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Experimental Research Article

Differential changes in the quantity of the hippocampal glial connexins mRNAs during memory consolidation

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ABSTRACT

Introduction: It is known that glial cells are crucial for memory formation. Glial cells and neurons interconnect via gap junction channels made of connexin (Cx) proteins. Glial connexins were shown to be involved in memory formation. However, the expression profile of different glial connexins was not measured during memory consolidation. Cx43 and Cx30 are expressed in astrocytes, whereas Cx32 is expressed in oligodendrocytes. We quantified the messenger RNA (mRNA) levels of the hippocampal Cx30, Cx32, and Cx43 throughout the consolidation stage of fear or spatial memory.

Methods: Male Wistar rats were distributed into eight groups of four each. To assess the spatial or fear memory consolidation, the Morris water maze and passive avoidance task were utilized. At different time intervals (one, three, and twenty-four hours) following the training sessions, rats were sacrificed and the hippocampi were isolated and frozen instantly in liquid nitrogen. A quantitative real-time polymerase chain reaction (PCR) was employed to measure mRNA levels of the target genes.

Results: The results revealed that Cx43 and Cx32 downregulated significantly, one or three hours after training in the inhibitory avoidance model. In the Morris water maze, Cx43 expression was upregulated three hours after training. The expression of Cx30 did not exhibit significant alterations in either of the experimental assays.

Conclusion: The results indicate the crucial, but differential role of the hippocampal Cx32 and Cx43 during fear or spatial memory consolidation. The exact outcomes of these potential changes need to be clarified.

Keywords:
Connexin
Glia
Gap junction
Hippocampus
Memory

Introduction

Memory is a crucial proficiency of animals that provides the potential change in their behavior after being exposed to novel experiences. Despite all the information regarding the cellular and molecular mechanisms of memory (Kandel 2012), there are still numerous questions to be addressed. This is somehow because of the

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neglect of the contribution of glial cells as important structural portions of the nervous system. All types of glia can react to and affect neurotransmission in several ways (Hertz and Chen 2016). Astrocytes and oligodendrocytes are the two main glial cells in the brain. These cells interconnect with each other and with neurons through neurotransmitters and gap junction channels (Nagy and Rash 2000). Gap junctions are channels between cells consisting of two hemichannels called connexons or pannexons. Each hemichannel is made of six subunit proteins named connexin (Cx) or pannexin (Panx) (Panchin 2005). Various genes encode connexin proteins (Willecke et al., 2005). Some of these connexins are expressed in the cells of the nervous system. Although different connexins do not show a cell-specific expression pattern in the nervous system, some of them are predominantly expressed in specific neural cells. For example, astrocytes express Cx30 and Cx43, whereas oligodendrocytes express Cx32 (Rash et al., 2001).

The process of memory formation occurs in some distinct steps (Abel and Lattal 2001). Of these, the consolidation step is central to the production of memory. The hippocampus has a vital role in processing learning and memory (Opitz 2014). It governs the consolidation stage of fear or spatial memory (Chaaya et al., 2018; Lorenzini et al., 1996). Diverse cell types of the hippocampus communicate via a network of gap junctions (Shiosaka et al., 1989). Meanwhile, glial cells influence the consolidation stage of memory (Hertz and Chen 2016), and their connexins are involved in this process. For example, the blockade of Cx43 hemichannels has been attendant with a major reduction in long-term potentiation (LTP) and deficiency in memory consolidation (Jammal et al., 2018). Also, microinjection of inhibitors of Cx43 hemichannels into basolateral amygdala (BLA) impaired memory in the course of fear conditioning (Stehberg et al., 2012).

Intercellular communication via gap junctions can be affected by alterations in the gating of the channels or changes in the number of channels. The number of channels depends on the expression levels of connexin proteins. The major stage for the regulation of connexins gene expression is the transcriptional level (Oyamada et al., 2005). It was previously shown that two of the hippocampal neuronal connexin proteins, Cx36 and Cx45 show a rapid upregulation during inhibitory avoidance memory consolidation (Beheshti et al., 2017). Considering the crucial role of glial gap junctions and connexins in memory formation, we studied the probable changes in the transcriptional patterns of three glial connexins, specifically Cx30, Cx32, and Cx43, within the hippocampus of rats in the course of consolidating either fear or spatial memory.

Material and methods

Laboratory Animals

The study was done on 32 adult male Wistar rats weighing 200±20 g. Animals were obtained from the breeding colony of the Faculty of Biological Science and Technology, University of Isfahan. Four rats were kept in each cage. Ambient conditions were standardized, maintaining animals at 22 ± 1◦C and a light cycle of 12:12.

Behavioral Trials

Animals were distributed into eight groups. Four groups of animals (n=4) were selected for each of the behavioral tests. Cx30, Cx32, or Cx43 mRNA levels were determined using quantitative real-time PCR. The experimental groups received the same treatment as described earlier (Beheshti and Dehestani, 2021).

Inhibitory avoidance task

The consolidation stage of memory is assessed by post-training manipulations in the inhibitory avoidance paradigm. The step-through passive avoidance was accomplished as in our previous study, with minor modifications (Beheshti and Aslani, 2018). First, the animals were placed in the white chamber contrasting the gliding door for habituation training. The door was then raised to allow the animals to enter the dark room. After entering, rats remained in the black chamber for 20 seconds. Thirty minutes later, rats were put in the white chamber for acquisition training and the door was raised. As soon as the animals went into the black chamber, the door was closed, and an electrical shock (0.8 mA, 3 seconds) was conveyed. The short-term memory was measured 2 min later. A successful memory formation was confirmed if the animal remained in the white chamber for two minutes. The sham group was treated as the test group without receiving any foot shocks. One hour following training, the sham group was sacrificed. Rats in the other groups were sacrificed one, three, and twenty-four hours after training. Connexins show rap-

