

In vivo MRS study of adolescent rhesus monkeys with early life stress experience

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Introduction: Early life stress (ELS) is a major risk factor in the development of anxiety, conduct, and mood disorders and other behavioral problems in children and adults. Nonhuman primates are ideal model for investigating the behavioral consequences of ELS in humans and the underlying neural mechanisms. In vivo Magnetic Resonance Spectroscopy (MRS) provides a non-invasive approach to detect brain metabolite changes, and has been used to detect ELS-induced alterations in brain metabolites both in maltreated children^[1] and monkeys with ELS^[2]. The common finding of reduced Nacetylaspartate/Creatine (NAA/Cr) ratio in both ELS studies, suggests common pathways, perhaps mediated by activation of stress-response pathways, as suggested by the fact that similar neuropathological changes have been detected by MRS in stress paradigms^[3]. Interestingly, alterations in early maternal care without evidence of stress-mediation results in similar reductions of NAA in frontal cortex via unknown mechanisms^[4]. In summary, the neurobiological alterations caused by ELS and their developmental course are not understood in non-human primates or in children. We suspect that cortico-limbic regions that underlie the control of emotional behavior, including the prefrontal cortex (PFC), amygdala (AMYG) and basal ganglia (BG) are impacted. In this study, we used MRS to investigate the metabolic changes in BG and anterior cingulate cortex (ACC) of adolescent rhesus monkeys with ELS.

Materials and Methods: 9 rhesus monkeys (6 years old; 4 male, 5 female) with ELS consisting of infant maltreatment by the mother^[5] and 11 age-matched control monkeys (4 male, 6 female) were used for this study. During MRI scans, animals were immobilized with a custom-built monkey head holder under anesthesia (1-1.5% isoflurane). End-tidal CO₂, inhaled CO₂, O₂ saturation, blood pressure, heart rate, respiration rate, and body temperature were monitored continuously and regulated. A single-loop custom-built receive-only surface coil (ID=7cm) was used for all MRS data acquisition. Single-voxel proton MRS sequence (TR/TE =1500ms/30ms, FA = 70°, 300 averages; voxel volume=0.2ml) was carried out with and without water suppression on a Siemens Trio 3T.

LC-model was used for all MRS data processing and analysis. NAA and choline (Cho) concentrations with %SD < 30% were selected for further statistical analysis. Two-Way ANOVA, followed by a *posteriori* comparison of the means with t-tests when applicable, was used for statistical analysis.

Results and discussion: Examples of single voxel placement in the BG and ACC together with the spectra are shown in Fig. 1. Two-Way ANOVA detected a significant interaction effect of ELS and gender for NAA concentrations in BG ($p < 0.05$). Thus, male monkeys with ELS had significantly lower NAA concentrations in BG (4.6 ± 0.7 mM) than control males (7.5 ± 0.3 mM) (t-test, $p < 0.01$), whereas no significant difference was observed between female ELS and control groups (Fig. 2). These findings suggest that ELS caused long-term alterations of neural integrity in BG and that males were more vulnerable to the detrimental effects of ELS than females. Two-Way ANOVA also detected that female monkeys had a significantly lower Cho concentration in BG (1.2 ± 0.4 mM) than males (1.6 ± 0.3 mM), suggesting potential sex differences in glial cell proliferation. As shown in Fig. 2, no significant changes were found in NAA and Cho concentrations in ACC.

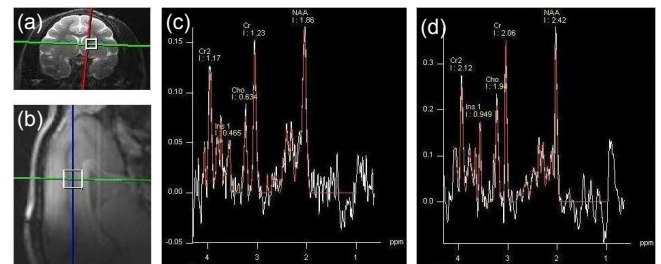


Fig. 1 Illustration of single voxel placement at (a) Basal ganglia (BG) and (b) anterior cingulate cortex (ACC); illustration of the spectra from (c) BG and (d) ACC.

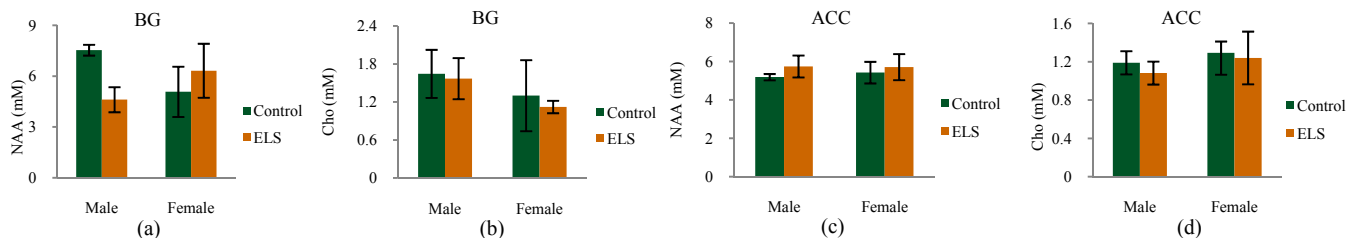


Fig. 2 NAA (a and c) and Cho (b and d) concentration in BG (a and b) and ACC (c and d).

Conclusion: The MRS findings suggest that ELS has an enduring impact on the brains of adolescent male non-human primates, potentially reflecting neuropathological alterations or even neuronal loss in their BG (striatum). Neuropathological alterations could have resulted from stress-induced excitotoxicity at the early ages, interpretation supported by evidence of NAA decreases in striatum after excitotoxic lesions. Males seem more vulnerable to these long-term alterations than females, supporting previous sex differences in vulnerability to ELS. The sex differences in Cho striatal concentrations could be due to differences in glial cell proliferation, although other explanations are plausible and the functional implications of these sex effects warrant further studies.

References: [1] De Bellis, et al., Am. J. Psychol. 157: 1175-1177, 2000. [2] Mathew, S.J., et al., Biol Psychiatry 54: 727-735, 2003. [3] Czeh, B., et al., PNAS 98: 12796-12801, 2001. [4] Poland, R.E., et al., J. Psychiatr. Res. 33: 41-51, 1999. [5] Sanchez, M.M., Horm. Behav. 50: 623-631, 2006.

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