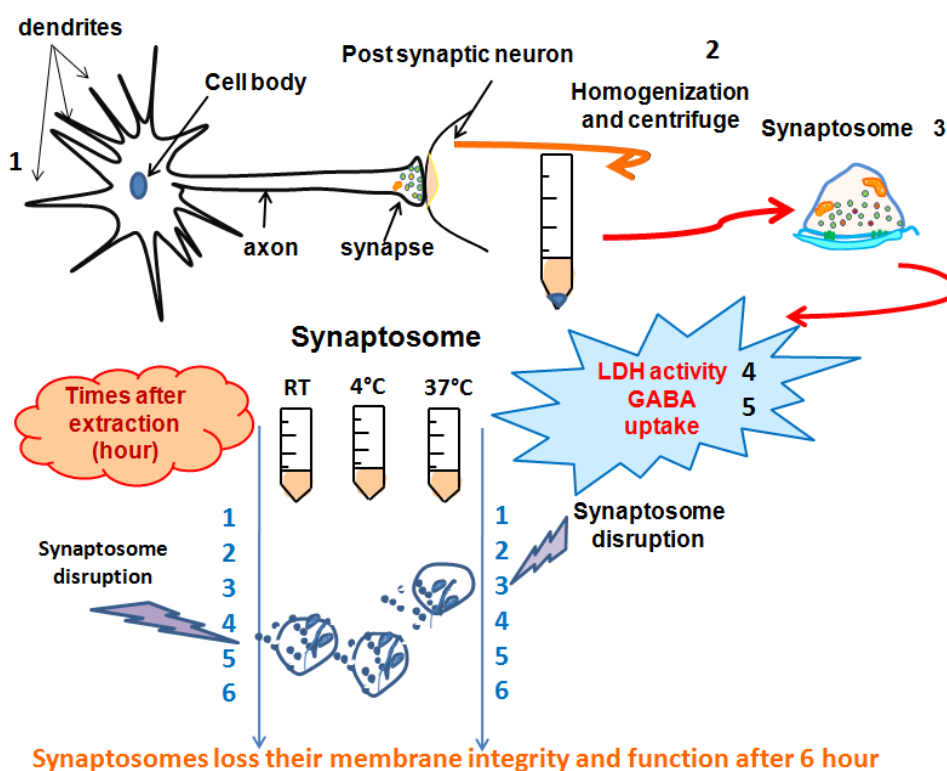


Original Article

High and low temperatures affect rat hippocampal synaptosome's viability and functions

Graphical Abstract



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Highlights

- LDH and GABA uptake assays are indexes of synaptosome membrane integrity and function.
- High and low temperatures affected synaptosome viability and functions during 6 hours after their extraction.
- Synaptosomes lost their membrane integrity and function in 3 hours at 37°C and 4 hours at 4°C and RT after synaptosome extraction.

Original Article

High and low temperatures affect rat hippocampal synaptosome's viability and functions

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Abstract

Introduction: Synaptosomes are sealed particles that contain mitochondria, cytoskeleton and vesicles which are necessary to synaptic events like neurotransmitter release and uptake in the nervous system. However, the effect of high and low temperatures on synaptosome membrane integrity and function during a time course after its extraction is less known. The purpose of this study was to assess synaptosome viability and function at 37, 4°C and room temperature (RT) during 6 hours after its extraction.

Methods: Hippocampi of 40 male Wistar rats were used for synaptosome preparation. To ensure synaptosome membrane integrity and function, lactate dehydrogenase activity (LDH) and GABA uptake were assessed during 6 successive hours after their extraction at 37, 4°C and RT.

Results: Our results showed that at 37°C, synaptosome membrane integrity was reduced 3 hours but at 4°C and RT, it occurred 5 hours following their extraction. The results of synaptosome function analysis coincide with LDH enzyme assay data, meaning that GABA uptake faced a 50% reduction from the initial value at 37°C after 3 hours and at RT after 5 hours. We also found that GABA uptake was reduced at 4°C in the first hour after extraction because the low temperature inhibits GABA transporters.

Conclusion: Synaptosomes preserved their viability and function at RT, 37 and 4°C at least for 3 hours after extraction and reduced over time. For long term application of synaptosomes, it is better to keep them at 4°C.