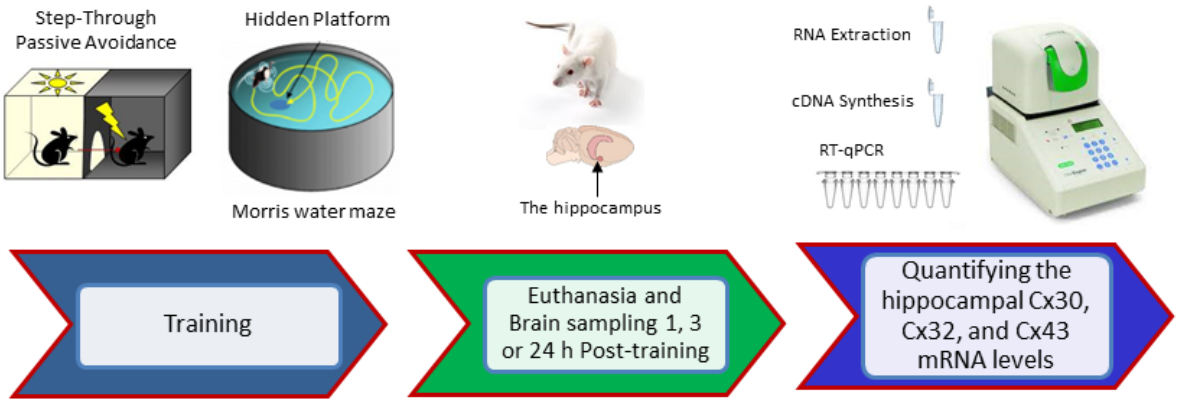

FIGURE 1. Illustration of the experimental procedure. Rats underwent the passive avoidance task or Morris water maze training. The hippocampi of the animals were removed one, three, and twenty-four hours after training. The expression levels of Cx30, Cx32, and Cx43 mRNAs were quantified by a quantitative real-time PCR.

id transcriptional changes even though they are membrane proteins (McCracken and Roberts 2006). For this reason, the hippocampal tissues were removed at these time intervals based on a previous study (Beheshti et al., 2017). Then the right hippocampi were isolated and set aside in a -70˚C freezer. The entire experiments were done between 9:00 and 15:00 h.

Morris water maze

The Morris water maze task was done in the same manner as in our previous study, with minor modifications to assess spatial memory consolidation (Beheshti et al., 2018). In brief, eight trials were performed in the training stage. Four starting points (east, west, north, and south) were used to place the animals in the water. The animals were given a maximum of 60 seconds to locate the invisible platform that was hidden in the southwest quadrant of the maze (also known as the target quadrant). When the animals found the platform, they were let to remain there for 20 seconds before moving on to the following trial, which took place after 30 seconds. Rats in the sham group had an equivalent swimming trial in the absence of the platform. One hour after the swimming trial, animals in the sham group were sacrificed. Rats in the other groups were sacrificed one, three, and twenty-four hours after training. Then the right hippocampi were isolated and set aside in a deep (-70˚C) freezer (Figure 1). The delay in reaching the platform was documented. The entire experiments were done between 9:00 and 15:00 h.

Quantitative Real-time PCR

The quantitative Real-time PCR was performed, as previously defined (Beheshti and Dehestani, 2021). In summary, the extraction of total RNA from the hippocampal tissue was conducted with RNX-PLUS solution (SinaClon, Iran). The RNAs were subjected to treatment with one unit of RNase-free DNase I to eliminate the likelihood of genomic DNA contamination. Quantification and qualification of the extracted RNAs were accomplished using a Nanodrop spectrophotometer. The mean absorbance ratio at 260/280 nm was 1.84 ± 0.05 and at 260/230 nm was 1.97 ± 0.17. Subsequently, a complementary DNA (cDNA) was made utilizing a commercially available cDNA synthesis kit (Takara, Japan).

Target genes were Cx30, Cx32, and Cx43. Beta-actin served as the housekeeping gene. The NCBI primer design tool was used to create primers (Table 1). With forward and reverse primers, 5µl of SYBR Green I Mastermix (Amplicon), 10 ng cDNA, and a reaction volume of 11µl, thermal cycling was performed using a Bio-Rad (Bio-Rad, USA) thermal cycler. To increase the reliability of the results, cDNAs were run in triplicate. The PCR was executed using the subsequent protocol: an initial denaturation step for 30 seconds at a temperature of 95°C, followed by 40 cycles each consisting of a denaturation stage at the same temperature and duration of 5 seconds, and an annealing stage at 55°C lasting 30 seconds. The Livak formula was used to determine the relative amount of mRNAs (Livak and Schmittgen 2001).

TABLE 1: The sequences of the specific primers and the relevant amplicon sizes.

Target gene	Sequences (5'→3')	Amplicon size	Accession Number
β-actin	Forward: CTGTGTGGATTGGTGGCTCT Reverse: CAGCTCAGTAACAGTCCGCC	135bp	NM_031144
Cx30	Forward: TGGTGGCTGTTATTGAAAGGTG Reverse: AACCACTGCAAAATCCCTCCT	142bp	NM_053388
Cx32	Forward: CAGGCAACCTCCCATCCAAC Reverse: AGTAATCCCTAGGAGGCAGAGG	96bp	NM_017251
Cx43	Forward: CTCACGTCCCACGGAGAAAA Reverse: CGCGATCCTTAACGCCTTTG	119bp	NM_012567

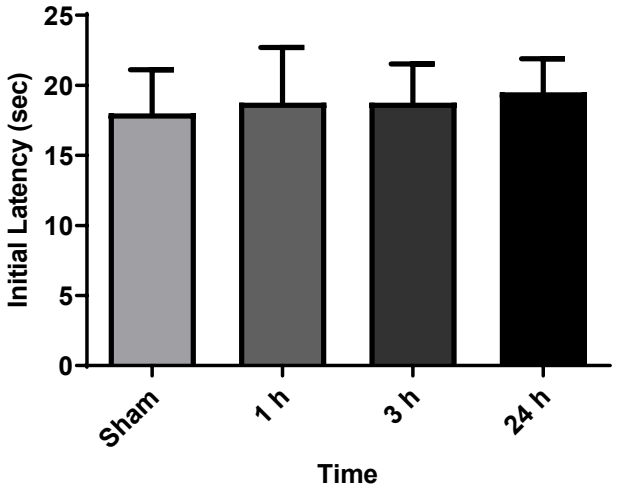

FIGURE 2. Initial latency to step into the black chamber in the passive avoidance task. Analysis of the data by one-way ANOVA showed no change in the initial latency amongst groups. Accordingly, rats had equivalent learning abilities at the beginning of the task. Data are shown as means ± S.E.M.

