Neurobiology of Learning and Memory 106 (2013) 334–342

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

# The use of sequential hippocampal-dependent and -non-dependent tasks to study the activation profile of the anterior cingulate cortex during recent and remote memory tests

Brianne C. Wartman *, Matthew R. Holahan

*Department of Neuroscience, Carleton University, Ottawa, ON K1S 5B6, Canada*

ARTICLE INFO

*Article history:*
Available online 29 August 2013

*Keywords:*
Remote memory
Hippocampus
Anterior cingulate
Spatial memory
Water maze
Radial arm maze
Multiple memories

ABSTRACT

Recent findings suggest that as time passes, cortical networks become recruited for memory storage. In animal models, this has been studied by exposing rodents to one task, allowing them to form a memory representation for the task then waiting different periods of time to determine, either through brain imaging or region-specific inactivation, the location of the memory representation. A number of reports show that 30 days after a memory has been encoded, it comes to be stored in cortical areas such as the anterior cingulate cortex. The present study sought to determine what factors, in addition to the passage of time, would influence whether memory retrieval was associated with cortical activation. To this end, rats were assigned to one of three behavioural groups: (1) Training on one hippocampal-dependent memory task, the water maze (WM); (2) Training on two, different hippocampal-dependent memory tasks, the WM followed by the radial arm maze; (3) Training on one hippocampal-dependent memory task (WM) followed by training on one, non-hippocampal-dependent task, operant conditioning. After training, each group received a recent (2 d) or remote (31 d) water maze probe test. The group trained on two different hippocampal-dependent tasks and tested 2 d later, showed the strongest preference for the platform location during the probe test. This group also displayed a pattern of c-Fos staining in the anterior cingulate cortex similar to the pattern of staining observed in the remotely-tested groups and different from that seen in the other recently-tested groups. These results suggest the formation of multiple hippocampal-dependent memories accelerate the speed at which cortical network recruitment is seen and leads to enhanced behavioural performance in the recent term.



## 1. Introduction

Consolidation is a naturally occurring process whereby a recently acquired memory becomes permanent over time. In 1900, Müller and Pilzecker (as described in Lechner, Squire, and Byrne (1999)) proposed that temporary reverberation of neural activity representing a memory or "trace" persists after an event and consolidation of the trace takes place during this period. The reverberating activity may promote synaptic changes thought to mediate more permanent memory representations (Hebb, 1955; McGaugh & Herz, 1972; Milner, 1957).

Current uses of consolidation refer to neural modifications that happen at the cellular and systems levels (Dudai, 1996, 2000, 2004; Frankland & Bontempi, 2005, 2006; McGaugh, 2000; McGaugh, ahill, Ferry, & Roozendaal, 2000). Systems consolidation appears to be a gradual process whereby the "permanent" storage of a memory may ultimately be represented by neural circuits that are linked to, but independent from, the brain circuit that initially encoded the memory. In this view, memory representations show a shift in the balance of neural locus for long-term or remote storage (Dudai, 2009; Frankland & Bontempi, 2005, 2006; Kandel, 2001; Kassardjian et al., 2005; Maviel, Durkin, Menzaghi, & Bontempi, 2004; Wiltgen, Brown, Talton, & Silva, 2004).

Evidence from animal models suggests that memories initially encoded by the hippocampus become increasingly dependent on cortical areas at remote time points (Bontempi, Laurent-Demir, Destrade, & Jaffard, 1999; Ding, Teixeira, & Frankland, 2008; Frankland, Bontempi, Talton, Kaczmarek, & Silva, 2004; Holahan & Routtenberg, 2007; Kim & Fanselow, 1992; Maviel et al., 2004; Teixeira, Pomedli, Maei, Kee, & Frankland, 2006). In this view, the anterior cingulate cortex (ACC) has been implicated as a site for remote memory storage. Work has consistently shown evidence of increased activity in the ACC on tests for remote memory (Bontempi et al., 1999; Frankland et al., 2004; Lopez et al., 2012; Maviel et al., 2004; Teixeira et al., 2006; Weible, Rowland, Monaghan, Wolfgang, & Kentros, 2012). Inactivation of the ACC has been shown to hinder performance on remote memory tests (Bontempi et al., 1999; Frankland et al., 2004; Holahan & Routten-

* Corresponding author. Address: Carleton University, 1125 Colonel By Drive, 325 Life Sciences Research Building, Ottawa, ON K1S 5B6, Canada. Fax: +1 613 520 3667.
*E-mail address:* briannewartman@cmail.carleton.ca (B.C. Wartman).

1074-7427/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nlm.2013.08.011

berg, 2007; Lopez et al., 2012; Maviel et al., 2004; Restivo, Vetere, Bontempi, & Ammassari-Teule, 2009; Teixeira et al., 2006) and evidence has shown structural changes indicative of memory storage within the ACC at remote time points (Restivo et al., 2009; Vetere et al., 2011; Weible et al., 2012).

Evidence also exists supporting a hippocampal-centric view of memory storage, whereby damage to the hippocampus at any time after memory formation results in a flat, ungraded, retrograde amnesia (Broadbent, Squire, & Clark, 2006; Clark, Broadbent, & Squire, 2005a, 2005b, 2007; Epp et al., 2008; Lehmann, Lacanilao, & Sutherland, 2007; Lehmann, Rourke, Booker, & Glenn, 2012; Sparks, Lehmann, Hernandez, & Sutherland, 2011; Sutherland, O'Brien, & Lehmann, 2008; Winocour, Moscovitch, & Bontempi, 2010). While various explanations have been put forth to address these differential findings (Sutherland & Lehmann, 2011; Sutherland, Sparks, & Lehmann, 2010), the current study explored an alternate possibility to explain why a memory may come to be represented in extra-hippocampal networks such as the ACC.

