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Longitudinal Metabolic Changes in the Thalamus of Macaque Brain During 42-Day Head Down Tilt

Xiao-Yun Zhang,[1] Hua-Song Ma,[2] Li Liu,[1] Zhong-Quan Dai,[3] Juan Du,[1] Zheng Wu,[1] Xu-Sheng Huang[4] and Yi-Ling Cai[1,*]

[1]*Department of Neurology, 306th Hospital of PLA. Beijing 100101, China*
[2]*Department of Orthopedics, 306th Hospital of PLA. Beijing 100101, China*
[3]*Department of Biomedical Engineering, the China Astronaut Research and Training Center. Beijing, 100101, China*
[4]*Department of Neurology, Chinese People's Liberation Army General Hospital, Beijing 100853, China.*

Xiao-yun Zhang and Hua-song Ma have contributed equally to this work.

ABSTRACT

Basic changes in environmental conditions are fundamental to understanding brain mechanisms. Several studies have reported impairment of central nervous processes during weightlessness. In this study, neurobiological alterations during 6°head down tilt (HDT) were investigated longitudinally using non-invasive proton magnetic resonance spectroscopy (^{1}H MRS) at 3T in region related to cognitive and movement, such as the thalamus. Scans were performed in five rhesus monkeys before HDT and at 7, 21 and 42 days after HDT. Spectra were processed using LC Model. Statistical analysis of the data obtained demonstrated no significant difference in the bilateral thalamus of macaques. Our MRS data showed reduced N-acetylaspartate (NAA) ($p<0.05$) glutamate/glutamine (Glx) ($p<0.05$) and elevated myo-inositol (mI) ($p<0.05$) in the bilateral thalamus during HDT, and all metabolites approached their baseline levels by the fourth scan. These results demonstrate that metabolic changes occur in the thalamus during HDT in rhesus monkeys. The ^{1}H MRS detection of ongoing neuro chemical changes induced by HDT, especially in the thalamus, may contribute to reduce motor control abilities and multiple executive functions in astronauts in a microgravity environment.

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Authors' Contributions
XYZ, XSH and YLC designed the experiment and wrote the article. HSM and ZW conducted the NMR scanning. LL and SQD worked with animal model. JD collected and analyzed the data.

Key words
Metabolic changes, thalamus, Macaque brain, head down tilt.

INRODUCTION

Microgravity has well-documented influences on cardiovascular, muscle and bone physiology (Adams *et al.*, 2003; Aubert *et al.*, 2005). There has been an increased focus on the interactions between central nervous activity and microgravity in recent years (Manzey *et al.*, 1993b; Fowler *et al.*, 2000). Microgravity have been related to decreases in both sensorimotor (Bock *et al.*, 2001) and cognitive abilities (Manzey, 2000). According to Newman and Lathan (1999) microgravity have effects on manual control movements, which indicated adisruption in fine motor control (Newmann and Lathan, 1999). In two studies, on microgravity by Manzey *et al*. (1993b, 1998) obvious decrements in visual motorfunction and tracking performance were observed. Additionally, several studies indicated that planning movements andgoal-directed actions, which require fine motor control, are also impaired in microgravity (Berger *et al.*, 1997; Sangals *et al.*, 1999; Watt, 1997). These degradations in behavioral and cognitive functions may cause potential risks in space exploration. So, to reduce the possibility of an accident, determining the mechanisms of behavioral and cognitive performance degradations in microgravity and the corresponding counter measures are of great significance and must be challengeable tasks for aerospace medicine researchers.

Changes have been observed in volumes of cerebrospinal fluid, cerebral blood flow, altered intracranial pressure and electroencephalograph (EEG) in astronauts in a weightless environment. According to Liao *et al.* (2012) and Zhengzhang *et al.* (2012) functional magnetic resonance imaging and voxel-based morphometry have shown that the thalamus is always involved in the progression of HDT. Their findings were encouraging; however, because of the limitations of their research, they could not clarify how the physiological changes in the brain contributed to the observed performance degradations. But now with the development of new experimental techniques, the application of imaging methods could complement the limitations of previous studies. Metabolic information provides intrinsic characteristics of neural development that may not be represented in structural and functional data as reported by Degnan *et al.* (2014). However, up to now there are few studies of brain alterations in

* Corresponding authors: caiyiling306@126.com
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weightlessness.