Statistical analysis

One-way analysis of variance (ANOVA) was used to evaluate the collected data. If the results were significant, we did a Tukey-Kramer test to compare different data points. The Graph Pad Prism version 9.1.0. program was used for the data analysis. The "p" values < 0.05 were set as the significance. Cohen's d-test was used to demonstrate effect sizes and to test the validity of statistics concerning sample size because the sample sizes were small and could not be used to calculate statistical power. Data were presented as mean ± S.E.M.

Results

The parameters of inhibitory avoidance

The initial latency to go into the black chamber is an important factor in the inhibitory avoidance task, which should not vary among experimental groups. Data analysis did not show a significant change in the initial latencies for learning acquisition between groups [$F(3, 12) = 0.22$; $P = 0.84$] (Figure 2).

The hippocampal Cx30, Cx32, and Cx43 mRNA levels during the inhibitory avoidance memory consolidation

There was no alteration observed in Cx30 mRNA levels in the hippocampus during the process of inhibitory avoidance memory consolidation, according to one-way ANOVA results [$F(3, 12) = 1.68$; $P=0.22$]. However, the hippocampal Cx32 mRNA levels showed a significant decrease 3 hours post-training. Cohen's d-test revealed a value of 0.9 between the sham and 3 h groups, which denotes a significant difference [$F(3, 12) = 4.972$; $P=0.01$; $d=0.9$]. Hippocampal Cx43 mRNA levels significantly decreased one hour after training, according to one-way ANOVA results. Cohen's d-test revealed a value of 1 between the sham and 1 h groups, which denotes a significant change [$F(3, 12) = 4.749$; $P= 0.02$; $d=1$] (Figure 3).

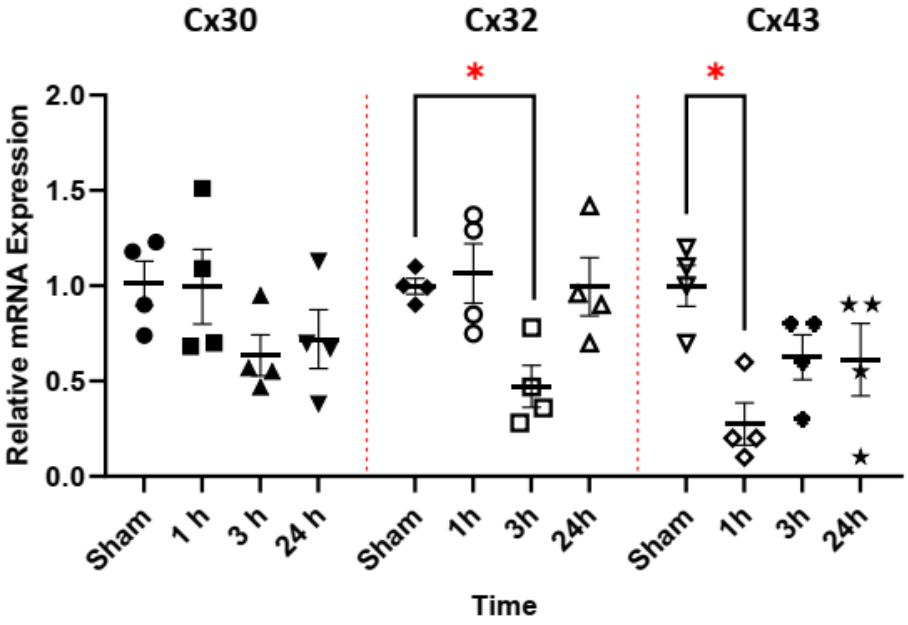

FIGURE 3. The comparative mRNA levels of Cx30, Cx32, or Cx43 in the rat hippocampus during the consolidation stage of memory in the passive avoidance task. Analysis of the data by one-way ANOVA followed by the Tukey-Kramer multiple comparisons test showed that Cx32 and Cx43 downregulated three or one hour after training, respectively. Cohen's d showed a large effect size for Cx32 or Cx43 amongst the sham and three or one-hour groups. Data are shown as means ± S.E.M. (*P<0.05).

Spatial memory performance

In the Morris water maze task, a decrease in escape latencies throughout the training trials indicates that memory acquisition has been successful. Our data showed that the time latency to discover the invisible platform declined during training trials significantly (data not presented). In the meantime, one-way ANOVA showed that during acquisition trials, the escape latencies between experimental groups did not change significantly from one another [F (3, 12) = 1.23; P = 0.32] (Figure 4).

The hippocampal Cx30, Cx32, and Cx43 mRNAs levels during the spatial memory consolidation

One-way ANOVA revealed that there was no significant change in the mRNA levels of the hippocampal Cx30 [F (3, 12) = 0.01; P=0.99] and Cx32 [F (3, 12) = 2.04; P=0.16] throughout the spatial memory consolidation. However, the levels of Cx43 mRNA altered significantly. The Tukey-Kramer comparison showed that compared with the sham group, the levels of Cx43 augmented 3 hours after training. Cohen's d-test revealed a value of 0.97 between the sham and 3 h groups, which denotes a significant change [F (3, 12) = 6.08; P=0.009; d= 0.97] (Figure 5).

Discussion

We indicated that the hippocampal Cx32 and Cx43 mRNAs downregulated during inhibitory avoidance memory consolidation, while Cx30 did not show a significant change. Conversely, Cx43 mRNA was upregulated during spatial memory consolidation, but Cx30 and Cx32 did not change significantly.