Keywords:

Synaptosome;
Viability;
LDH activity;
GABA uptake;
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Introduction

The human brain consists of approximately 10^{12}

heterogeneous neurons that communicate through the chemical synapses (Pocklington et al., 2006). The term "Synaptosomes" was first used in 1964 (Whittaker et al., 1964) which was primarily isolated

by Whittaker in the late 1950s and detected as detached synapses by electron microscopy (Whittaker, 1993; Whittaker and Gray, 1962). Synaptosomes are acquired by homogenization of fresh brain tissue in an appropriate isotonic solution which causes the nerve terminals to be separated from their axon stalks. Then, the membrane of presynaptic terminals is resealed, which contains cytoplasm, cytoskeleton, mitochondria and synaptic vesicles with the presynaptic and occasionally postsynaptic membranes (Dunkley et al., 2008; Whittaker et al., 1964). Synaptosomes have widely been applied as an *in vitro* model to investigate the molecular mechanisms of brain synapses specially storage, release, and uptake of neurotransmitters. As a result, our knowledge about the function of synapse ending at a physiological, cellular and molecular level was enhanced (Breukel et al., 1997; Dunkley et al., 1988; Dunkley et al., 2008; Whittaker, 1993).

Different procedures have been developed to isolate synaptosomes including ultrafiltration, electrophoresis, sucrose and percoll gradients; however, a median-speed centrifuge technique seems to be more appropriate (Dunkley et al., 1988; Dunkley et al., 2008; Enriquez et al., 1990; Kamat et al., 2014; Stadler and Tashiro, 1979). Synaptosomes can be obtained from any part of the brain tissues. In addition, the nerves of the non-neurological tissue are also involved in the synaptosomes preparation (Jonakait et al., 1979). If a chemical active ingredient is found abundantly in synaptosomes, it can be assumed as a neurotransmitter or co-neurotransmitter. Moreover, mixed fraction synaptosomes can be applied to assess the mechanisms of neurotransmitters release (Breukel et al., 1997).

Exposure of adult albino rats to the high ambient temperature at 35 °C for 2-12 hours or at 45 °C for 1-2 hours increased the acetylcholinesterase activity of their brain synaptosomes (Mukhopadhyay and Poddar, 1990). Several studies have investigated the effects of organophosphate compound on the GABA uptake of synaptosomes in cerebral cortex, cerebellum and hippocampus, suggesting that the GABA uptake was optimal one hour after synaptosome extraction (Ghasemi et al., 2007; Pourabdolhossein et al., 2009; Shahroukhi, et al., 2007). Furthermore, Hosseini et al. reported 35 minute is the optimal time for GABA release by rat

cerebral synaptosomes (Hosseini et al., 2004). Incubation of synaptosomes at room temperature (22-25 °C) gradually increases the percentage of microtubule containing synaptosomes (41-47%) while its stabilization at low temperature leads to a significant reduction in the number of synaptosomes (Hajos et al., 1979).

Despite the significance of synaptosomes in pharmaceutical, structural and functional studies, there is no empirical data on synaptosomes viability and function at different temperatures and time points after their extraction. Thus, the main ambition behind this study was to examine the synaptosomes survival and function at different temperatures and time points by measuring lactate dehydrogenase enzyme (LDH) and GABA uptake. We used hippocampal synaptosome due to its high synaptic contacts ratio compared to the other brain regions (Cragg, 1975).

Materials and methods

Animals

A total of 70 male Wistar rats, weighing 220–250 g, were included in this study. The animals were kept at 22±2 °C and an automatic light/dark cycle (12-h). They were fed standard laboratory diet and water ad libitum throughout the study. Permissions for conducting experiments were taken from Institutional Animal Care and Use Committee of the Babol University of Medical Sciences.

Chemicals

Gamma-aminobutyric acid and aminoxyacetic acid were obtained from the Sigma company. Tritiated GABA [³H GABA (86 Ci/mmol)] was supplied by Amersham Bioscience company. Bovine serum albumin (BSA) was acquired by Fluka and other materials from the Merck company.

Isolation of synaptosomes

Synaptosomes were prepared as explained before (Raiteri et al., 2003). In brief, the procedure was to dissect the hippocampi (0.6±0.05 g) quickly and homogenized in 0.32 M sucrose buffered with 100 mM phosphate at 4°C, pH 7.4. Centrifugation of the homogenate was made 5 min at 1000 g to remove cell debris. Then, the supernatant was spun at 12000 g for 20 min at 4°C. The isolated synaptosomes obtained from pellets, were resuspended in buffer

solution containing: 22mM NaHCO₃, 1mM NaH₂PO₄, 125mM NaCl, 3mM KCl, 1.2mM MgSO₄, 1.2mM CaCl₂, pH 7.4 and saturated with O₂ (95%) and CO₂ (5%). The protein concentration of synaptosomes was adjusted to 1 mg/ml.

Biochemical assays

We quantified protein concentration of isolated synaptosomes by Bradford's method and used BSA as the standard (Bradford, 1976). To assess synaptosomes membrane integrity, LDH activity test was done with reduction of pyruvate to lactate (Edward and Powsner, 1999; Pourabdolhossein et al., 2009). Total synaptosomal LDH activity was measured in the presence of 1% Triton X-100 and free synaptosomal LDH activity was determined in the absence of 1% Triton X-100. Subtraction of total LDH activity with and without Triton X-100 is called LDH occluded activity which is expressed as a percentage of the total. Aliquots of 0.5ml synaptosomes (triplicate) were incubated at 37, 4 °C and RT for 6 hours and LDH activity was assessed every hour in the first 6 hours after their extraction.

