



# The time course of systems consolidation of spatial memory from recent to remote retention: A comparison of the Immediate Early Genes Zif268, c-Fos and Arc



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## ABSTRACT

Systems consolidation is a process involving the stabilisation of memory traces in the neocortex over time. The medial prefrontal cortex becomes increasingly important during the retrieval of older memories, however the timescale of its involvement is unclear, and the contribution of other neocortical brain regions to remote memory have received little attention. The Immediate Early Genes (IEGs) Zif268, c-Fos and Arc have been utilised as markers of neural activity during spatial memory retrieval, however the lack of a direct comparison between them hinders the interpretation of results. To address these questions, we examined the expression of Zif268, Arc and c-Fos protein in the medial prefrontal cortex, as well as the hippocampus, and the entorhinal, perirhinal, retrosplenial and parietal cortices of male Wistar rats following a probe trial of the Morris water maze either one day, seven days, 14 days or 30 days after acquisition. Activity of the medial prefrontal cortex during retrieval, as measured by all three IEGs, increased in correspondence with the age of the memory, reaching significance between 14 and 30 days. Similar increases in c-Fos and Arc were observed over the course of consolidation in other neocortical and parahippocampal areas, however this pattern was not observed with Zif268. Activity of the hippocampus remained largely unchanged across retention intervals. These findings suggest that systems consolidation of spatial memory takes at least two weeks, are consistent with an ongoing role for the hippocampus in the retrieval of spatial memory, and suggest that c-Fos and Arc may be a more sensitive measure of neural activity in response to behavioural tasks than Zif268.

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## 1. Introduction

Newly acquired memories are thought to depend primarily on the hippocampus for their successful retrieval, whereas the neocortex assumes this responsibility over the course of time, in a process known as systems level consolidation (Frankland & Bontempi, 2005). However, whether or not the role of the hippocampus in retrieving detailed episodic and spatial memory is time-limited (Squire, 1992) or permanent (Nadel & Moscovitch, 1997) remains an open debate. There exists neuropsychological evidence to support both views (Squire & Bayley, 2007; Winocur & Moscovitch, 2011), and studies of spatial memory in animals have also proven inconclusive. Hippocampal lesions spare remote spatial discrimination memory (Maviel, Durkin, Menzaghi, & Bontempi, 2004), but not allocentric spatial memory in the Morris water maze (Broadbent, Squire, & Clark, 2006). Contextual fear memories can

be abolished by pharmacological inactivation (Cullen, Gilman, Winiecki, Riccio, & Jasnow, 2015) or optogenetic inhibition (Goshen et al., 2011) of area CA1 in the hippocampus for prolonged retention intervals of up to 12 weeks. Furthermore, the precision of remote spatial memory in the water maze, and contextual fear memory, is associated with the extent of long-term structural plasticity in area CA3 (Ruediger et al., 2011).

The neocortical structures supporting remote spatial memory have not been clearly defined, although inactivation of the anterior cingulate cortex disrupts remote memories while leaving recent memories intact (Teixeira, Pomedli, Maei, Kee, & Frankland, 2006). However, this region may play a role in both recent and remote recall of spatial memory in the water maze (Leon, Bruno, Allard, Nader, & Cuello, 2010). Therefore it is unclear how long it takes for newly acquired spatial memories to become dependent on extrahippocampal structures. Studies investigating systems consolidation in animals normally regard one and 30 days as recent and remote retention, respectively. However, attempts to disrupt spatial memory consolidation in first few weeks following

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learning suggest that this process may take place over a shorter timescale (Riedel et al., 1999; Shimizu, 2000; Frankland, O'Brien, Ohno, Kirkwood, & Silva, 2001).

The expression of Immediate Early Genes (IEGs) such as Zif268, c-Fos and Arc is rapidly induced following neuronal activity, leading to structural and functional changes to the neuron which are essential for memory formation (Tischmeyer & Grimm, 1999; Knapska & Kaczmarek, 2004; Plath & et al., 2006). IEG imaging allows for the visualisation of patterns of neural activity following learning, and has also been employed to investigate the activity of brain regions during memory retrieval (see Barry & Commins, 2011). Hippocampal IEG expression decreases from recent to remote memory in a spatial discrimination task (Maviel et al., 2004), but remains elevated during allocentric remote spatial memory retrieval in the Morris water maze (Teixeira et al., 2006). Increases in IEG expression are observed from recent to remote retention in the anterior cingulate cortex for both tasks, however (Maviel et al., 2004; Teixeira et al., 2006). To date, one study has attempted to chart the increase in activity of cortical sites over the course of spatial memory consolidation using the IEG c-Fos as a marker of neural activity (Bonaccorsi et al., 2013), finding activity of the anterior cingulate cortex during a probe trial significantly increased as early as 10 days following learning.