Glial cells were shown to affect both the encoding and consolidation stages of memory. Microglia, oligodendrocytes, and astrocytes were indicated to play crucial roles in synaptic plasticity like late stages of LTP and memory consolidation (Sancho et al., 2021). Additionally, the signaling of astrocytes played a contributing role in LTP induction in cholinergic neurons of the hippocampus and cortex (Navarrete et al., 2012). Glial cells interconnect with each other and with neurons via gap junction channels made of connexin proteins. This communication is argued to be vital for memory formation. Astrocytes contribute to neuronal transmission and synaptic plasticity by releasing gliotransmitters into the synaptic cleft (Lalo et al., 2014). There are diverse pathways for gliotransmitter release from astrocytes, which include Cx43 hemichannels (Cotrina et al., 1998). There is some data in the literature which has investigated the

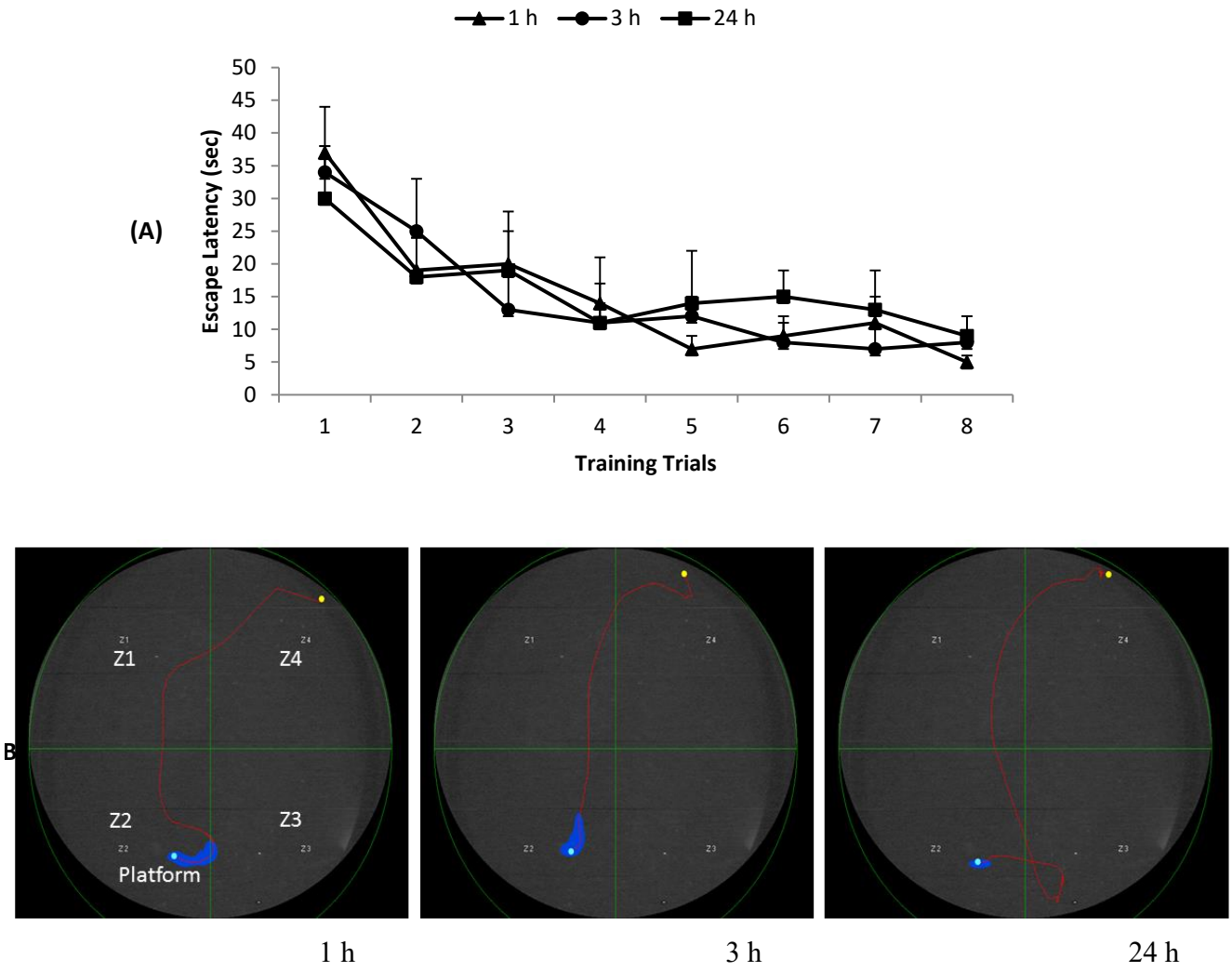

FIGURE 4. The acquisition of spatial memory. (A) The average time to discover the invisible platform is presented. (B) Typical swim path traces during the last training trial displayed that the animals discovered the platform shortly after placing into the pool. Analysis of the data by one-way ANOVA showed that the escape latencies did not differ between groups.

effects of Cx43-made gap junctions or hemichannels on different models of learning and memory (He et al., 2020).