Uptake assay

Mantz protocol was used to set up GABA uptake assays (Mantz et al., 1995). To find the best incubation time in our experiments, we added 0.5 ml aliquots of synaptosomes (containing 0.5mg protein) to tubes and incubated with 400nM GABA (1.5% of which was [3H] GABA) in different times between 5 to 30 min at 37 °C. We stopped the reaction every 5 minutes by addition of 2ml of ice-cold buffer solution, transferred synaptosomes tube content to the superfusion chambers and then washed three times with ice-cold buffer. Since GABA uptake was time-dependent, the incubation time was set on 15 minutes in the linear portion. Aminooxyacetic acid (10 μM) was applied in all experiments to avoid GABA catabolism. To measure GABA uptake, we stopped the reaction by addition of 2ml of ice-cold buffer after the incubation time and transferred the synaptosomes to superfusion chambers. The synaptosomes were placed onto 0.65μm pore Millipore filters at the base of a set of parallel superfusion chambers (Raiteri et al., 2003; Raiteri and Raiteri, 2000). To remove washing buffer from the synaptosomes filter we connected a peristaltic pump to the bottom of the superfusion chambers with

the flow rate of 0.5 ml/min. The filters containing the remains of synaptosomes were put in scintillated liquid and their radioactivities were counted with a beta liquid scintillation counter (Betamatic, Kontron, France). Synaptosome samples were incubated at 37, 4°C and RT during 6 hours and their GABA uptake was measured every hour during 6 hours after their extraction. In some experiments, we substituted sodium chloride by lithium chloride (equimolar) to elucidate transporter-dependency of uptake (Lu and Hilgemann, 1999).

Statistical analysis

Our data were shown as mean±SEM. For data analysis we used paired t-test or two way repeated measures (RM) ANOVA followed by Bonferroni post-tests with Graph pad PRISM software (version 7). The *P*-values less than 0.05 were considered as significant.

Results

Synaptosome membrane integrity

After extracting the synaptosome, its protein concentration was measured by Bradford protocol, and the final concentration was adjusted to 1mg/ml (Bradford, 1976). The changes in occluded LDH activity were used to show synaptosomes viability (Fig. 1). Interruption of synaptosome particles with 1% Triton X-100 increased enzyme activity significantly compared to the samples obtained from the same intact synaptosomes. Free and occluded LDH activities in our experiments were 16±4.2% and 84±3.7%, respectively (*P*<0.001, n=18, Fig. 1). It was concluded that if the occluded LDH activity decreases following the synaptosome membrane disruption, the synaptosomes do not function effectively any more.

The effect of different temperatures on synaptosome viability and membrane integrity at several time points after its extraction

Our results showed that synaptosome LDH occluded activity in all temperatures in the first three hours preserved 70% of control, which indicates that synaptosome membrane was intact. However, at 37 °C synaptosome LDH activity was significantly reduced at 3 hours compared to the first hours after its extraction, a finding which shows synaptosome membrane disruption at this temperature after 3

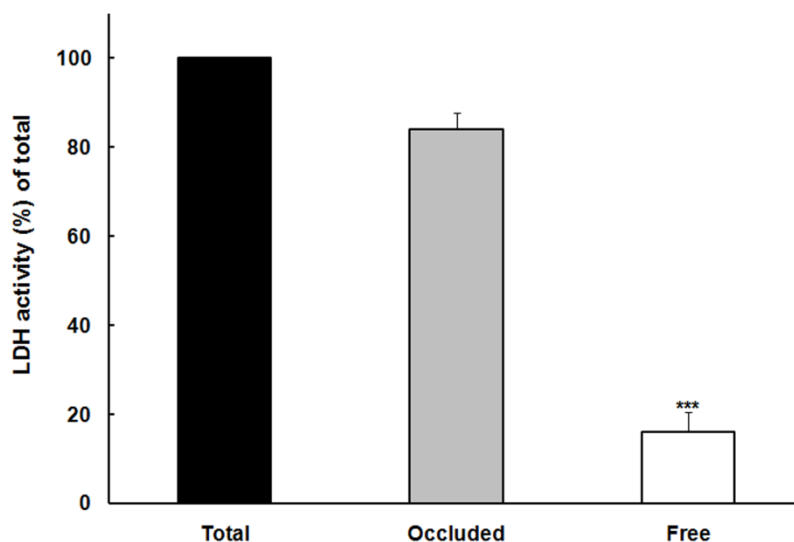


Fig.1. LDH activity (percent of total) of rat hippocampal synaptosomes. Total LDH activity was measured in the presence and free activity in the absence of 1% Triton X-100. Occluded activity was obtained by the subtraction of the total and free activity. Results are shown as mean±SE for 12 experiments (***) $P < 0.001$, paired t test).

