex on NAA/Cr \( [F(1,44) = 7.355, p = 0.008] \) 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.

<table>
<thead>
<tr>
<th>Laterality</th>
<th>Metabolites</th>
<th>CON</th>
<th>CON + saline</th>
<th>CON + ESC</th>
<th>MS</th>
<th>MS + saline</th>
<th>MS + ESC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left</td>
<td>NAA/Cr</td>
<td>1.19±0.07</td>
<td>1.20±0.08</td>
<td>1.24±0.11</td>
<td>1.18±0.07</td>
<td>1.18±0.04</td>
<td>1.24±0.08</td>
</tr>
<tr>
<td></td>
<td>Cho/Cr</td>
<td>0.18±0.03</td>
<td>0.17±0.04</td>
<td>0.16±0.03</td>
<td>0.16±0.02</td>
<td>0.16±0.03</td>
<td>0.16±0.03</td>
</tr>
<tr>
<td></td>
<td>Glu/Cr</td>
<td>1.12±0.08</td>
<td>1.03±0.19</td>
<td>1.09±0.15</td>
<td>1.07±0.09</td>
<td>1.15±0.12</td>
<td>1.09±0.10</td>
</tr>
<tr>
<td></td>
<td>MI/Cr</td>
<td>0.64±0.05</td>
<td>0.71±0.08</td>
<td>0.74±0.08</td>
<td>0.69±0.05</td>
<td>0.67±0.06</td>
<td>0.70±0.12</td>
</tr>
<tr>
<td>Right</td>
<td>NAA/Cr</td>
<td>1.19±0.08</td>
<td>1.17±0.07</td>
<td>1.28±0.13 #</td>
<td>1.13±0.04</td>
<td>1.13±0.05</td>
<td>1.30±0.10 ***</td>
</tr>
<tr>
<td></td>
<td>Cho/Cr</td>
<td>0.16±0.03</td>
<td>0.17±0.02</td>
<td>0.19±0.04</td>
<td>0.18±0.03</td>
<td>0.19±0.04</td>
<td>0.20±0.04</td>
</tr>
<tr>
<td></td>
<td>Glu/Cr</td>
<td>1.24±0.10</td>
<td>1.15±0.17</td>
<td>1.18±0.12</td>
<td>1.13±0.13</td>
<td>1.06±0.11</td>
<td>1.21±0.13 *</td>
</tr>
<tr>
<td></td>
<td>MI/Cr</td>
<td>0.68±0.12</td>
<td>0.71±0.09</td>
<td>0.76±0.10</td>
<td>0.62±0.06</td>
<td>0.64±0.05</td>
<td>0.71±0.12</td>
</tr>
</tbody>
</table>
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 (Banerjee 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 1H-MRS measurements

The NAA signal in 1H-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, synaptic 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 1H-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 Kimmelberg, 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 Nie and Baoci Shan 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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