Proton magnetic resonance spectroscopy (1H MRS) is a non-invasive technique for the characterization and measurement of in vivo brain metabolites. N-acetyl-aspartate (NAA) isone of the most prominent peaks and reflects neuronal integrity, with decreasedconcentrations indicating neuronal loss and dysfunction (Klar *et al.,* 2010; Reynolds and Reynolds, 2011). The creatine (Cr) signal includes concentrations of intracellular Cr and phosphocreatine (PCr), providing a measure of bioenergetic metabolism as stated by Kraguljac *et al.* (2012). By Cr actively facilitate energy transport and metabolism and also plays a key role in maintaining steady brain energy production and consumption levels (Kraguljac *et al.,* 2012; Uhl *et al.,* 2011). Myo-Inositol (mI) is an astroglial marker that aids in the regulation of cell volume, neuronal metabolism and energy consumption (Fisher *et al*., 2002; Oz *et al*., 2010). Other prominent metabolites, albeit at a lower limit of detection, include excitatory neurotransmitter glutamate (Glu) and glutamine (Gln). Detection and quantification of these peaks are better resolved at high field and benefit from the use of specialized spectroscopic sequences.

The entire thalamus is defined as a single region of interest (ROI). However, neurochemical changes of the thalamus in vivo remain largely unexplored. The current study was designed to examine possible alteration in thalamus metabolism during HDT. The results of this study may support the findings of previous studies and serve as a foundation for additional studies of individual altered brain activities in a microgravity environment.

MATERIALS AND METHODS

All experimental procedures with animals in this study were approved by the ethical committee of China Astronaut Research and Training Center. The entire procedure was performed strictly according to the guidelines of the institution.

Animals

The ground-based macaque HDT and the Wronski and Morey-Holton tail-suspension rat models are two widely accepted, reliable, and effective animal models to study the influence of gravitational unloading on brain function. The brain of the macaque is the most similar to that of humans; therefore, HDT on macaques as a model in the present study was selected. Five (2-5 years old) macaques were laid on a bed, which was tilted backward 6° from the horizontal. The head down animals wore a special cloth, which allowed us to fix them to the bed, but their arms and legs were free to move and access food and water. The model reduces mechanical loading on the hind limbs and produces acephalad fluid shift similar to that encountered during spaceflight. Macaques were provided a regular dietary program, which included primate biscuits, necessary fresh fruits and additional vitamin syrup. Animals were housed one per cage or bed in the macaques rooms maintained at 23±2°C, and on a standard 12:12-h dark-light cycle. The animal general health condition was monitored carefully.

Preparation for MR studies

All animals were scanned at baseline (scan 1), and then 7 (scan 2), 21(scan 3) and42 days (scan 4) after HDT. Prior to being removed from their beds, animals were tranquilized with intramuscular ketamine hydrochloride (15–20 mg/kg). All animals were intubated, and their lungs were mechanically ventilated with a mixture of 0.8% isoflurane and nitrous oxide–oxygen gas (7:3) over the duration of the MR procedure. Intravenous atropine (0.4 mg/kg) was administered to prevent bradycardia. The heart rate, oxygen saturation, end-tidal CO_2 and respiratory rate were monitored and recorded continuously. A heated water blanket was used and the rectal temperature was monitored to prevent hypothermia.

MRS

Imaging was performed on a 3-T MR scanner (Trio 3 T, Siemens Healthcare, Erlangen Germany) using a 12-channel head coil for both MRI and MRS measurements.T1-weighted images of the rhesus brain were performed in three orthogonal planes (sagittal, coronal and transversal) to permit the localization of the voxels of interest. Single-voxel point-resolved spectroscopy data were collected (TR, 2000 ms; TE, 30 ms; average, 128; spectral bandwidth, 1200 Hz, 1024 data points). For MRS, volumes of interest with a size of 10mm x 10mm x 10mm were positioned in the right and left thalami (Fig. 1). During MRS scanning, zones of saturation were used to avoid cerebrospinal fluid and other tissue contamination. The water signals were acquired from the same voxels of the right and left thalami.