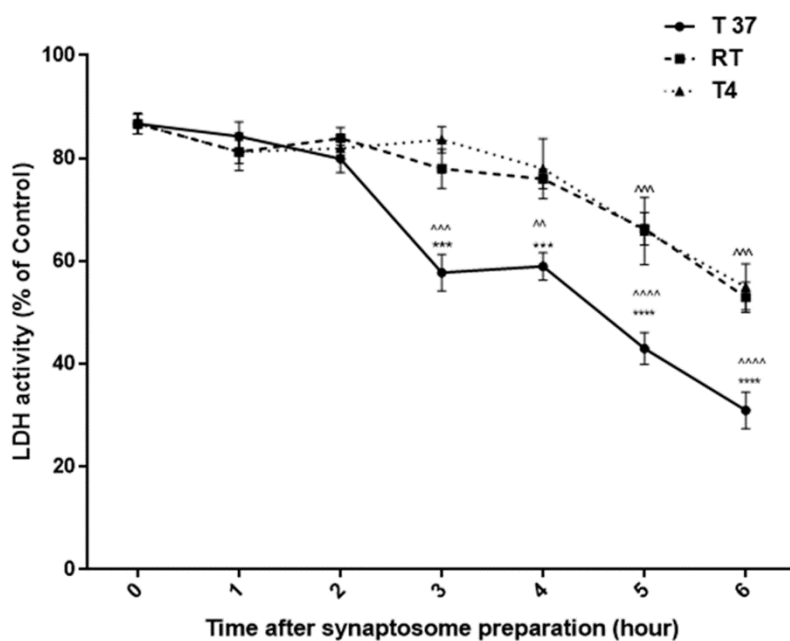


Fig.2. Synaptosome membrane integrity during 6 hours after extraction at 37, 4 °C and RT. There was a significant reduction of synaptosome membrane integrity at 37 °C, three hours after extraction and for other temperatures membrane integrity was preserved up to 4 hours. Data are shown as mean±SE for 12 experiments. (^^ $P < 0.001$, ^^ $P < 0.0001$ compared to the initial value within groups, *** $P < 0.001$, **** $P < 0.0001$ comparison between groups, two way RM ANOVA, bonferroni post, $n=12$). RT: room temperature

hours ($P < 0.001$, $n=12$, Fig. 2). Moreover, synaptosome membrane integrity at RT and 4 °C was conserved up to 5 hours after extraction and reduced over time ($P < 0.001$, $n=12$, Fig. 2). LDH activity at 37°C from 3 to 6 hour after synaptosome extraction was significantly reduced compared to 4 °C and RT ($P < 0.001$, $P < 0.0001$, Fig. 2). These results suggest that synaptosome can conserve the membrane integrity and its function 3 hours after extraction in the

concerned temperatures and if the experimenter needs to keep them more than 3 hours, it is better to maintain them at 4 °C or RT.

Uptake assays as a synaptosome functional test

To study the synaptosome function, we measured GABA uptake in rat hippocampal synaptosomes in different conditions. The best incubation time in our experiments was 15 min because [3H] GABA uptake

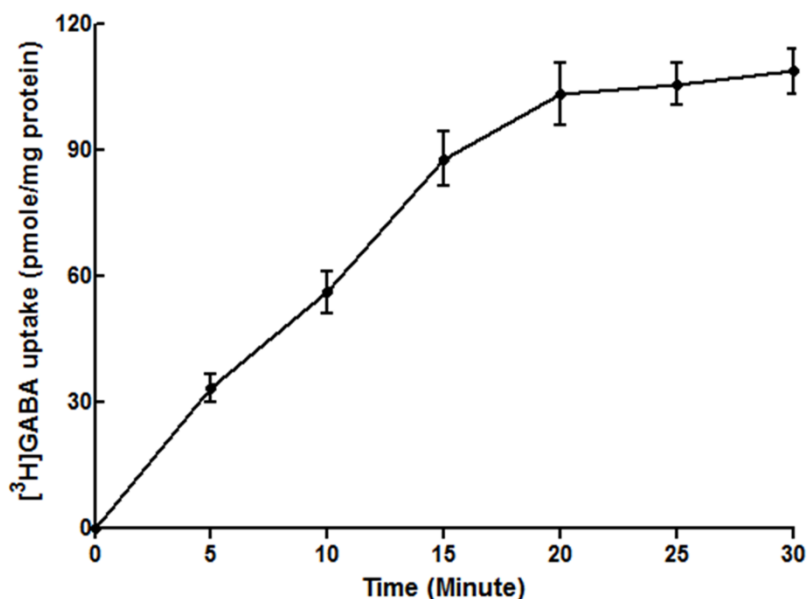


Fig.3. Time-dependency of synaptosome GABA uptake. It reaches the maximum level at 20 min after beginning of incubation.

