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Research report

Rapid upregulation of the hippocampal connexins 36 and 45 mRNA levels during memory consolidation

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HIGHLIGHTS

- Gap junction channels coupling is required for cognitive processes.
- Cx36 and Cx45 mRNAs upregulated in the hippocampus during memory consolidation.
- Hippocampal electrical synapses are crucial for memory encoding.

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ABSTRACT

Gap junction channels are implicated in learning and memory process. However, their role on each of the particular stages of memory formation has been studied less. In this study, the time profile of the expression levels of hippocampal connexins 36 and 45 (Cx36 and Cx45) mRNAs was measured during memory consolidation, in a passive avoidance paradigm. Totally 30 adult male rats were distributed into 5 groups of each 6. At different times profiles (30 min, 3, 6 and 24 h) following training, rats were decapitated and their hippocampi were immediately removed and frozen in liquid nitrogen. Total RNA was extracted and cDNA was synthesized, using oligo-d_t primers. A quantitative real-time PCR was used to measure the levels of each of Cx36 and Cx45 mRNAs. Both connexins showed a rapid upregulation (30 min) at the transcriptional level, which declined in later times and reached to the control level at 24 h. The rapid up-regulation of Cx36 and Cx45 mRNAs might be accompanied with increasing intercellular coupling via gap junction channels and neuronal oscillatory activities required for memory consolidation. The results highlight the role of gap junctional coupling between hippocampal neurons during memory consolidation in the physiological conditions.

1. Introduction

Gap junctions are composed of intercellular channels gathered together in a gap junction plaque. They are expressed throughout the nervous system and in various cells including astrocytes, oligodendrocytes and neurons [1]. The major proteins that form gap junctions are termed connexins (Cxs). Different Cx subtypes have been identified in the vertebrate CNS, which are defined by their molecular mass (in kilo Daltons) [2]. In the adult brain, neurons form gap junctions with other neurons, but not with astrocytes. Nevertheless, some studies reported significant neuron-glia coupling in a few brain areas [3]. Gap junction channels connecting neurons are also called electrical synapses. These synapses connect soma-to-soma, soma-to-dendrite, dendrite-to-dendrite or axon-to-axon. There are possibly five different connexins (Cx26, Cx30.2, Cx31.1, Cx36 and Cx45) that are expressed in neurons of the brain [4]. Cx36 and Cx45 are more abundant than other neuronal connexins in the brain [6] and play important roles in physiological conditions in the central nervous system [7–13].

There is growing body of evidence indicating the role of brain gap junctions in physiological conditions, including memory formation [10,12,14–20]. However, the role of intercellular coupling via gap junctions for learning and memory is not well known. Earlier studies using behavioral genetics and behavioral pharmacology approaches have investigated the impact of gap junctions on learning and memory [9,10,12,16,19,21–23].

Different neuronal and astrocytic connexin-deficient rodents were used for behavioral correlates of learning and memory. Cx43-deficient mice showed a steeper learning course in the water maze

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[22]. The deletion of the Cx31.1 coding DNA in the mouse led to impaired one-trial object recognition at all delays tested [19]. Conditional neuronal Cx45-deficient mice showed impaired one-trial object recognition after delays of 10, 30 and 60 min [12]. Cx36-deficient mice exhibited one-trial object recognition after retention intervals of 45 and 90 min, but not at a delay of 15 min between the sample and the test trial [20]. Also, Cx36-deficient mice showed impaired behavioral habituation and rewarded spatial alternation behavior in the Y-maze [10,20]. However, spatial learning in the water maze was shown to be unaffected by the Cx36 knockout in the mouse [10].

The behavioral relevance of gap junctions has also been studied in a few pharmacological studies using gap junction uncouplers. For example, intraperitoneal or dorsal hippocampal injection of carbenoxolone or mefloquine in rats impaired acquisition and consolidation of contextual fear memory and accelerated its extinction without having a detrimental effect on the acquisition and expression of cued fear [14]. Also, the bilateral infusion of carbenoxolone into the hippocampus of rats impaired memory performance in a water maze [16] and memory consolidation in the passive avoidance task (PAT) [23]. Meanwhile, blocking the hippocampal CA_1 area gap junction channels by carbenoxolone disrupted morphine state-dependent learning [17].

Intercellular coupling via gap junction channels can increase in two ways. One way is the rise in the number of open channels at a given time. The other way is to increase the number of gap junction channels at a gap junction plaque, which can be done via increased expression of connexin family of proteins. Therefore, if the gap junction channels are involved in memory processing, the expression of connexins might upregulate and thereby increase the number of gap junction channels at a gap junction plaque and the intercellular communication between neuronal cells in the brain areas responsible for memory formation.