The MRS data were post-processed with LCModel software (Version 6.1, Stephen Provencher Inc., Oakville, Ontario, Canada). The metabolites NAA, glutamate + glutamine (Glx), mI and Cr were analyzed using the internal water calibration method in LCModel.

RESULTS

The metabolite concentrations of all voxels investigated are presented in Table I.

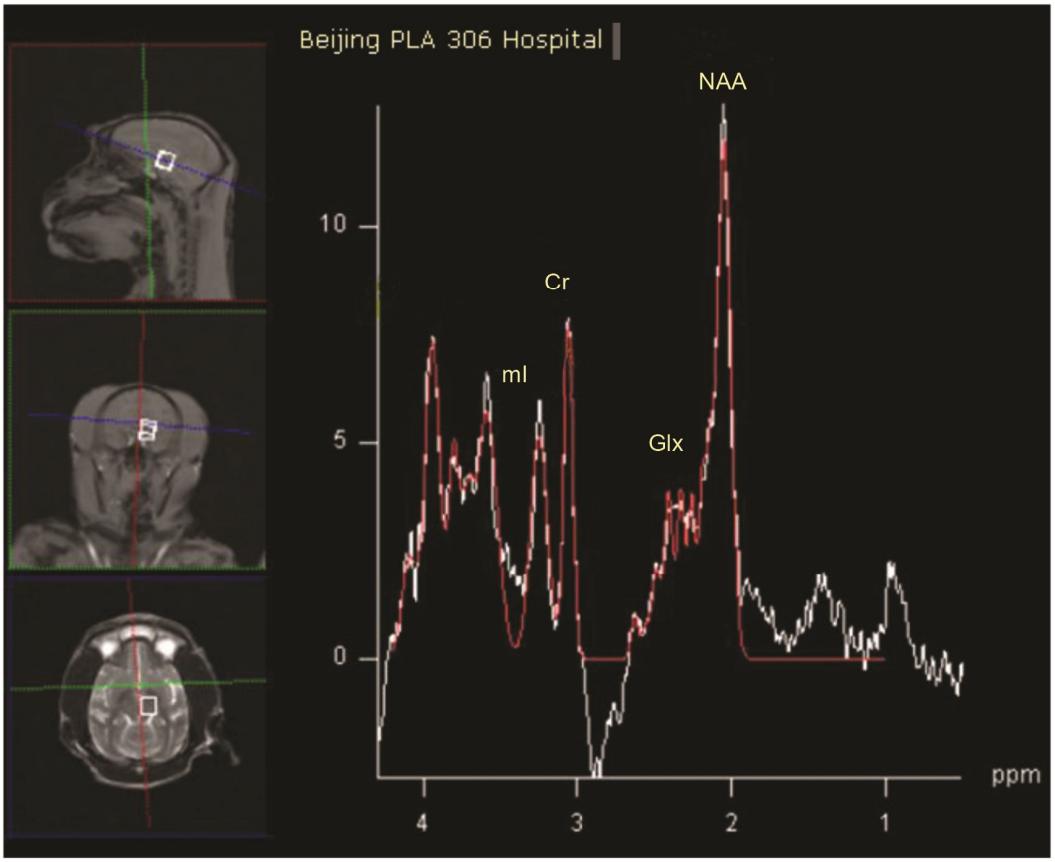

Fig. 1 .Voxels (white frame) in thalami and related spectra in a rhesus monkey (No. 1). The thin red line shows the LCModel fit to the spectra. Cr, creatine; Glx, glutamate + glutamine; mI, myo-inositol; NAA, N-acetyl aspartate.

Table I.- Mean metabolite concentrations (mM)±SD in the left and right sides of the thalamus at different time points.