Inhibition of Cx43 hemichannels in the BLA by the peptide TAT-Cx43L2 during the consolidation stage of memory induced loss of memory in the auditory fear conditioning, but did not disturb short-term memory. However, synaptic transmission or gap junctional communication between astrocytes was unaffected. Interestingly, the injection of TAT-Cx43L2 along with some of the gliotransmitters recovered memory (Stehberg et al., 2012). Meanwhile, the secretion of D-serine and glutamate from the BLA astrocytes through Cx43 hemichannels was shown to be crucial for the development of short and long-term memory (Linsambarth et al., 2022). These data show that modulation of Cx43 hemichannels might have a different impact from Cx43 gap junctional communication. Nevertheless, our results do not show whether the downregulation of Cx43 in the hippocampus relates to Cx43 hemichannels or gap junctions. Meanwhile, we measured the levels of Cx43 in the hippocampus, which has a less important role in fear memory formation than the amygdala. Therefore, the downregulation in the hippocampal Cx43 expression levels might relate to the different neuro-glial circuitry required for fear memory consolidation. Interestingly, downregulation of the hippocampal Cx43 expression or blocking Cx43 hemichannels reduced infrasound-in-

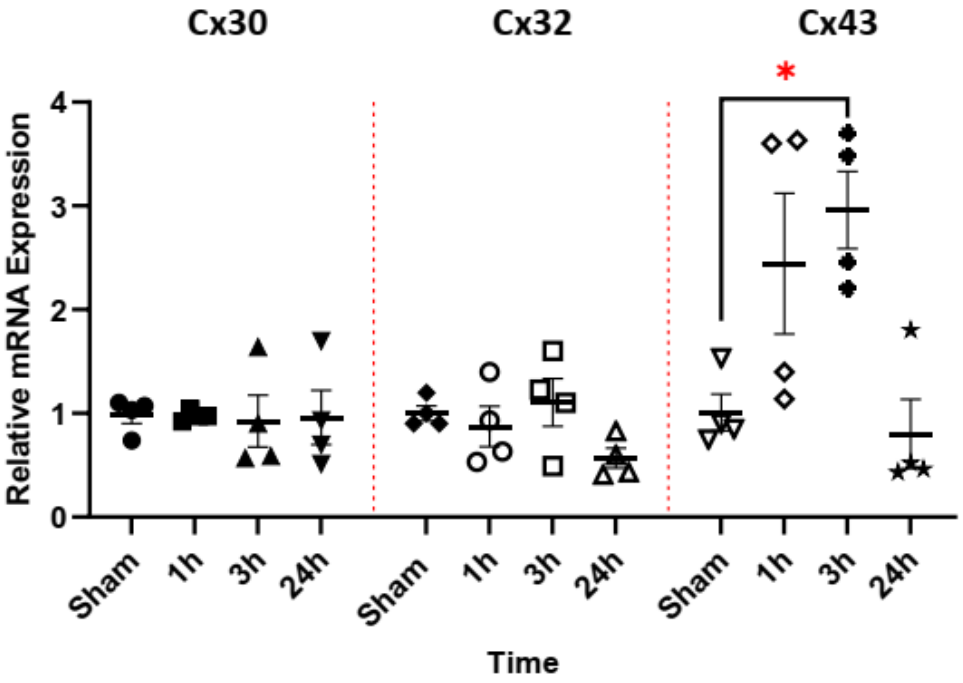

FIGURE 5. The comparative mRNA levels of Cx30, Cx32, or Cx43 in the rat hippocampus during the consolidation stage of spatial memory. Analysis of the data by one-way ANOVA followed by Tukey-Kramer multiple comparisons post-hoc test showed that the hippocampal Cx43 upregulated three hours after training. Cohen's d showed a large effect size for Cx43 amongst the sham and three-hour groups. Data are shown as means ± S.E.M. (*P<0.05).

duced memory impairment (Zhang et al., 2023).

Our results indicated that the hippocampal Cx32 and Cx43 show a somehow associated reduction in the expression levels during inhibitory avoidance memory consolidation. Astrocytes and oligodendrocytes showed gap junction-mediated communication in co-culture studies (Theis and Giaume 2012). This type of communication was shown in the hippocampus, the neocortex, and the thalamus (Griemsmann et al., 2015). It may have a crucial impact on the intercellular exchange of metabolic substrates amongst astrocytes and oligodendrocytes (Niu et al., 2016), which confounds neuroglial networking communications. Meanwhile, the intercellular connection between astrocytes critically diminished in cells missing Cx43 and stopped when both Cx30 and Cx43 did not express (Naus et al., 1997; Rouach et al., 2008; Velazquez et al., 1996; Wallraff et al., 2006). Thus, the existence of these two Cxs is crucial for the occurrence of direct intercellular communication within astrocytes.

We revealed that the hippocampal Cx43 increased during the consolidation stage of spatial memory. Blocking Cx43 made hemichannels impaired spatial short-term memory in the Y maze task (Walrave et al., 2016). In Cx43 deficient mice, the acquisition stage of memory formation was augmented in the water maze, while escape latencies during the probe test were like the control mice (Frisch et al., 2003). Inhibition of Cx43 hemichannels reduced the LTP of excitatory synaptic currents in the prefrontal cortex of the mouse (Meunier et al., 2017). In the resting state, the basal excitatory synaptic transmission of CA1 pyramidal neurons of the hippocampus was regulated by the activity of astrocytic Cx43 hemichannels via ATP signaling (Chever et al., 2014). It was revealed that neurons adjust the expression of Cx30 and Cx43 in cortical astrocytes (Koulakoff et al., 2008). In addition, neuronal activity upregulated the expression levels of the astrocytic Cx43 (Rouach et al., 2000a). Meanwhile, continual stimulation of neurons made enduring alterations in the calcium-wave activity of astrocytes, which affected the calcium waves of neurons (Pasti et al., 1997). Thus, it seems that neurons and astrocytes might communicate with each other during spatial memory consolidation. Double KO Cx43/Cx30 mice showed spatial memory deficiency, which indicates the role of astrocytic networking for proper brain functioning (Lutz et al., 2009). Meanwhile, inactivat-

ing Cx43 and Cx30 decreased synaptic transmission in neurons of the hippocampus (Pannasch et al., 2011). Accordingly, the upregulation of the hippocampal Cx43 expression levels might be a physiologic response to the need for enhancement of Cx43-related increase in synaptic plasticity during spatial memory consolidation.