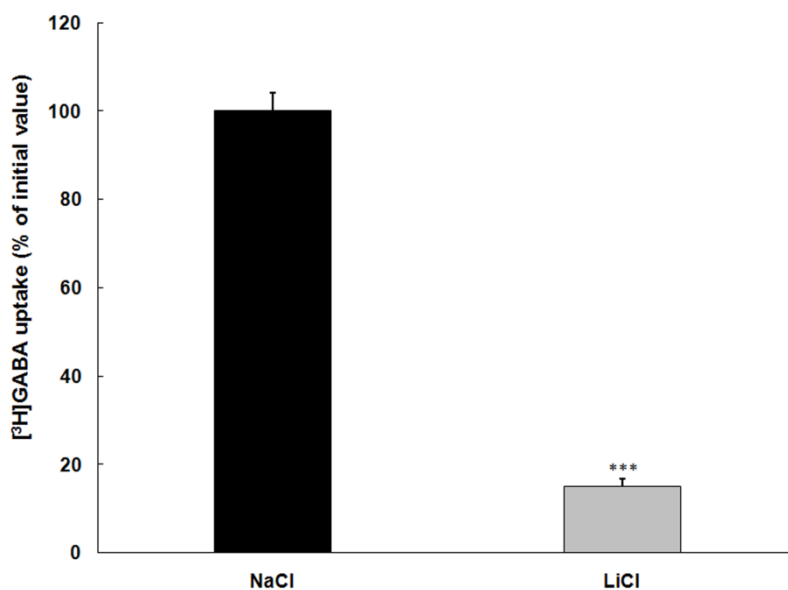


Fig.4. Effect of substituting lithium chloride for sodium chloride on GABA uptake by rat hippocampal synaptosome. Data demonstrated that GABA uptake was sodium dependent (***) $P < 0.001$, t test). Data are presented as mean \pm SE.

peaked at 20 min after incubation and was 33, 56, 88, 103, 104 and 107 pmol/mg protein at 5, 10, 15, 20, 25 and 30min after incubation, respectively (Fig. 3). We found that GABA uptake in synaptosomes was time-dependent; hence, we chose 15 min as a suitable incubation time in order to signify GABA uptake changes more precisely.

Since the GABA uptake is sodium dependent, we substituted the same amount of sodium chloride with lithium chloride and the result demonstrated that GABA uptake was reduced significantly to 21% of the original value ($P < 0.001$, $n=8$, Fig. 4). This result

confirms that GABA uptake occurs in our synaptosomes and was sodium dependent.

LDH enzyme assay results confirmed that our synaptosomes are viable and their membrane integrity was conserved at least 3 hours after its extraction in different temperatures. To evaluate the synaptosome functionality, we measured GABA uptake in synaptosome incubated at 37, 4 °C and RT multiple times after its extraction. Our results showed that synaptosome function at RT and 37 °C in the first three hours was acceptable but it was significantly decreased over time especially at 37 °C ($P < 0.01$,

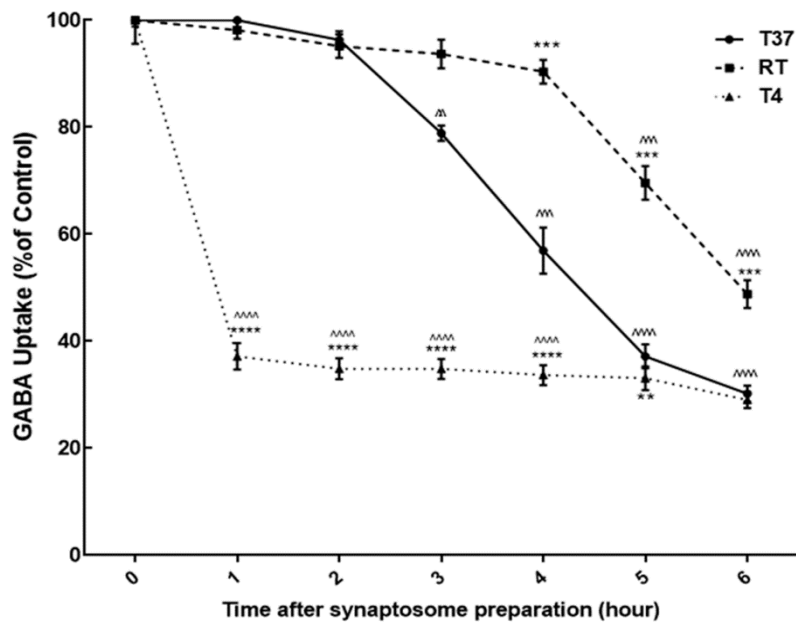


Fig.5. Synaptosome function changes at 37, 4 °C and RT during 6 hours after extraction. GABA uptake was significantly reduced in 3 and 4 hours after synaptosome extraction compared to their initial value at 37 °C and RT respectively ($^{^^}P<0.0001$, $^{^^}p<0.01$, Two way RM ANOVA, Bonferroni post test). At 4 °C GABA uptake was drastically decreased one hour after synaptosome extraction compared to its initial value ($^{^^^}P<0.0001$) but afterwards it remained constant till the end of the experiment. Comparison between the groups (37 °C and RT) indicated a significant reduction of GABA uptake after 3 hours till 6 hours. ($^{****}P<0.0001$, Two way RM ANOVA, Bonferroni post test).