		NAA	Glx	mI	Cr
Baseline	Left	6.42±0.54	12.21±1.63	5.86±0.23	6.61±0.24
	Right	6.13±0.21	11.97±1.42	6.09±0.55	6.69±0.36
Time after HDT					
7 days	Left	5.53±1.01*	10.67±1.07*	5.99±0.42	6.29±0.25
	Right	5.43±0.82*	10.92±1.11*	5.85±0.37	6.43±0.21
21 days	Left	5.49±0.83*	11.08±1.21*	6.85±0.31*	6.35±0.18
	Right	5.65±0.89*	10.81±0.96*	6.51±0.21*	6.47±0.29
42 days	Left	6.34±0.47	12.11±1.01	5.95±0.19	6.55±0.33
	Right	6.04±0.64	11.89±1.22	5.81±0.38	6.46±0.41

* Significant analysis of variance effects compared with baseline
Cr, creatine; mI, myoinositol; Glx, glutaminet glutamate; NAA, N-acetyl aspartate.

NAA

There was no difference in NAA concentration between the right and left thalami in monkeys. Seven days after HDT in the bilateral thalamus, the NAA concentration dropped significantly of the baseline concentration (left, F = 9.15, p = 0.0032; right, F=5.42, p = 0.0192), and reached a plateau level by 21days (Fig. 2). By 42 days after HDT (scan 4), the NAA levels were indistinguishable from the baseline level (F = 1.05, p = 0.32).