There might also be another probable mechanism for the increased levels of the hippocampal Cx43 throughout the consolidation stage of spatial memory. Adult neurogenesis was suggested as a mechanism to mediate spatial memory. The targeted reduction of neurogenesis in the dentate gyrus during adulthood was found to have a detrimental effect on the capacity to retain spatial long-term memories during a water maze task, as well as during an object recognition task (Jessberger et al., 2009). Furthermore, the ablation of adult-generated neurons within the hippocampus has exhibited a detrimental effect on spatial memory (Dupret et al., 2008). The creation of new neurons during training was shown to be crucial for long-term memory in the water maze (Snyder et al., 2005). In this regard, the hippocampal Cx43 was shown to promote the persistence of newborn neurons in mice (Liebmann et al., 2013). Meanwhile, Cx43 but not Cx30 exhibited a significant contribution to the process of adult neurogenesis within the dentate gyrus (Zhang et al., 2018). Consequently, upregulation of the hippocampal Cx43 during spatial memory consolidation might contribute to neurogenesis required for memory formation.

Interestingly, neurons increased the expression of Cx43 in cell cultures. Neurons also induce Cx30 expression in astrocytes. Some mechanisms have been suggested to mediate the increase in neuronal-induced gap junctional interconnection. The enhancement of gap junctional coupling in astrocytes was observed as a result of activity-dependent K+ release mediated depolarization (Kristian Enkvist and McCarthy 1994), with the involvement of Cx43 phosphorylation by calcium-calmodulin protein kinase II (De Pina-Benabou et al., 2001). Glutamate was shown to be involved in this modulation (Escartin and Rouach 2013; Giaume 2010). The control of astrocytic Cx43 expression may be due to the influence of neuronal glutamate (Rouach et al., 2000b). As glutamatergic transmission plays a crucial role during the consolidation of memory, it is worth noting the impact of glutamate-induced alterations in Cx43 expression levels on mechanisms of memory consolidation. Therefore, it seems that neuronal functioning during spatial memory consolidation upregulates Cx43 gene expression, which in turn might have crucial roles for spatial memory consolidation.

The expression level of connexins was indicated to be changed rapidly, despite the fact that they are membrane proteins (McCracken and Roberts 2006). Cx36 and Cx45 mRNA levels were upregulated 30 min after training in the inhibitory avoidance task (Beheshti et al., 2017). Here, the expression levels of Cx43 mRNA showed a somehow rapid change (1 and 3 h after training) in two experimental models of memory. This finding is consistent with previous studies.

Though the expression levels of Cx43 mRNA were downregulated during inhibitory avoidance memory consolidation, their levels were upregulated during spatial memory consolidation. The differential change might somehow be due to the different neuronal circuitry involved in memory consolidation in these two learning and memory models. While the hippocampus has a crucial role in spatial memory consolidation (Redish and Touretzky 1998), the main role in fear memory is played by the amygdala (Slotnick 1973). However, the outcomes of such differential changes should be elucidated in upcoming studies. This research adds to the body of evidence highlighting the potential role of glial gap junctions in the physiological state of the nervous system.

Conclusion

To sum up, the results of the present investigation displayed a differential alteration in the quantity of two hippocampal glial connexins, Cx32, and Cx43 during the consolidation stage of the inhibitory avoidance or spatial memory. The differential change might be somehow due to the different neuronal circuitry involved in memory consolidation in these two learning and memory models. The findings underscore the significance of glial gap junction channels on memory consolidation and indicate that they could potentially play a notable role in this process.

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Conflict of interest

The authors take full responsibility for the writing and content of this article and confirm that there are no conflicts of interest associated with this academic publication.

Ethics approval

Animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals, and reported according to the ARRIVE Guidelines. They were approved by the ethics committee of the University of Isfahan (Code No.IR.UI.REC.1398.078).

References

Abel T, Lattal K M. Molecular mechanisms of memory acquisition, consolidation and retrieval. Current Opinion in Neurobiology 2001; 11: 180-187. https://doi.org/10.1016/S0959-4388(00)00194-X

Beheshti S, Aslani N. Local injection of d-lys-3-GHRP-6 in the rat amygdala, dentate gyrus or ventral tegmental area impairs memory consolidation. Neuropeptides 2018; 67: 20-26. https://doi.org/10.1016/j.npep.2017.11.002

Beheshti S, Dehestani H. Differential expression levels of the hippocampal ghrelin and its receptor mRNA during memory consolidation. Behav Brain Res 2021; 408: 113270. https://doi.org/10.1016/j.bbr.2021.113270

Beheshti S, Ghorbanpour Skakakomi A, Ghaedi K, Dehestani H. Frankincense upregulates the hippocampal calcium/calmodulin kinase II-α during development of the rat brain and improves memory performance. Int J Dev Neurosci 2018; 69: 44-48. https://doi.org/10.1016/j.ijdevneu.2018.06.011

Beheshti S, Zeinali R, Esmaeili A. Rapid upregulation of the hippocampal connexins 36 and 45 mRNA levels during memory consolidation. Behav Brain Res 2017; 320: 85-90. https://doi.org/10.1016/j.bbr.2016.11.048

Chaaya N, Battle A R, Johnson L R. An update on contextual fear memory mechanisms: Transition between Amygdala and Hippocampus. Neurosci Biobehav Rev 2018; 92: 43-54. https://doi.org/10.1016/j.neubiorev.2018.05.013

Chever O, Lee C-Y, Rouach N. Astroglial connexin43 hemichannels tune basal excitatory synaptic transmission. The Journal of Neuroscience 2014; 34: 11228-11232. https://doi.org/10.1523/JNEUROSCI.0015-14.2014

Cotrina M L, Lin J H, Alves-Rodrigues A, Liu S, Li J, Azmi-Ghadimi H, et al. Connexins regulate calcium signaling by controlling ATP release. Proc Natl Acad Sci U S A 1998; 95: 15735-40. https://doi.org/10.1073/pnas.95.26.15735

De Pina-Benabou M H, Srinivas M, Spray D C, Scemes E. Calmodulin kinase pathway mediates the K+-induced increase in Gap junctional communication between mouse spinal cord astrocytes. Journal of Neuroscience 2001; 21: 6635-6643. https://doi.org/10.1523/JNEUROSCI.21-17-06635.2001

