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EXPERIENCE-DEPENDENT EXPRESSION OF RAT HIPPOCAMPAL *ARC* AND *HOMER 1A* AFTER SPATIAL LEARNING ON 8-ARM AND 12-ARM RADIAL MAZES

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Abstract—The expression of *Arc* and *Homer 1a* (*H1a*) depends on neural activity. This study was designed to determine hippocampal *Arc* and *H1a* mRNA expression levels after spatial learning with differing behavioral task demands. Forty-four male rats were distributed into 11 groups of four. One group received no training or trial sessions. Of the ten remaining groups, three were tested on the 8-arm maze, three on the 12-arm maze, two on the 8-arm maze and then the 12-arm maze, and two on the 12-arm maze and then the 8-arm maze. Each animal was sacrificed 30 min after the last session of maze testing and its hippocampus was immediately dissected and stored at −80 °C. The level of mRNA expression at different stages of maze learning was determined using real-time reverse-transcription polymerase chain reaction (qRT-PCR). Significantly elevated expression of both *Arc* and *H1a* was observed. The orchestrated expression levels of both genes were correlated with the behavioral task demand level and behavioral performance.

Key words: learning, memory, immediate early genes, radial-arm maze, hippocampus, qRT-PCR.

INTRODUCTION

Memory storage depends profoundly on neural plasticity at synaptic connections (Lynch, 2004; Bramham et al., 2008). The molecular mechanisms of induction and stabilization of plasticity-dependent changes are of great importance in molecular neuroscience (Bredt and Nicoll, 2003). Activity-dependent immediate early genes (IEGs) expression is a critical component of molecular cascades underlying synaptic plasticity and long-term memory formation (Robertson, 1992; Dragunow, 1996; Tischmeyer and Grimm, 1999). Among IEGs, activity-regulated cytoskeleton-associated protein (abbreviated as *Arc* (Lyford et al., 1995), also known as *Arg3.1* (Link et al., 1995)) has been widely implicated in hippocampal memory consolidation due to its highly regulated expression and localization at activated synapses (Link et al., 1995; Lyford et al., 1995; Guzowski et al., 2000; Dynes and Steward, 2007; Ploski et al., 2008). Intrahippocampal injection of *Arc* antisense oligodeoxynucleotides impairs maintenance of long term potentiation and consolidation of long-term memory (Guzowski et al., 2000). *Arc* gene expression has been found to be significantly elevated by behaviors inducing long term potentiation, for example Guzowski et al. (2001) reported a correlation between *Arc* expression in the hippocampus and performance on a spatial water maze task. The importance of *Arc* in synaptic plasticity arises from its critical role in regulating AMPA receptor trafficking and homeostatic scaling of synapses containing AMPA receptors (Chowdhury et al., 2006; Plath et al., 2006; Shepherd et al., 2006; Kessels and Malinow, 2009). *Homer 1a* (*H1a*) is another important IEG involved in modifying glutamatergic signaling pathways (Xiao et al., 2000). After a behavioral experience, *H1a* and *Arc* are expressed by a common set of neurons (Vazdarjanova et al., 2002). Alterations in both *Arc* and *H1a* mRNA and protein levels correlate with the task parameters in several behavioral and experimental paradigms such as high frequency stimulation (Lyford et al., 1995; Steward et al., 1998), novel exploration of the environment and spatial learning and memory formation (Guzowski et al., 1999; Pinaud et al., 2001; Vazdarjanova and Guzowski, 2004).

Spatial navigation in rodents, particularly in rats, has been widely applied as a paradigm for recognizing brain structures involved in spatial learning and memory as well as in investigations of genes and proteins associated with these types of processes (Griffin et al., 2007). Several researchers have studied the regulation of *Arc* and *H1a* expressions in different behavioral tasks such as the Morris water maze (Guzowski et al., 2001; Guzowski, 2002) and the lever pressing task (Kelly and Deadwyler, 2002; Rapanelli et al., 2009). These investigations have revealed an increased expression of these two genes in newly trained compared with over-trained rats. However, the correlation between the expression levels of IEGs in

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Abbreviations: ANOVA, analysis of variance; Arc, activity-regulated cytoskeleton-associated protein; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; H1a, Homer 1a; IDD, infrared diode detector; IEG, immediate early gene; INF, intranuclear foci; qRT-PCR, real-time reverse-transcription polymerase chain reaction; RAM, radial-arm maze; RME, Reference Memory Errors; SEM, standard error of the mean; WME, Working Memory Errors.

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general, particularly those of *Arc* and *H1a*, and the level of behavioral task demand and behavioral performance has not yet been clearly studied. This project was designed to study the concerted expression of both genes in question and the link between gene expression level, the level of behavioral task demand and the behavioral performance.

EXPERIMENTAL PROCEDURES

Animals

Forty-four 2-month old male Wistar rats weighing 220 g on average were acclimatized to the animal room conditions for one week. The animals were housed in pairs in standard rat cages with food and water available *ad libitum*. The animal room was maintained with a reverse 12 h of light/dark cycle and the temperature was set at 22 °C. All handling, shaping, training and testing were performed during the dark phase from 7:00 AM to 7:00 PM. After the acclimatization period, the animals were semi-randomly distributed into 11 groups, each consisting of four animals. These groups were named according to the maze and number of sessions completed by the group. Animals in the control group (C) completed no training or trial sessions. The animals in groups 8F, 8M and 8L were trained and tested on the 8-arm maze for 1, 12 or 25 sessions, respectively. Likewise, the animals in groups 12F, 12M and 12L were trained and tested on 12-arm maze for 1, 12 or 25 sessions, respectively. The animals in the remaining groups completed 25 sessions on one maze and then were tested on the other maze. Groups 8–12F and 8–12L began with the 8-arm maze and then completed 1 or 4 sessions, respectively, on the 12-arm maze. Similarly, groups 12–8F and 12–8L began with the 12-arm maze and then completed 1 or 4 sessions, respectively, on the 8-arm maze. In all cases, one training or testing session was completed each day. All experimental procedures reported here were approved by the Regional Bioethics Committee in Isfahan.