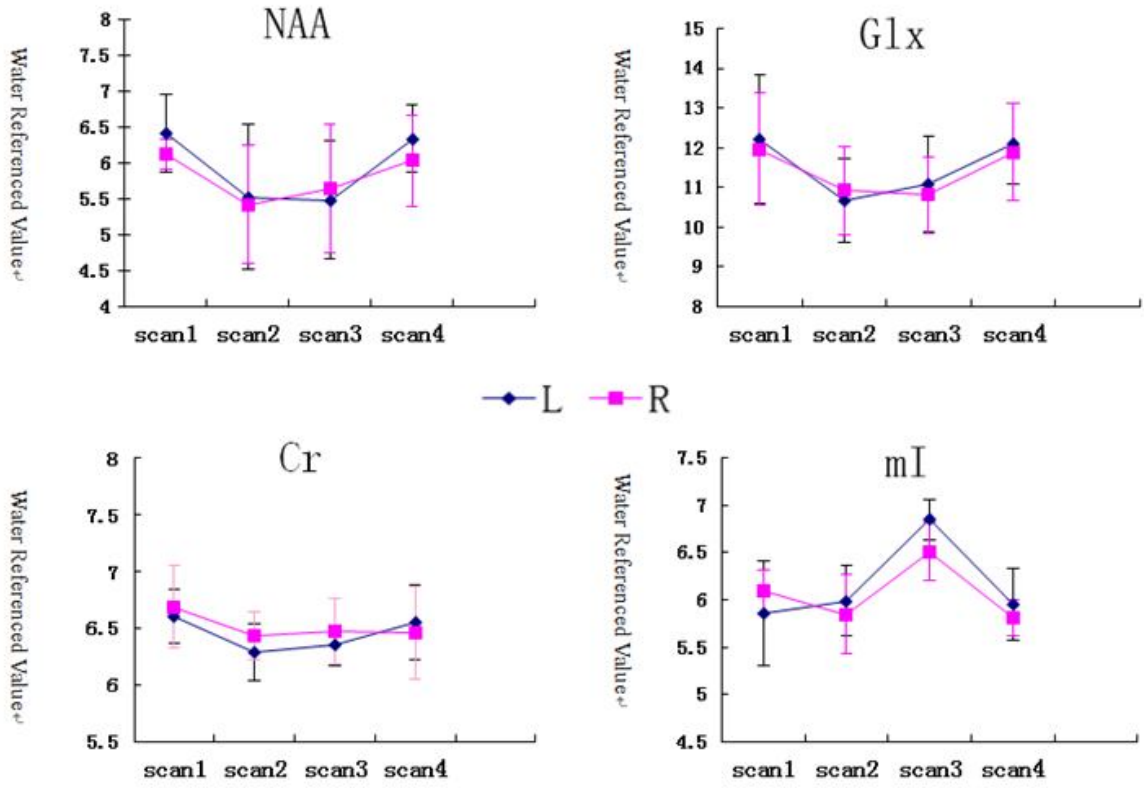

Fig. 2. Water-referenced metabolite levels in the left (L) and right (R) thalami at baseline (scan 1), and 7 (scan 2), 21 (scan 3) and 42 days (scan 4) after HDT. Cr, creatine; Glx, glutamate + glutamine; mI, myo-inositol; NAA, N-acetylaspartate.

Glx

In the bilateral thalamus, a significant decrease in Glx was observed7 day following HDT (left, F = 12.05, p = 0.0032; right, F=6.79,p = 0.0192). By the third scan, 21 days after HDT, the Glx levels in the thalamus were still decreased compared with the first scan (left, F=8.760,p = 0.0092; right, F = 8.98, p = 0.0085) (Fig. 2).The decrease was followed by a significant increase in Glx, as seen in the scan performed 42 days following HDT (left, F=6.31, p=0.0631; right, F = 6.35, p = 0.1227).

mI

mI increased21 day after HDT in both the left and right thalami compared with the baseline measurement in the healthy macaque (left, F=8.45, p = 0.0103; right, F=31.16, p<0.0001). There were no significant differences in mI concentrations between scan 1, scan 2 (left,F=1.03, p = 0.3261; right, F=2.70, p = 0.1201) and scan 4 (left, F=0.05, p = 0.8256; right, F=2.49, p = 0.1338).

Cr

HDT did not affect significantly Cr in the thalamus.

When comparing the symmetry of the analyzed parameter distribution between the right and left thalami, statistical analysis showed that there were no significant differences in the levels of NAA (F = 6.34, p = 0.12), Glx (F = 2.04, p = 0.45), mI (F = 5.91, p = 0.66) and Cr (F = 4.58, p = 1.03).

DISCUSSION

The aim of this study was to identify metabolic brain alterations in HDT that relate to cognitive decline and performance degradation. The thalamus was focused on as a brain region centrally and tested NAA and a broad range of other brain metabolites during HDT. The primary findings were reduced concentrations of neuronal metabolites NAA and Glx, accompanied by an elevation of mI in the thalamus. Metabolic changes in these regions

would provide neurobiological information related to sensory input, cortical arousal, memory and language as per stated by Katz and Shatz (1996).

This is the first study to perform MRS on the thalamus in HDT. This region was mainly selected because of its central role in cognitive functions particularly affected in HDT (Liao *et al.,* 2012), but also because of the fact that the thalamus is only affected by subtle volume loss compared to other brain areas such as frontal lobes or striatum (Zhengzhang *et al.,* 2012), which simplifies MRS analysis.

NAA is primarily located in neuron bodies, axons, and dendrites and it is a sensitive marker for neuronal density or viability (Kantarci, 2013). Therefore, the reduction and recovering in bilateral thalamic NAA following HDT, likely indicated a dysfunction in local neurons. As for the remarkable decrease in NAA, an alternative interpretation may be as follows: measures of the higher cognitive function correlated strongly with the thalamus metabolites, which correlated only with NAA (Unschuld *et al.,* 2012); previous study had reported reduced NAA and glutamate levels relate to short/long-term memory (Sharma *et al.,* 2011) decreased cognitive performance and possibly precede structural brain changes (Kreis *et al.,* 2011). Other interpretations of our results may be due to abnormal dopaminergic neurons. It is suggested that the function of the medial portion of the thalamus is mainly modulated by dopaminergic afferents Rizzo *et al.* (2012). Indeed, an extensive mesothalamic and nigrothalamic system originates as collaterals from A8–A9–A10 dopaminergic neurons (Freeman *et al.,* 2001). Thus, dopaminergic axons directly innervate thalamic components of several basal ganglia-thalamo-cortical loops in nonhuman primates' and humans (Rye, 2004). Accordingly, the thalamic metabolic alteration may lead to abnormal affective motivational processing of the sensory inputs.