Dupret D, Revest J M, Koehl M, Ichas F, De Giorgi F, Costet P, et al. Spatial relational memory requires hippocampal adult neurogenesis. PLoS One 2008; 3: e1959. https://doi.org/10.1371/journal.pone.0001959

Escartin C, Rouach N. Astroglial networking contributes to neurometabolic coupling. Frontiers in neuroenergetics 2013; 5: 4-4. https://doi.org/10.3389/fnene.2013.00004

Frisch C, Theis M, De Souza Silva M A, Dere E, Söhl G, Teubner B, et al. Mice with astrocyte-directed inactivation of connexin43 exhibit increased exploratory behaviour, impaired motor capacities, and changes in brain acetylcholine levels. European Journal of Neuroscience 2003; 18: 2313-2318. https://doi.org/10.1046/j.1460-9568.2003.02971.x

Giaume C. Astroglial wiring is adding complexity to neuroglial networking. Frontiers in neuroenergetics 2010; 2: 129. https://doi.org/10.3389/fnene.2010.00129

Griemsmann S, Höft S P, Bedner P, Zhang J, von Staden E, Beinhauer A, et al. Characterization of panglial gap junction networks in the thalamus, neocortex, and hippocampus reveals a unique population of glial cells. Cereb Cortex 2015; 25: 3420-33. https://doi.org/10.1093/cercor/bhu157

He J-T, Li X-Y, Yang L, Zhao X. Astroglial connexins and cognition: memory formation or deterioration? Bioscience reports 2020; 40: BSR20193510. https://doi.org/10.1042/BSR20193510

Hertz L, Chen Y. Editorial: All 3 types of glial cells are important for memory formation. Frontiers in Integrative Neuroscience 2016; 10. https://doi.org/10.3389/fnint.2016.00031

Jammal L, Whalley B, Barkai E. Learning-induced modulation of the effect of neuroglial transmission on synaptic plasticity. J Neurophysiol 2018; 119: 2373-2379. https://doi.org/10.1152/jn.00101.2018

Jessberger S, Clark R E, Broadbent N J, Clemenson G D, Jr., Consiglio A, Lie D C, et al. Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. Learn Mem 2009; 16: 147-54. https://doi.org/10.1101/lm.1172609

Kandel E R. The molecular biology of memory: cAMP, PKA, CRE, CREB-1, CREB-2, and CPEB. Molecular Brain

2012; 5: 14. https://doi.org/10.1186/1756-6606-5-14

Koulakoff A, Ezan P, Giaume C. Neurons control the expression of connexin 30 and connexin 43 in mouse cortical astrocytes. Glia 2008; 56: 1299-311. https://doi.org/10.1002/glia.20698

Kristian Enkvist M, McCarthy K D. Astroglial gap junction communication is increased by treatment with either glutamate or high K+ concentration. Journal of neurochemistry 1994; 62: 489-495. https://doi.org/10.1046/j.1471-4159.1994.62020489.x

Lalo U, Rasooli-Nejad S, Pankratov Y. Exocytosis of gliotransmitters from cortical astrocytes: implications for synaptic plasticity and aging. Journal 2014. https://doi.org/10.1042/BST20140163

Liebmann M, Stahr A, Guenther M, Witte O W, Frahm C. Astrocytic Cx43 and Cx30 differentially modulate adult neurogenesis in mice. Neurosci Lett 2013; 545: 40-5. https://doi.org/10.1016/j.neulet.2013.04.013

Linsambarth S, Carvajal F J, Moraga-Amaro R, Mendez L, Tamburini G, Jimenez I, et al. Astroglial gliotransmitters released via Cx43 hemichannels regulate NMDAR-dependent transmission and short-term fear memory in the basolateral amygdala. Faseb j 2022; 36: e22134. https://doi.org/10.1096/fj.202100798RR

Livak K J, Schmittgen T D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25: 402-8. https://doi.org/10.1006/meth.2001.1262

Lorenzini C A, Baldi E, Bucherelli C, Sacchetti B, Tassoni G. Role of dorsal hippocampus in acquisition, consolidation and retrieval of rat's passive avoidance response: a tetrodotoxin functional inactivation study. Brain Res 1996; 730: 32-9. https://doi.org/10.1016/0006-8993(96)00427-1

Lutz S E, Zhao Y, Gulinello M, Lee S C, Raine C S, Brosnan C F. Deletion of astrocyte connexins 43 and 30 leads to a dysmyelinating phenotype and hippocampal CA1 vacuolation. Journal of Neuroscience 2009; 29: 7743-7752. https://doi.org/10.1523/JNEUROSCI.0341-09.2009

McCracken C B, Roberts D C. A single evoked afterdischarge produces rapid time-dependent changes in connexin36 protein expression in adult rat dorsal hippocampus. Neuroscience letters 2006; 405: 84-88. https://doi.org/10.1016/j.neulet.2006.06.025

Meunier C, Wang N, Yi C, Dallerac G, Ezan P, Koulakoff A, et al. Contribution of astroglial Cx43 hemichannels to the modulation of glutamatergic currents by d-serine in the mouse prefrontal cortex. J Neurosci 2017; 37: 9064-9075. https://doi.org/10.1523/JNEUROSCI.2204-16.2017

Nagy J I, Rash J E. Connexins and gap junctions of astrocytes and oligodendrocytes in the CNS. Brain Research Reviews 2000; 32: 29-44. https://doi.org/10.1016/S0165-0173(99)00066-1

Naus C C, Bechberger J F, Zhang Y, Venance L, Yamasaki H, Juneja S C, et al. Altered gap junctional communication, intercellular signaling, and growth in cultured astrocytes deficient in connexin43. Journal of neuroscience research 1997; 49: 528-540. https://doi.org/10.1002/(SICI)1097-4547(19970901)49:5<528::AID-JNR3>3.0.CO;2-D

Navarrete M, Perea G, de Sevilla D F, Gómez-Gonzalo M, Núñez A, Martín E D, et al. Astrocytes mediate in vivo cholinergic-induced synaptic plasticity. PLoS biology 2012; 10: e1001259. https://doi.org/10.1371/journal.pbio.1001259