$P<0.001$, $P<0.0001$, Fig. 5). We found that at 4 °C GABA uptake was considerably reduced 1 hour after extraction ($P<0.0001$, Fig. 5). Synaptosomes' GABA uptake at 4 °C was at the same level up to 5 hours after extraction; however, after 6 hours it seems to have been reduced compared to the first hour ($P<0.001$, Fig. 5).

Discussion

Synaptosomes are one of the best *in vitro* model to study the molecular mechanisms of brain synapses function such as release, uptake and storage of neurotransmitters (Evans, 2015; Sugimura et al., 2001; Whittaker, 1993). The objective of this study was to evaluate synaptosome viability and function in the long term after its extraction for researchers who plan to extend their experiment for more than one hour after synaptosome preparation. We used the centrifuge technique to extract hippocampal synaptosome, which has a high percentage of synaptic ending relative to the weight of the tissue (Cragg, 1975). Succinic dehydrogenase, LDH and acetylcholinesterase enzyme activities can be used for synaptosomal membrane integrity assessment

(Edward and Powsner, 1999; Gilbert and Wyllie, 1976). LDH enzyme is one of the best cytosolic markers as a valid index to show synaptosomes membrane integrity and viability (Gilbert and Wyllie, 1976; Legrand et al., 1992; Taupin et al., 1994). Analysis of the LDH activity confirmed that the synaptosome at the time of extraction preserved the membrane integrity. Synaptosome morphology and structure was confirmed by electron microscopy earlier (Shahroukhi et al., 2007). GABA transporters (GATs) belong to a large family of sodium symporters and are broadly expressed throughout the central nervous system. GAT1 and some other GABA transporters are highly expressed in the hippocampus and dentate gyrus (Scimemi, 2014). In GABA uptake assay we replaced lithium with sodium and confirmed the dependency of GATs on sodium, verifying the effectiveness of synaptosomes.

In response to the research question as to how long the synaptosomes are viable and usable after extraction and what temperature is suitable for their survival and effective function, we incubated synaptosomes in 4, 37 °C and RT for 6 hours. We also studied the LDH activity and GABA uptake to verify the synaptosome function in the mentioned

temperature and time points.

The result confirmed that the synaptosomes have intact membrane and high effectiveness at least 3 hours after extraction at mentioned temperatures and afterwards especially in 37 °C their functions and membrane integrity was significantly reduced.

Since the discovery of synaptosomes in 1964, many researchers have used the synaptosome model for different purposes, each selecting special temperatures (Evans, 2015; Whittaker, 1993; Whittaker et al., 1964). The present study is the first attempt to study the effect of different temperature and time lapses on the synptosome viability and function.

Ghasemi and colleagues (2007) reported the effect of paraoxon on GABA uptake using cerebral synaptosomes one hour after extraction at 37 °C. Hosseini and collaborators (2004) showed GABA release in cerebral synaptosomes one hour after extraction. Shahroukhi and Cecchini indicated GABA uptake in rat cerebellar and cerebral synaptosomes one hour after extraction at RT (Shahroukhi et al., 2007, Cecchini et al., 2004). We also proved that rat hippocampal synaptosomes at 37 °C and one hour after their extraction had optimum function (Pourabdolhossein et al., 2009). So we can conclude that synaptosomes from any part of the brain one hour after extraction are viable and functional. In the present study we analyzed the LDH activity of synaptosomes during 6 hours, i.e. every hour after their extraction at 37, 4 °C and RT.

Our results showed that temperatures affect synaptosome membrane integrity during time lapses. After 3 hours of extraction, at 37 °C synaptosome membrane integrity was reduced over time but this time for RT and 4 °C was prolonged for 5 hours. It has been reported that heat stress condition induced increase of cell metabolic enzyme activity, disturbance acid-base status and reduced membrane integrity (Sandercock et al., 2001). These reports can explain why synaptosome viability at 37 °C was less than other temperatures. Noiles and colleagues (1995) have reported that low temperatures (0-4°C) reduced membrane permeability and increased the constancy of membrane. Also Stokes et al. reported cold stress altering membrane integrity (Stokes et al., 2016). Temperature over time reduced synaptosome membrane integrity. We also checked synaptosome function by measuring GABA uptake. Our pervious

study confirmed that a major portion of the GABA uptake into the synaptosomes occurred via transporters using selective inhibitor of the GABA transporter, nipecotic acid (Pourabdolhossein et al., 2009; Shahroukhi et al., 2007).

