Longitudinal 1H MRS of Hamster Superior Colliculus following Retinotectal Deafferentation

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INTRODUCTION: This study aims to employ *in vivo* proton magnetic resonance spectroscopy (^{1}H MRS) to evaluate the trans-synaptic effect of acute axonal injury to the superior colliculi (SC) by monitoring longitudinally the metabolic changes in the SC following deafferentation of the optic tract in adult hamsters. In addition, a self-designed, self-assembly peptide nanofiber scaffold (SAPNS) was applied to the lesion (2), and the metabolic changes in the SC were compared with saline sham treatment.

METHODS: ***Animal Preparation:*** Adult male Syrian hamsters (130-175 g, N = 17) were divided into 3 groups. In the first 2 groups, the optic tract was deafferented at the brachium of the left superior colliculus (BSC) (1) as in Figure 1. 30µL of saline (Group 1, n = 6) or 1% SAPNS solution (Group 2, n = 5) was applied to the site of transection respectively right after BSC cut, and ^{1}H MRS was performed on each side of the SC at 3, 14, and 28 days after treatment. Six age-matched normal hamsters were scanned as controls in Group 3.

MR Imaging and Spectroscopy: All MR measurements were acquired utilizing a 7T Bruker PharmaScan 70/16 scanner. Under inhaled isoflurane anaesthesia (3% induction and 1.5% maintenance), animals were kept warm at 37°C on the heating pad and were imaged using a 38 mm rat brain quadrature resonator. RARE T1WI and T2WI were acquired for morphological evaluations and subsequently for accurate placements of single voxels for ^{1}H-MRS. After shimming with FASTMAP, ^{1}H-MRS was performed using a PRESS sequence with TR/TE = 2000/15 ms and NEX = 128. A 3x3x3 mm^3 voxel was placed over the ipsilesional superior colliculus as in Figure 1, and another contralateral to it.

Data Analysis: Metabolite ratios were calculated and compared between the intact and cut superior colliculi using Sub-QUEST method in jMRUI (2), with apodization by a 15Hz Gaussian filter, removal of residual water component by HLSVD using 25 spectral components, and 22 truncated initial data points. The numerical time-domain modal functions of 11 metabolites were quantum mechanically simulated in NMR-SCOPE for the *in vivo* experimental protocol and were used as prior knowledge in QUEST. NAA:Cr, Cho:Cr, Glu:Cr, Lac:Cr and mI:Cr ratios were statistically evaluated using Students' two-tailed paired t-tests. Results were considered to be significantly different when $p<0.05$. Cramer-Rao lower bounds were below 15% of estimated amplitudes for most of the metabolites of interests quantitated.

RESULTS: In the saline-treated group as in Figures 2 and 3, a significant decrease in both NAA:Cr and Glu:Cr was observed in the ipsilesional SC compared to the intact SC at Day 3, which was then normalized at the later time points. Significant increase in Cho:Cr was observed in the transected side of SC at Day 3, whereas significantly elevated but gradually decreasing Lac:Cr ratios were observed throughout the 4-week experimental period. mI:Cr was indifferent bilatearlly early after deafferentation, but increased at Day 14 when the extent of Lac:Cr increase was reduced at the same time. Similar patterns were found in the Group 2 which had been treated with SAPNS as in Figure 3, except that no significant differences were obtained in NAA:Cr, Cho:Cr and Glu:Cr ratios at Day 3. No statistically significant difference was observed in the metabolite ratios between the unlesioned side of SC and the normal SC in Group 3.