Niu J, Li T, Yi C, Huang N, Koulakoff A, Weng C, et al. Connexin-based channels contribute to metabolic pathways in the oligodendroglial lineage. J Cell Sci 2016; 129: 1902-14. https://doi.org/10.1242/jcs.178731

Opitz B. Memory function and the hippocampus. Front Neurol Neurosci 2014; 34: 51-9. https://doi.org/10.1159/000356422

Oyamada M, Oyamada Y, Takamatsu T. Regulation of connexin expression. Biochim Biophys Acta 2005; 1719: 6-23. https://doi.org/10.1016/j.bbamem.2005.11.002

Panchin Y V. Evolution of gap junction proteins - the pannexin alternative. Journal of Experimental Biology 2005; 208: 1415-1419. https://doi.org/10.1242/jeb.01547

Pannasch U, Vargová L, Reingruber J, Ezan P, Holcman D, Giaume C, et al. Astroglial networks scale synaptic activity and plasticity. Proceedings of the national academy of sciences 2011; 108: 8467-8472. https://doi.org/10.1073/pnas.1016650108

Pasti L, Volterra A, Pozzan T, Carmignoto G. Intracellular calcium oscillations in astrocytes: a highly plastic, bidirectional form of communication between neurons and astrocytes in situ. J Neurosci 1997; 17: 7817-30. https://doi.org/10.1523/JNEUROSCI.17-20-07817.1997

Rash J E, Yasumura T, Dudek F E, Nagy J I. Cell-specific expression of connexins and evidence of restricted gap junctional coupling between glial cells and between neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience 2001; 21: 1983-2000. https://doi.org/10.1523/JNEUROSCI.21-06-01983.2001

Redish A D, Touretzky D S. The role of the hippocampus in solving the morris water maze. Neural Computation 1998; 10: 73-111. https://doi.org/10.1162/089976698300017908

Rouach N, Glowinski J, Giaume C. Activity-dependent neuro-

nal control of gap-junctional communication in astrocytes. J Cell Biol 2000a; 149: 1513-26. https://doi.org/10.1083/jcb.149.7.1513

Rouach N, Glowinski J, Giaume C. Activity-dependent neuronal control of gap-junctional communication in astrocytes. The Journal of cell biology 2000b; 149: 1513-1526. https://doi.org/10.1083/jcb.149.7.1513

Rouach N, Koulakoff A, Abudara V, Willecke K, Giaume C. Astroglial metabolic networks sustain hippocampal synaptic transmission. science 2008; 322: 1551-1555. https://doi.org/10.1126/science.1164022

Sancho L, Contreras M, Allen N J. Glia as sculptors of synaptic plasticity. Neuroscience Research 2021; 167: 17-29. https://doi.org/10.1016/j.neures.2020.11.005

Shiosaka S, Yamamoto T, Hertzberg E L, Nagy J I. Gap junction protein in rat hippocampus: correlative light and electron microscope immunohistochemical localization. J Comp Neurol 1989; 281: 282-97. https://doi.org/10.1002/cne.902810210

Slotnick B M. Fear behavior and passive avoidance deficits in mice with amygdala lesions. Physiology & Behavior 1973; 11: 717-720. https://doi.org/10.1016/0031-9384(73)90258-8

Snyder J S, Hong N S, McDonald R J, Wojtowicz J M. A role for adult neurogenesis in spatial long-term memory. Neuroscience 2005; 130: 843-52. https://doi.org/10.1016/j.neuroscience.2004.10.009

Stehberg J, Moraga-Amaro R, Salazar C, Becerra A, Echeverria C, Orellana J A, et al. Release of gliotransmitters through astroglial connexin 43 hemichannels is necessary for fear memory consolidation in the basolateral amygdala. Faseb j 2012; 26: 3649-57. https://doi.org/10.1096/fj.11-198416

Theis M, Giaume C. Connexin-based intercellular communication and astrocyte heterogeneity. Brain research 2012; 1487: 88-98. https://doi.org/10.1016/j.brainres.2012.06.045

Velazquez J P, Frantseva M, Naus C, Bechberger J, Juneja S, Velumian A, et al. Development of astrocytes and neurons in cultured brain slices from mice lacking connexin43. Developmental brain research 1996; 97: 293-296. https://doi.org/10.1016/S0165-3806(96)00156-3

Wallraff A, Köhling R, Heinemann U, Theis M, Willecke K, Steinhäuser C. The impact of astrocytic gap junctional coupling on potassium buffering in the hippocampus. Journal of Neuroscience 2006; 26: 5438-5447. https://doi.org/10.1523/JNEUROSCI.0037-06.2006

Walrave L, Vinken M, Albertini G, De Bundel D, Leybaert L, Smolders I J. Inhibition of connexin43 hemichannels impairs spatial short-term memory without affecting spatial working memory. Frontiers in cellular neuroscience 2016; 10: 288. https://doi.org/10.3389/fncel.2016.00288

Willecke K, Eiberger J, von Maltzahn J. Connexin and pannexin genes in the mouse and human genome. In: Winterhager E, editor. Gap Junctions in Development and Disease. Berlin, Heidelberg: Springer Berlin Heidelberg, 2005: 1-12. https://doi.org/10.1007/3-540-28621-7_1

Zhang J, Griemsmann S, Wu Z, Dobrowolski R, Willecke K, Theis M, et al. Connexin43, but not connexin30, contributes to adult neurogenesis in the dentate gyrus. Brain Res Bull 2018; 136: 91-100. https://doi.org/10.1016/j.brainresbull.2017.07.001

Zhang W, Yin J, Gao B Y, Lu X, Duan Y J, Liu X Y, et al. Inhibition of astroglial hemichannels ameliorates infrasonic noise-induced short-term learning and memory impairment. Behav Brain Funct 2023; 19: 23. https://doi.org/10.1186/s12993-023-00226-7