Systems consolidation models suggest that as time passes, there is a shift in the balance of neural activity from the hippocampus to the ACC in the representation of a remote memory. This has typically been studied by exposing rats to one task, allowing them to form a memory for the task, then waiting different periods of time to assess the location of the memory representation based on neural activity. Some reports indicate 30 days after encoding, the ACC becomes a critical node in storing the memory (Bontempi et al., 1999; Ding et al., 2008; Frankland et al., 2004; Holahan & Routtenberg, 2007; Kim & Fanselow, 1992; Maviel et al., 2004; Teixeira et al., 2006), while other cases have reported the hippocampus remains engaged (Broadbent et al., 2006; Clark et al., 2005a, 2005b, 2007; Epp et al., 2008; Lehmann et al., 2007, 2012; Sparks et al., 2011; Sutherland, O'Brien, & Lehmann, 2008). We sought to determine if the shift in the balance of neural activity favouring the ACC could be influenced by the number of memories being sequentially processed. It was hypothesized that multiple hippocampal-dependent memories may impact the time course at which the ACC becomes part of a critical node in storing memory representations.

## 2. Materials and methods

### 2.1. Subjects

A total of 49 male Long Evans rats (190–250 g) from Charles River, Quebec were used. Rats were housed individually in clear plastic cages (26 × 20 × 45 cm) and given water *ad libitum* under a 12-h light/dark cycle (lights on at 8:00 a.m.; rats tested during the light phase). Rats received no nesting material and no direct enrichment of any kind in their home cage environment. Efforts were made to minimize enrichment and experiences outside of those outlined in the experimental procedure to minimize the impact of housing variables. Food was restricted until rats reached 90% of their free-feeding baseline, which was maintained throughout the experiment. Prior to behavioural training, rats were given 5 chocolate pellets (45 mg) in their home cage and handled for 5 min daily. Principles of laboratory animal care were followed and all procedures were conducted in accordance with the Canadian Council on Animal Care and protocols approved by the Carleton University Animal Care Committee.

### 2.2. Apparatus

#### 2.2.1. Water maze (WM)

The water maze was located in a room within the animal housing area. The opaque, white, polypropylene pool measured 155 cm in diameter and 60 cm in height and was filled to a depth of 37.5 cm with water that remained at approximately 21 °C. The platform was submerged 2 cm below the water surface and made from clear Plexiglas. Visual cues such as posters and geometric shapes were placed on the walls at distances of 48 cm, 188 cm, 54 cm and 104 cm. The experimenter remained in the same position throughout all trials.

#### 2.2.2. Radial arm maze (RAM)

RAM testing was done in a different room than WM testing located across the hall within the animal housing area. The maze was positioned 98.5 cm off the floor. Each arm measured 59 cm long and 11 cm wide. The distance between the ends of arms, where food (chocolate pellet-BioServe, New Jersey) reward was located was 32.5 cm. Plastic inserts were located on the sides of maze arms to stop animals from jumping across arms. Visual cues such as posters and geometric shapes were located on the walls around the room. The experimenter remained in the same position throughout all trials.

#### 2.2.3. Operant conditioning (OP) apparatus

Rats were tested in groups of six using six operant chambers (Habitest Operant Cage, Coulbourn Instruments; $12''W \times 10''D \times 12''H$). Each chamber was housed in an insulated box to minimize external noise. Each chamber possessed a pellet dispensing system, two levers separated by a food hopper, a houselight, a grid floor and a three light-panel. OP training took place in a room outside of the animal housing area.

### 2.3. Behavioural procedure

An overview of the behavioural procedure is shown in Fig. 1. Rats were assigned to one of 3 behavioural groups: (1) Training on one hippocampal-dependent task, the WM; (2) Training on two, hippocampal-dependent tasks, the WM followed by the RAM; (3) Training on one hippocampal-dependent task (WM) and training on one non-hippocampal-dependent task, OP. Sequential training on two tasks was separated by a 24-h rest period. Following this training, rats received either a recent WM probe test (8 days after the end of WM training, $n = 25$) or a remote WM probe test (37 days after the end of WM training, $n = 25$). Six experimental groups resulted: (1) WM:recent ($n = 8$), (2) WM:remote ($n = 8$), (3) WM/RAM:recent ($n = 8$), (4) WM/RAM:remote ($n = 7$), (5) WM/OP:recent ($n = 9$) and (6) WM/OP:remote ($n = 9$).

#### 2.3.1. Water maze

Rats received 5, 60 s training trials per day for 5 days with a different starting location for every trial within a day and randomized starting locations across days. The hidden platform was in a fixed location within and across days. Rats were placed in the pool facing the wall and given a maximum of 60 s to locate the hidden platform. Rats that did not find the hidden platform within 60 s were guided to the platform by the experimenter. All rats remained on the platform for 15 s then given a 15 s rest period in a holding cage before the next trial. All movement within the pool was tracked using HVS Image 2100 Tracking System (HVS Image, Buckingham, UK). Following the final trial of each day, rats were dried with a towel and placed in a holding cage on a heating pad in the housing room for 10–15 min after which they were returned to the home cage.

#### 2.3.2. Radial arm maze

Rats received one day of pretraining and 4 days of testing on the RAM. On the first trial of pretraining, chocolate pellets were located in the starting area, at the entrance to arms, within the arms as well as in the food holes located at the end of each arm. On trial 2 of pretraining, pellets were located within the arms and in food