Memory formation consists of several phases, including acquisition, consolidation, retention and retrieval, which have different mechanisms [25]. The hippocampus, which is the main component of the limbic system, plays a crucial role in governing learning and memory [26]. It is involved in the consolidation of memory in the passive avoidance task [27]. It is well known that there is a wide network of gap junction communication between different cell types within the hippocampus [5].

Though the results of most researches have indicated a relationship between gap junctions and memory formation [12,14,17,19,20,23], there are not any reports indicating the expression levels of any of the connexins during each of the particular stages of memory formation. The aim of the present study was to evaluate the time profile of two neuronal connexins, Cx36 and Cx45 mRNA expression levels in the hippocampus, during the process of memory consolidation.

2. Materials and methods

2.1. Animals

Totally 30 three months old naive male Wistar rats weighing 230–280 g were obtained from the breeding colony of Department of Biology, University of Isfahan and randomly distributed into 5 groups of 6 each. Rats were housed four per cage in a temperature (24 °C) controlled room that was maintained on a 12:12 light cycle (light on at 07:00 A.M.). Rats had unrestricted access to food and water in their home cage. The animals were handled evenly and habituated with the experimenter and were placed in the test room, 30 min before the experiment. All experiments were carried out in accordance with the guide for the care and use of laboratory animals (USA National Institute of Health publication No. 80-23, revised 1996) and were approved by the graduate studies committee of the Department of Biology, University of Isfahan. The experiments were performed between 9 A.M. and 13 P.M.

2.2. Passive avoidance task

A step-through passive avoidance apparatus (Tajhiz Gostar Co, Iran, 2013) with two opaque white and black chambers was used in the behavioral experiments. The white chamber was illuminated by a lamp. The two distinct chambers, each with the interior dimensions of $30 \times 25 \times 25\,cm^3$ were separated by a sliding door of $8 \times 25\,cm^2$. The floors of both chambers were made of stainless steel rods with 3 mm diameter and 1 cm space between the rods. The experiments were performed in a silent room.

Each rat was placed in the white chamber of the PAT apparatus facing the sliding door. After 5 s the door was raised. When the animal stepped into the dark chamber with all four paws, the door was closed and the rat remained there for 20 s. Then the animal was removed to be placed in a temporary cage (habituation training). Thirty min later, the rat was again placed in the white chamber for 5 s, then the door was raised to let the animal enter the dark chamber and following entrance, the door was closed, but this time a controlled electrical shock of 0.3 mA lasting for 1 s was delivered. After 20 s, the rat was placed into the temporary cage (acquisition training). 2 min later, the same testing procedure was repeated. When the rat remained in the white compartment for a 2-min time period, the training was terminated. The control group was treated the same as the test groups, but did not receive any foot-shock. After completion of the training session, rats were decapitated at different time profiles (30 min, 3, 6 and 24 h after training) and their hippocampi were immediately removed and frozen in liquid nitrogen, then stored in a −70 °C freezer.

2.3. Gene expression assay

2.3.1. Tissue preparation

The frozen hippocampal samples were pulverized completely and mixed with 200 μL chilled phosphate-buffered saline (in mmol/L: 137 NaCl, 2.7 KCl, 4.3 Na2HPO4.7H2O, and 1.4 KH2PO4), vortexed for 30 s and then divided into aliquots [28].

2.3.2. RNA extraction

Total cellular RNA was isolated from the hippocampus using RNX-PLUS reagent (SinaClon, Iran). The RNA was treated with 1 U RNase-free DNase I (Thermo Fisher Scientific Inc, United States) to avoid DNA contamination. The integrity of the RNA samples was determined using denaturing agarose gel electrophoresis. The concentration and purity of the RNAs were determined by spectrophotometry (Eppendorf, Germany). The mean absorbance ratio at 260/280 nm was 1.7 ± 0.2 and at 260/230 nm was 1.8 ± 0.1.

2.3.3. Complementary DNA (cDNA) synthesis

The reverse transcription reaction was performed with a cDNA synthesis kit (Takara, Japan) using Oligo-dT primer, MULV reverse transcriptase and 500 ng total RNA as template, according to the manufacturer's instructions.

2.3.4. Real-time PCR and comparative threshold cycle method

Cx36 and Cx45 were chosen as target genes and GAPDH was used as an internal reference gene. All primers were designed using the NCBI primer design tool (Table 1). The specificity of the primers for their target sequences was checked on the NCBI website (www.ncbi.nlm.nih.gov/blast). The SYBR Green I real-time PCR assay was carried out in a final reaction volume of 10 μL with 5 μL SYBR Green I Master mix (Takara, Japan), 100 nmol/L forward and reverse primers and 10 ng cDNA. Thermal cycling was performed on the ABI