# T2 MR Relaxometry Study of Acute Heat Stress induced changes in Rat Brain at 7T

Sunil Koundal[1], Sonia Gandhi[1], Rajendra P Tripathi[1], and Subash Khushu[1]
[1]*NMR Research Centre, INMAS, Delhi, Delhi, India*

**Introduction:** Thermal condition influences the development of living organisms in a wide variety of ways triggering various adaptive responses[1]. Exposure to thermal stress poses a significant problem as it affects physiological and cognitive performance in humans[2], alters the concentration of selected neurotransmitters and hormones, affects the development of neural pathways and hippocampal activities in rats[3], causes hypohydration, and effects on gene expression. Hyperthermia associated with heat stroke is a life threatening illness, particularly when the body temperature reaches beyond 40°C. Severe hyperthermia induces central nervous system (CNS) dysfunction, such as delirium, convulsion and coma[4]. Many hypotheses have been proposed regarding heat induced neurotoxicity in brain caused due to oxidative stress[5]. Hyperthermia has been reported to enhance numerous forms of reactive oxygen species (ROS) including superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide ($H_2O_2$), hydroxyl radical •OH, nitric oxide •NO and peroxynitrite ($OONO^-$) in various cell types[6] which plays an important role in heat induced neuronal cell degeneration[7]. MRI has been used as a powerful tool to trace abnormality in brain caused due to heat stroke[8]. T2 relaxometry studies due to heat stress are almost negligible in literature & can prove to be a very useful tool in tracking initiation of heat induced changes in brain.

**Aim & Objective:** To study the effects of heat stress on rat brain using T2-weighted MR Imaging technique at 7Tesla.

**Materials and Methods**: 8-10 week old male Sprague-Dawley rats (200±20g, n=5) were housed in polypropylene cages and fed with a certified standard rat chow and tap water *ad libitum*. Room temperature & humidity were regulated at 24±1$^0$C & 30±1%, respectively. Rats were acclimatized in cages & animal room was maintained on a 12h light/12h dark cycle. Rats were exposed to acute heat stress of 40±1$^0$C for 1hr on day 1 in customized climatic chamber (Sevenstar Pvt. Ltd, India). Rats were anaesthetized by Intraperitonial (IP) route and baseline T2 weighted images were acquired before heat treatment using RARE-VTR pulse sequence (TR = 2500 ms and 6 echoes with TE = 11–99 ms, two signal averages ,matrix =256 X 128, FOV=35mm, resolution =0.137 X 0.237, slice thickness =2.0mm). Similarly, T2 weighted images were acquired on same group of rats after 1 hr heat treatment on day 1 & thereafter on day 3 to check whether rats were recovering back from heat stress. Bruker Paravision software was used to fit the decay curves to single exponential function & region of interest (ROI, 0.00224 cm$^2$) were placed over cortex, hippocampus, thalamus & hypothalamus (Fig. 1).Statistical analysis of the data was performed using one way ANOVA & the regions showing significant difference ($P< 0.05$) were calculated to understand the changes between control group before & after heat stress.

**Results:** T2 relaxometry studies on various regions of rat brain pre & post heat stress (1 hr at 40°C) showed a significant decrease in T2 values for Hippocampus & Thalamus regions (Table 1). T2 weighted images were acquired 72 hrs post heat exposure to study the recovery phase but the results (Table 1) showed further significant decrease in T2 values for these regions indicating a possible slow recovery or permanent changes induced due to heat exposure. Cortex & Hypothalmus also showed a decrease in T2 values but they were not significant up to $P< 0.05$.

**Discussion:** T2 values for hippocampus & thalamus regions in rat brain showed significant changes on heat exposure of 40°C for 1 hr. These changes can possibly be attributed to the fact that heat stress induces inhibition of superoxide dismutase and accumulation of reactive oxygen species (ROS) which leads to neuronal cell death[1]. This oxidative stress induces increased Heme oxygenase activity (HO-1 or HSP-32), which converts free Heme into iron, Carbon monoxide & Biliverdin (antioxidant) thus contributing to protection against oxidative stress[9]. From previous literature, induction of HO-1 by hyperthermia has been examined in intact rat brain & it was found that the HO-1 mRNA increases 30-40-fold as compared to that of the control value[10]. It has been studied that stress induced HO-1 expression leads to accumulation of iron in thalamic regions[11]. Our results suggests that heat stress may lead to iron accumulation in some areas of Thalamus & Hippocampus in rat brain as a result of oxidative stress induced HO-1 gene response & significant decrease in T2 relaxation time in these areas may be due to deposition of iron which causes local magnetic field inhomogeneities, which is proportional to the square of the field strength. These accumulated iron & ROS reacts with lipids, proteins, & DNA causing lipid peroxidation, altered protein conformations, & DNA damage respectively. Such damage leads to induction of apoptosis (programmed cell death) or necrosis[6]. This accumulated ROS plays an important role in heat induced neuronal cell degeneration[7]. Hence, heat stress induced inhibition of superoxide dismutase enzyme in the hippocampus region plays important role in controlling cellular ROS levels particularly superoxide anion radicals. Further, our results showed a significant decrease in T2 values 72 hrs post heat exposure indicating possibly a slow/insignificant recovery phase or permanent cell/tissue damage.

| Brain Region | Cortex (mean±SD) | Hippocampus (mean±SD) | Thalami (mean±SD) | Hypothalamus (mean±SD) |
|---|---|---|---|---|
| (a) Pre-treated | 48.3±0.43 | 56.4±2.0 | 49.6±0.9 | 57.2±1.5 |
| (b) Post-treated (1hr post exposure) | 47.9±1.4 | 55.2±2.0 | 48.1±0.2 | 57.0±0.3 |
| (c) Recovery phase (72 hrs post exposure) | 47.1±1.4 | 53.8±1.2* | 47.3±1.4* | 56.9±1.5 |

*Significant at 0.05 level

*Table 1: T2 relaxation values of rat brain regions (a) Before heat stress (b) Post heat stress (1hr post exposure) (c) Recovery phase (72 hrs post exposure).*

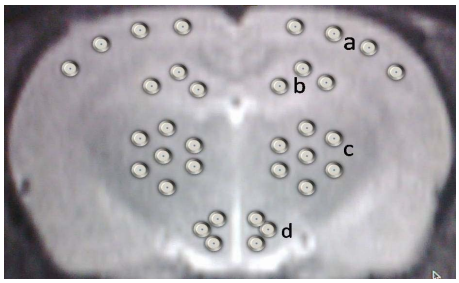


*Fig 1: T2-weighted image of rat brain. Region of interest (ROI) were placed on (a) Cortex (b) Hippocampus (c) Thalamus (d) Hypothalamus.*

**Conclusion:** T2 MR relaxometry studies is a powerful tool to give an insight of heat induced changes in brain. The results of this work show significant decrease in T2 values in rat brain regions on heat exposure indicating changes in tissue architecture at micro level & possibly an initiation of apoptosis or necrosis. Extending this work further at different time point & correlation with other MR techniques such as diffusion & spectroscopy studies will be useful in non-invasive tracking & evaluation of neuronal cell degeneration due to heat stress at an early stage.

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