Glutamate (Glu), Glutamine (Gln) and gamma-aminobutyric acid (GABA) result in a complex of peaks (Glx) between 2.05 and 2.5 ppm. Glu and Gln play a role in detoxification and regulation of neurotransmitters. Glu, which is viewed as an important neurotoxin is also a participant in the redox cycle (Danielsen and Ross, 1999). Although ^{1}H-MRS may reliably detect the complex signal Glx at short echo times (Danielsen and Ross, 1999). Further studies are needed to determine whether GLx elevation directly represents elevation of glutamate or glutamine or an increase in glutamatergic neurons, and thus, Glx values should be interpreted with caution (Simister *et al.,* 2003).While we find reduced levels of Glx during HDT, earlier literature supports glutamatergic excitotoxicity as a significant mechanism in the pathogenesis of cognitive dysfunction (Khema *et al.,* 2011). For neurons glutamatergicexcito toxicity has been suggested to result from increased levels of glutamate as a neurotransmitter but also possibly through increased sensitivity of glutamate receptors in a context of generally lower abundance of glutamate (Estrada-Sanchez, 2008; Roze *et al*., 2008). The glutamate levels measured in present study by MRS approach however, are not specific for synaptic transmission, but rather reflect cellular integrity of viable neurons which may decrease with progressing cognitive dysfunction.

mI increased in the thalamus. mI is major osmolytes and is also known as an astrocytemarker. Brain cells are sensitive to extracellular tonicity; hence changes in the ion concentration could alter the excitability of the brain (Robertson *et al.,* 2001). Several cellular processes in the central nervous system (CNS) involve mI, such as the intracellular signal transduction, cell membrane structure, cell adhesion, vesicular trafficking and, more importantly, modulation of cell volume during persistent osmotic stress (Berridge, 1993; Thurston *et al.,* 1989). Both changes in mI/Cr and Lac/Cr ratios could be associated with Na+/myo-inositol cotransporter (SMIT) (Yamashita *et al.,* 1996, 1997). The increase in mI in the thalamus could be ascribed to the regulation of osmolalityvia SMIT, which prevents excitotoxic damage to neuronal cells. Previous HMRS studies showed that mI might play an essential role in the mechanisms of brain adaptation and plasticity (Bernabeu *et al.,* 2009*;* Rango *et al*., 2008). Thus, sustained higher levels of mI in the thalamus may provide new insights into the plasticity of the maternal brain, although it will require further investigation to understand the detailed mechanisms.

The findings of this study are discordant with the results of previous studies, one of which found no differences in metabolite concentrations between the right and left thalami. In the present study, there was not ipsilateral to the handedness in the majority in our nonhuman primates. However, further studies are needed to determine the exact mechanisms underlying the neuroanatomical and neurophysiological changes in this region.

Some potential limitations of the present study should be taken into consideration. Firstly, the relatively small sample size may have limited our ability to detect minor changes in metabolite concentrations. Nonetheless, as suggested by the reported effect sizes from studies that examined the brain ^{1}H MRS metabolites, present study with 5 subjects had adequate statistical power. Secondly, only the thalamus was investigated in this study. Therefore, even though function and structure only changed in the thalamus from previous study, caution should be taken when findings are extrapolated to other brain areas. However, the small sizes of brain region,

such as amygdala which plays a primary role in the processing of memory and emotional reactions, in macaques make it difficult to acquire reliable spectra with a sufficient signal-to-noise ratio to detect metabolic changes. Thirdly, it might be better if the study could be added in further longitudinal study.

In conclusion, this study demonstrates metabolic differences in the macaques during HDT by using *in vivo* ^{1}HMRS. This non-invasive approach enables the longitudinal monitoring of biochemical alterations during different stages of HDT. The findings of this study provide neurochemical information from the macaques brain, which may underlie the behavioral changes associated with HDT. Such detailed information may also be important when assessing cerebral pathology during HDT with ^{1}H MRS.

Conflict of interest

The authors declare no conflict of interest.

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