IEG imaging studies often use just a single marker of neural activity, which can potentially hinder comparisons between findings and their interpretation. Here, we directly compared three IEGs, Zif268, c-Fos and Arc, to investigate the activity of hippocampal, parahippocampal and neocortical brain regions following a water maze probe trial at either one day, seven days, 14 days or 30 days following acquisition. By examining the extent of hippocampal and cortical engagement from recent to remote retention, we aimed to chart the time-course of systems consolidation of spatial memory, and investigate whether or not these three well-established markers of neural activity revealed similar patterns of expression.

## 2. Materials and methods

### 2.1. Subjects

Thirty-three male Wistar rats, obtained from Charles River Laboratories, UK, were used as subjects in this experiment. Subjects were approximately three months old and weighed 200–300 g at the beginning of experimentation. All animals were housed three per cage, in a temperature controlled environment ( $21 \pm 1$  °C), which was maintained on a fixed 12:12 h light–dark cycle (07:00–19:00). All rats were given ad libitum access to food and water. Experimentation took place during the light phase and all subjects were well handled before experimentation began. Guidelines for the maintenance and experimentation of animals conformed to the Department of Health and Children (Ireland) guidelines under statutory instrument (S.I.) No. 543 of 2012 and the European directive 2010/63/EU.

### 2.2. Spatial task

The Morris water maze (Morris, 1981), was chosen as the spatial task as it is an extensively studied and particularly demanding task of allocentric spatial memory. The water maze was made of black fibreglass 1.7 m in diameter and 36 cm in depth, mounted on a platform 70 cm above the floor. The maze was filled with water to a depth of 20 cm and maintained at a temperature of  $20 \pm 1$  °C. The escape platform was 13.5 cm in width and 18 cm in height, and was placed in the centre of the northeast quadrant of the maze, submerged 2 cm below the surface of the water. The

water maze was surrounded by a black curtain which obscured the rest of the room from view. Three distal cues were available, two 25 W bulbs which were suspended from the ceiling at a distance of 75 cm from the edge of the pool and at an angle of 60°, one in the north-east and the other in the south-east, and a rectangular piece of white card (55 cm × 81 cm) which was also suspended from the ceiling on the west side of the maze. The animal's movements for each trial were recorded by a camera positioned directly above the centre of the maze. This information was collected by the digital tracking software EthoVision (Noldus Information Technologies, Wageningen, Netherlands).

Rats were randomly allocated to one of four experimental groups ( $n = 7$  per group). Animals were trained for five consecutive days in the water maze, with four trials per day (see Harvey et al., 2008). Animals were placed into the water maze from one of four starting positions, either north, south, east or west, with each starting position used just once per day. Animals were allowed 60 s to locate the escape platform, after which they would be guided to the platform by the experimenter, and allowed to remain there for 15 s. Following an inter-trial interval of 10 s, the animal was placed back into the maze to begin the next trial. The limitations of using “free-swimming”, as well as task-based controls as a comparison group in the water maze have been previously reported, with these control groups often displaying higher IEG expression than experimental groups in widespread brain regions (Shires & Aggleton, 2008). This neural activity is likely due to factors such as increased stress (Duncan, Johnson, & Breese, 1993; Cullinan, Herman, Battaglia, Akil, & Watson, 1995; Ons, Marti, & Armario, 2004) and incidental learning about the environment through exploration (Guzowski, McNaughton, Barnes, & Worley, 1999), which can hinder interpretation of results. The present experiment circumvented these challenges by comparing relative levels of IEG expression from one time-point to another, as all experimental groups would be affected equally by any factor extraneous to the task which may influence expression. A naïve control group ( $n = 5$ ) which was not exposed to the water maze apparatus was included to provide a baseline measure of IEG expression. The four spatially-trained groups were given a single retention probe trial, however the length of time between the final acquisition day and retention differed for each group. Probe trials took place either one day, seven days, 14 days or 30 days post-acquisition. The animals were placed back into the water maze from a south-west starting position, with the escape platform removed from the maze. Animals were allowed 60 s to search the maze for an escape. Successful retention of the maze was assessed by analysis of time spent searching in a circular area around where the platform was previously located, comprising 7% of the total searchable area of the maze, compared to equivalent areas of the maze. This target area was chosen for analysis as it was deemed a more accurate measure of successful retention than quadrant analysis (Moser, Moser, & Andersen, 1993).

### 2.3. Immunohistochemistry

Immunohistochemical protocol for the detection of Zif268, c-Fos and Arc protein was carried out on all groups of animals. For the experimental groups, 90 min after the retention probe trial, rats were deeply anaesthetised with an intraperitoneal injection of sodium pentobarbital (100 mg/kg, Euthatal), and subsequently perfused transcardially with ice cold 0.9% phosphate buffered saline (PBS, Ph7.4), followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, Ph7.4). Brains were then rapidly removed and post-fixed in 4% paraformaldehyde overnight, and then transferred to a 30% sucrose solution and stored at 4 °C. Coronal sections were cut at 40 µm using a freezing microtome, with every fourth section taken for subsequent immunohistochemical analysis. Rats in the

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