Behavioral procedures

Behavioral training and testing were conducted using a standard 12-arm radial-arm maze (RAM) which could be easily converted to an 8-arm maze. This apparatus, with its multiple behavioral challenges due to different configurations, provided the possibility to evaluate the expression of *Arc* and *H1a* during different stages of maze learning. Exposure to a novel environment, incomplete and complete learning of the task, and stable memory retrieval were considered for evaluation. Since rats typically complete learning on RAM after 18–25 daily sessions (Olton, 1979), a total of 25 sessions were considered for achieving the stable performance. These 25 sessions were systematically performed during five weeks. No handling or testing was performed on weekends.

All rats tested on the maze had five days of handling. Each animal was taken out of the cage, kept on the tester's arm, weighed and then it was gently put back into its cage. Following the handling sessions, the rats had five days of shaping prior to testing. This procedure involved leaving each animal inside an opaque cylindrical ring, which had a diameter of 25 cm and a height of 30 cm and was located in the central arena of the RAM, for four minutes for the 8-arm maze and six minutes for the 12-arm maze or until all the baits present (4 or 6) were eaten. As the rats were shaped, their daily food intake was gradually limited to decrease their weight to 85% of their *ad libitum* weight in order to be more motivated to eat the baits in the maze. They were also food restricted throughout the experiment to maintain this body weight. A small piece of popcorn (50 mg) was used as the bait or the food reinforcement during shaping and maze testing.

The behavioral apparatus used in this study was a custom-made automated RAM elevated 50 cm above the floor. It consisted of twelve identical 60-cm arms radiating from a central circular platform, which had a diameter of 60 cm. All surfaces of the maze that the animals were exposed to were made of opaque white Plexiglass sheets installed on wooden boards. The maze was designed so that it could be easily converted into an 8-arm maze by removing four arms and adjusting the remaining arms to equalize the distance between the arms. Each arm was equipped with three pairs of infrared diode detectors (IDDs). The first pair was located 15 cm into each arm and the second pair was placed at the middle point of each arm. These pairs were positioned so that the infrared light beam was 2 cm above the maze floor. Together these pairs provided an assured registry of when the animal entered each arm. The entrance of an animal to an arm was detected by the interruption of the infrared light beam between a pair of IDDs as the hind paws of the rats entered the arm. The third IDD set was located by the food cup, which was at the end of each arm. This set maintained an infrared light beam diagonally through the circular food cup, which had a diameter of 2 cm. When baited, this light beam was interrupted. However, after the bait was taken by a rat, the light beam was reset, registering this action. Automation of the RAM and the input signals from the interruptions or resets of the light beams were controlled by a custom-made software. While a maze test was in progress, the shape of the maze and the location of the animal being tested were both shown schematically on a computer monitor. The software was also capable of processing and saving several variables, including total time period of the session, the number of arms visited, the time spent on each arm, and the number of reference and working memory errors committed. In the exceptional case of unregistered arm entry or food intake, the software let the experimenter to manually register these events. The whole session was monitored with a camera placed on top of the apparatus.

Two 100-watt regular incandescent light bulbs were used to provide suitable visible light with a mean intensity equal to 300 lux at the level of the maze. Several black and white posters showing simple geometrical shapes were installed at fixed positions on all four walls surrounding the maze. Prior to each session of maze testing, the maze was wiped out with a clean moist towel. The rat was gently put inside the central opaque ring for 5 s and then the ring was taken away allowing the animal to freely explore the maze. The tester sat on a chair in front of the computer in the same corner of the room. Half of the maze arms (6 of the 12-arm or 4 of the 8-arm) were baited semi-randomly (no more than two adjacent arms were baited). The same baiting pattern was consistent for each rat throughout all the testing sessions. For those rats that completed both the 8-arm and 12-arm mazes, two baited and two unbaited arms were either removed or added (depending on the direction of change), leaving the remaining arms in the same spatial location. No two consecutively tested rats had the same baiting pattern. An arm of the maze was recorded as visited when both infrared light beams of the entrance IDDs were interrupted consecutively. Each session of testing continued until the rat ate all 4 or 6 baits or until the maximum time of either 4 or 6 min (for 8-arm and 12-arm mazes, respectively) expired.

Molecular procedures

Tissue dissection and RNA extraction for qRT-PCR. In all cases except the control group, rats were sacrificed exactly 30 min after their last testing session on the maze. The rats were first anesthetized with halothane then sacrificed using a rodent guillotine. Their brains were quickly and cautiously removed. The hippocampi were dissected and frozen using liquid nitrogen, and kept at −80 °C for further processing. Total hippocampal RNA was extracted using RNX plus kit (Cinnagen, Tehran, Iran). First-strand cDNA was then synthesized from a 1-μg total RNA aliquot in a reaction mixture containing random hexamer primer by performing the RevertAid reverse transcription protocol (K1622; Fermentas, Hanover, MD, USA) and stored at −20 °C.