So far, no research has been reported effects of time passage after synaptosome extraction and temperature on their survival and function. In the present study, the results of synaptosome function analysis coincide with LDH enzyme assay data, meaning that the synaptosome function in different temperatures across time correlated with their membrane integrity. We found that the amount of GABA uptake as an index of synaptosome function faced a 50% reduction from the initial value at 37 °C after 3 hours and at RT after 4 hours. At 4 °C, after one hour, GABA uptake had a significant reduction. Considering that at this initial time, synaptosome membrane integrity was confirmed by LDH activity test, the reduction of synaptosome function could be related to the inhibitory effect of low temperature on GABA transporters activity. GABA transporters are temperature, metabolic energy, Na⁺ and cl⁻ dependent while low temperature (4 °C) inhibits GATs and markedly diminishes GABA uptake (Zhang and Liu, 1998); so the reduction of GABA uptake at 4 °C in the first hour after extraction does not mean our synaptosomes are not functional. Also cold buffer is used for stopping neurotransmitter uptake in the laboratories (Evans, 2015; Ghasemi et al., 2007; Ng and Ong, 2002; Pourabdolhossein et al., 2009).

Conclusion

The present data demonstrated that synaptosome prepared by centrifuge technique preserved their membrane integrity and function at the time of extraction. We further confirmed that temperature had an effect on synaptosome membrane integrity and function during 6 hours after their extraction. Synaptosome viability and function preserved at least 3 hours after extraction in all temperatures but afterwards they lost their membrane integrity and function especially at 37°C.

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Conflict of interest

Authors declare no conflicts of interest.

References

- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72:248-54.
- Breukel AI, Besselsen E, Ghijsen WE. Synaptosomes. A model system to study release of multiple classes of neurotransmitters. *Methods Mol Biol* 1997; 72: 33-47.
- Cecchini AL, Soares AM, Giglio JR, Amara S, Arantes EC. Inhibition of L-Glutamate and GABA synaptosome uptake by crotoxin, the major neurotoxin from *Crotalus durissus terrificus* venom. *J Venom Anim Toxins incl Trop Dis* 2004; 10: 260-79.
- Cragg BG. The development of synapses in the visual system of the cat. *J Comp Neurol* 1975; 160: 147-66.
- Dunkley PR, Heath JW, Harrison SM, Jarvie PE, Glenfield PJ, Rostas JA. A rapid Percoll gradient procedure for isolation of synaptosomes directly from an S1 fraction: homogeneity and morphology of subcellular fractions. *Brain Res* 1988; 441: 59-71.
- Dunkley PR, Jarvie PE, Robinson PJ. A rapid Percoll gradient procedure for preparation of synaptosomes. *Nat Protoc* 2008; 3:1718-28.
- Edward R, and Powsner MD. Lactate dehydrogenase activity and its measurement, In: *Tietz Text Book of Clinical Chemistry*, Edited by CA Burtis and ER Ashwood, Third edition, 1999; Philadelphia Saunders, p. 812-17.
- Enriquez JA, Sanchez-Prieto J, Muino Blanco MT, Hernandez-Yago J, Lopez-Perez MJ. Rat brain synaptosomes prepared by phase partition. *J Neurochem* 1990; 55: 1841-49.
- Evans GJ. The synaptosome as a model system for studying synaptic physiology. *Cold Spring Harb Protoc* 2015; 2015: 421-4.
- Ghasemi A, Sadidi A, Mohammadi M, Khoshbaten A, Asgari A. Paraoxon inhibits GABA uptake in brain synaptosomes. *Toxicol In Vitro*. 2007; 21(8): 1499-1504.
- Gilbert JC, Wyllie MG. Effects of anticonvulsant and convulsant drugs on the ATPase activities of synaptosomes and their components. *Br J Pharmacol* 1976; 56: 49-57.
- Hajos F, Csillag A, Kalman M. The morphology of microtubules in incubated synaptosomes. Effect of low temperature and vinblastine. *Exp Brain Res* 1979; 35: 387-93.
- Hosseini S, Asgari A, Mehrani H, Koshbaten A. Non-cholinergic effects of paraoxon on [³H]-GABA release from rat cerebellar giant synaptosomes. *Iran J Pharm Res* 2004; 3: 95-95.
- Jonakait GM, Gintzler AR, Gershon MD. Isolation of axonal varicosities (autonomic synaptosomes) from the enteric nervous system. *J Neurochem* 1979; 32: 1387-1400.
- Kamat PK, Kalani A, Tyagi N. Method and validation of synaptosomal preparation for isolation of synaptic membrane proteins from rat brain. *MethodsX* 2014; 1: 102-7.
- Legrand C, Bour JM, Jacob C, Capiaumont J, Martial A, Marc A, et al. Lactate dehydrogenase (LDH) activity of the cultured eukaryotic cells as marker of the number of dead cells in the medium [corrected]. *J Biotechnol* 1992; 25: 231-43.
- Lu CC, and Hilgemann DW. GAT1 (GABA:Na⁺:Cl⁻) cotransport function. Steady state studies in giant *Xenopus* oocyte membrane patches. *J Gen Physiol* 1999; 114: 429-44.
- Mantz J, Lecharny JB, Laudénbach V, Henzel D, Peytavin G, Desmots JM. Anesthetics affect the uptake but not the depolarization-evoked release of GABA in rat striatal synaptosomes. *Anesthesiology* 1995; 82: 502-11.
- Mitchell MA, Sandercock DA. Possible mechanisms of heat stress induced myopathy in the domestic fowl. *J Physiol Biochem* 1997; 53: 75.
- Mukhopadhyay S, Poddar MK. Higher environmental temperature-induced change in synaptosomal acetylcholinesterase activity of brain regions. *Neurochem Res* 1990; 15: 231-6.
- Ng CH, Ong WY. Increased synaptosomal [³H] GABA uptake in the rat brainstem after facial carrageenan injections. *Pain* 2002; 98: 259-68.
- Noiles EE, Bailey JL, Storey BT. The temperature dependence in the hydraulic conductivity, L_p, of the mouse sperm plasma membrane shows a discontinuity between 4 and 0 degrees C. *Cryobiology* 1995; 32: 220-38.
- Pocklington AJ, Armstrong JD, Grant SG. Organization of brain complexity--synapse proteome form and function. *Brief Funct Genomic Proteomic* 2006; 5: 66-73.
- Pourabdolhossein F, Ghasemi A, Shahroukhi A, Sherafat MA, Khoshbaten A, Asgari A. In vitro assessment of paraoxon effects on GABA uptake in rat hippocampal synaptosomes. *Toxicol In Vitro* 2009; 23: 868-73.
- Raiteri L, Giovedi S, Benfenati F, Raiteri M, Bonanno G. Cellular mechanisms of the acute increase of glutamate release induced by nerve growth factor in rat cerebral cortex. *Neuropharmacology* 2003; 44: 390-402.
- Raiteri L, Raiteri M. Synaptosomes still viable after 25 years of superfusion. *Neurochem Res* 2000; 25: 1265-74.
- Sandercock DA, Hunter RR, Nute GR, Mitchell MA, and Hocking PM. Acute heat stress-induced alterations in blood acid-base status and skeletal muscle membrane integrity in broiler chickens at two ages: implications for meat quality. *Poult Sci* 2001; 80: 418-25.
- Scimemi A. Structure, function, and plasticity of GABA

