Alteration of Metabolites in the lateral septum of peripubertally stressed rats

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Introduction: For a better understanding of the mechanisms implicated in the long-lasting emotional effects of stress during adolescence, an animal model was used to examine the impact of stress in the lateral septum (LS), a brain region associated with emotional processes using proton magnetic resonance spectroscopy ([1]HMRS). The neurochemical profile of the lateral septum (LS) of male rats subjected to short unpredictable episodes of peripubertal stress (PS) from perinatal day 28 to 42 was investigated. Previous tests showed that PS rats exhibited more aggression than control rats when faced with an unfamiliar male. In this project we aimed to search for potential neurobiological mechanisms (altered metabolites in the lateral septum at basal levels and after corticosterone challenge; molecular changes in synapse-associated proteins; epigenetic modifications) involved in the pathogenesis and maintenance of abnormal aggression displayed by rats submitted to PS and their offspring.

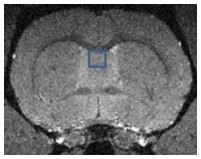

Figure 1:3D-GRE image showing the location of lateral septum within the rat brain

Methods: Animals: 24 Male Wistar rats were subchronically exposed to stress (predator odor and open elevated spaces) during peripuberty (7 days of stress across the P28-P42 period) while 24 male Wistar rats were left undisturbed. The offsprings of these male rats were then further subdivided into 4 groups as described in Table1. The long term effects of peripubertal stress were examined when animals were 3 to 4 months-old. For [1]HMRS, rats (n=6 per group, 495 ± 56 g) were anesthetized using isoflurane (2-3% in O_2). Each subject was placed in a dedicated stereotactic rat holder under continuous isoflurane anesthesia (2% in O_2). The rat body temperature was monitored continuously by a rectal probe and maintained under physiological temperature (37±1°C) using warm circulating water around the animal. **1H-MRS** All the experiments were performed on an actively shielded 9.4T/31cm bore magnet (Magnex, Varian) with 12cm gradients (400mT/m in 120µs). A quadrature Transmit/Receive 17mm surface coil was used. First and second order shims were adjusted using FASTMAP (1) in a 27-45µl volume placed over the lateral septum by reference to the Paxinos atlas (2) and using with a 3D- Gradient Echo sequence (Fig.1). Localized Proton spectroscopy was performed using SPECIAL (3). 40 blocks of 16 FIDs were acquired for a total acquisition time of 40 minutes. Metabolite concentrations were calculated using LCmodel (4). Absolute metabolite concentrations were obtained using unsuppressed water signal as a reference.

Table1: Groups of rats analyzed in the study (n=6/per group)

Control- Control **(C-C)**	Offsprings of control Males left undisturbed
Control- PS **(C-S)**	Offsprings of control Males submitted to peripubertal stress
PS-C **(S-C)**	Offsprings of peripubertal stressed Males left undisturbed
PS-PS **(S-S)**	Offsprings of peripubertal stressed Males submitted to peripubertal stress

Results and Discussion: In general, shimming in the LS was easy, the typical linewidth of the water resonance was 12-15Hz. Spectra exhibited good signal to noise ratio and differences in metabolites signals were noticeable (Fig.1).The neurochemical profile (mean± SEM) measured in the lateral septum of control and peripubertally stressed offsprings is shown in Fig.3. Statistical analysis of metabolite concentrations by two-way ANOVA revealed a significant effect of the stress condition on rats ($p<0.0008$). In particular, GABA levels significantly decreased (-50% in C-S relative to C-C, -30% in S-S relative to S-C) while NAA and Glu levels increased in C-S and S-S relative to both C-C and S-C groups of rats. Follow-up post- mortem immunohistochemical analyses also revealed a reduction of the GABA-synthesizing enzyme gad67 emphasizing the potential role of GABAergic mechanisms in the lateral septum of the peripubertally stressed rats (data not shown). Moreover, GABA levels were negatively correlated with adult aggression challenge associated plasma corticosterone levels in PS groups but not in control groups (data not shown). After an I.P injection of 2.5mg/kg of corticosterone in each rat, LS GABA levels were significantly enhanced in both C-S (+80%) and S-S (+44%) groups compared to basal levels. The present results demonstrate the potential role of GABA in the lateral septum for modulating stress during puberty. Evidence that LS is involved in the hypothalamo-pituitary-adrenocortical (HPA) axis regulating the release of stress hormones was shown in previous studies (5). Here, in addition to GABA changes, Glutamate and N-Acetyl-Aspartate were modulated in PS groups. [Glu] was elevated in other studies of mood disorders and decreased upon antidepressant treatment (5). NAA is considered reflective of neuronal density/functional integrity. In psychiatric disorders, an [NAA] increase is often viewed as a marker of restoration of neuronal viability (6). However, upregulation of NAA also leads to oxidative stress and upregulation of nitric oxide according to recent studies (7).

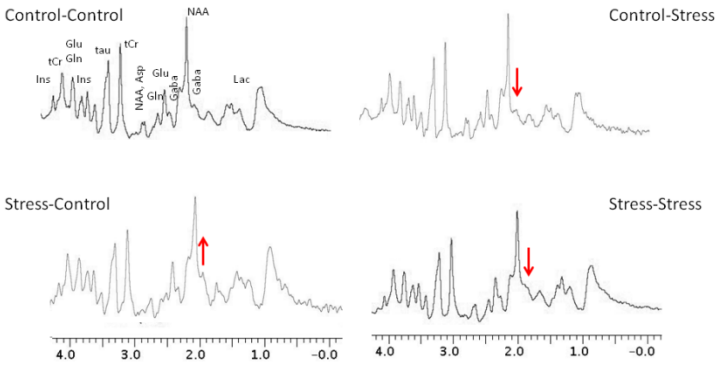

Figure 2 : SPECIAL 1H spectra in the lateral septum of a C-C rat, a C-S rat, a S-C rat and a S-S rat.

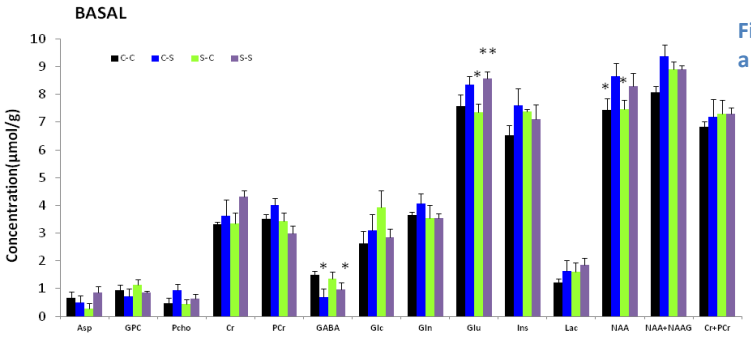

Figure 3:Neurochemical profile (Mean ± SEM) in the Lateral septum of Control and peripubertally stressed rats. **<0.004, *<0.05

In conclusion, 1H-MRS in vivo is capable of detecting metabolic changes in the lateral septum of peripubertally stressed rats and may provide additional information into GABAergic and glutamatergic alterations caused by peripubertal stress disorders and their consequences in adulthood.

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