DISCUSSIONS: Previous *in vitro* ^{1}H MRS studies demonstrated a transient decrease in NAA of SC following stretch injury of guinea pig optic nerve at Day 3 (3), likely due to changes of NAA metabolism caused by functional neuronal inactivity rather than neuronal loss, injury or dysfunction. It was also reported in other neurochemical studies that glutamate content was transiently reduced in the rat superior colliculus after afferent lesions (4-6). Lac:Cr was significantly higher in the transected side of SC at all time points possibly due to acute inflammation and demyelination (7). Disruption of the blood-brain barrier and cerebral edema after injury might trigger upregulation of the sodium/myo-inositol transporter and facilitate mI transport into neural cells at later time points. mI was also found in astrocytes and might increase as a result of inflammation or reactive gliosis (8). Though the exact mechanisms of SAPNS treatment to the site of injury were still uncertain (1), it has been demonstrated that SAPNS could provide immediate hemostasis (9) and permit axonal growth through the site of treated lesion (1). Given the current observations, it is possible that upon SAPNS treatment, functional neuronal activity and glutamate neurotransmission along the retinotectal pathway could be partially preserved. Further investigations are currently undergoing to address this issue. Limitation of the current study included partial volume effect of the voxel.

CONCLUSION: ^{1}H MRS may help monitor metabolic changes in the superior colliculi upon BSC transections, and is a potential tool for the study of functional effect of CNS lesions in vivo.

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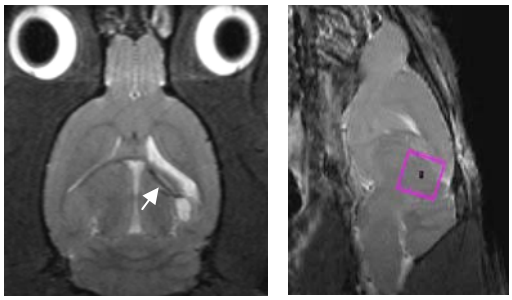

Figure 1: (Left) Typical axial T2WI of the brain, showing the transection site at the brachium of left superior colliculus (arrow). (Right) Localization of the 3x3x3 mm^3 volume of interest on the superior colliculus in sagittal T2WI.

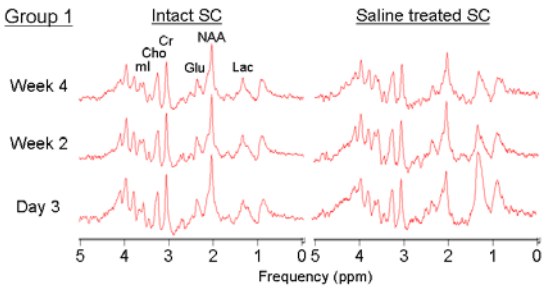

Figure 2: Averaged spectra of Group 1 animals on both sides of SC.

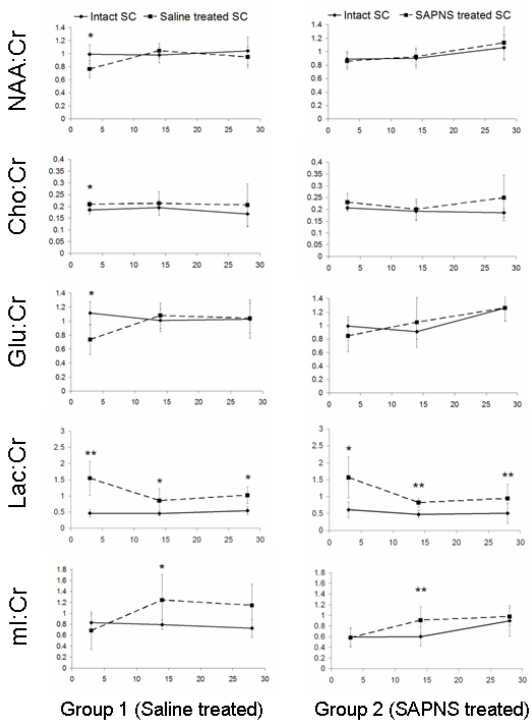

Figure 3: Metabolite ratios in the intact (solid lines) and transected (dashed lines) sides of the SC. (paired t-test between contralateral superior colliculi, * $p<0.05$, ** $p<0.01$). y-axes: Means of Ratios ± 1 standard deviation; x-axes: number of days after BSC cut.