- transporters. *Front Cell Neurosci* 2014; 8: 161.
- Shahroukhi A, Ghasemi A, Poorabdohossein F, Asgari A, Khoshbaten A. The effect of paraoxon on GABA uptake in rat cerebellar synaptosomes. *Med Sci Monit* 2007; 13: BR194-199.
- Stadler H, Tashiro T. Isolation of synaptosomal plasma membranes from cholinergic nerve terminals and a comparison of their proteins with those of synaptic vesicles. *Eur J Biochem* 1979; 101: 171-8.
- Stokes JM, French S, Ovchinnikova OG, Bouwman C, Whitfield C, Brown ED. Cold Stress Makes *Escherichia coli* Susceptible to Glycopeptide Antibiotics by Altering Outer Membrane Integrity. *Cell Chem Biol* 2016; 23: 267-77.
- Sugimura M, Kitayama S, Morita K, Irifune M, Takarada T, Kawahara M, et al. Effects of volatile and intravenous anesthetics on the uptake of GABA, glutamate and dopamine by their transporters heterologously expressed in COS cells and in rat brain synaptosomes. *Toxicol Lett* 2001; 123: 69-76.
- Taupin P, Zini S, Cesselin F, Ben-Ari Y, Roisin MP. Subcellular fractionation on Percoll gradient of mossy fiber synaptosomes: morphological and biochemical characterization in control and degranulated rat hippocampus. *J Neurochem* 1994; 62: 1586-95.
- Whittaker VP. Thirty years of synaptosome research. *J Neurocytol* 1993; 22: 735-42.
- Whittaker VP, Gray EG. The synapse: biology and morphology. *Br Med Bull* 1962; 18: 223-8.
- Whittaker VP, Michaelson IA, Kirkland RJ. The separation of synaptic vesicles from nerve-ending particles ('synaptosomes'). *Biochem J* 1964; 90: 293-303.
- Zhang Y, Liu GQ. Sodium and chloride-dependent high and low-affinity uptakes of GABA by brain capillary endothelial cells. *Brain Res* 1998; 808: 1-7.