Neurobiology of Learning and Memory xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Activity of the anterior cingulate cortex and ventral hippocampus underlie increases in contextual fear generalization

Patrick K. Cullen, T. Lee Gilman, Patrick Winiecki, David C. Riccio, Aaron M. Jasnow *

Department of Psychological Sciences, Kent State University, Kent, OH 44242, United States

ARTICLE INFO

Article history:
Received 28 May 2015
Revised 30 June 2015
Accepted 2 July 2015
Available online xxxx

Keywords:
Context fear conditioning
Remote memory
Fear generalization
Hippocampus
Anterior cingulate cortex
Memory precision

ABSTRACT

Memories for context become less specific with time resulting in animals generalizing fear from training contexts to novel contexts. Though much attention has been given to the neural structures that underlie the long-term consolidation of a context fear memory, very little is known about the mechanisms responsible for the increase in fear generalization that occurs as the memory ages. Here, we examine the neural pattern of activation underlying the expression of a generalized context fear memory in male C57BL/6J mice. Animals were context fear conditioned and tested for fear in either the training context or a novel context at recent and remote time points. Animals were sacrificed and fluorescent in situ hybridization was performed to assay neural activation. Our results demonstrate activity of the prelimbic, infralimbic, and anterior cingulate (ACC) cortices as well as the ventral hippocampus (vHPC) underlie expression of a generalized fear memory. To verify the involvement of the ACC and vHPC in the expression of a generalized fear memory, animals were context fear conditioned and infused with 4% lidocaine into the ACC, dHPC, or vHPC prior to retrieval to temporarily inactivate these structures. The results demonstrate that activity of the ACC and vHPC is required for the expression of a generalized fear memory, as inactivation of these regions returned the memory to a contextually precise form. Current theories of time-dependent generalization of contextual memories do not predict involvement of the vHPC. Our data suggest a novel role of this region in generalized memory, which should be incorporated into current theories of time-dependent memory generalization. We also show that the dorsal hippocampus plays a prolonged role in contextually precise memories. Our findings suggest a possible interaction between the ACC and vHPC controls the expression of fear generalization.

1. Introduction

The formation of a new memory involves the reorganization of information across the neural systems that encode and store memory as the memory trace is transferred from temporary to more permanent locations within the brain, a process known as consolidation (Dudai, 2004; Frankland et al., 2006; Kim & Fanselow, 1992; McGaugh, 1966; Squire & Alvarez, 1995; Vetere et al., 2011; Zola-Morgan & Squire, 1990). Specifically, it has been demonstrated that expression of recent contextual memories are dependent on activity of the hippocampus whereas expression of remote contextual memories involves increased activity of the infralimbic (IL) and prelimbic (PL) areas of the medial prefrontal cortex (mPFC) and becomes dependent on activity of the anterior cingulate cortex (ACC) (Frankland, Bontempi, Talton, Kaczmarek, & Silva, 2004; Gafford, Parsons, & Helmstetter, 2013; Goshen et al., 2011; Kim & Fanselow, 1992). These results suggest that as a context memory ages, it is transferred from the hippocampus to a distributed cortical network for long-term storage. While studies on the neural structures involved in the long-term storage and consolidation of contextual memory provide valuable insight into the time-dependent role of the hippocampus and neocortex, they do not explain the loss of context-specificity (i.e. increase in generalization) that occurs as a result of long-term consolidation.

In the case of context fear conditioning, placing animals in a novel context (i.e., distinctly different from the training context) shortly following training results in little expression of learned fear (lower levels of freezing behavior) (Feinberg & Riccio, 1990; Jasnow, Cullen, & Riccio, 2012; Riccio, Ackil, & Burch-Vernon, 1992; Ruediger et al., 2011; Wiltgen & Silva, 2007; Wiltgen et al., 2010; Zhou & Riccio, 1996). However, as the retention interval between training and testing increases, there is a loss of memory precision (i.e., fear responses to the training context generalize over time) such that animals exhibit equivalent levels of fear expression in both the training context and the novel context

* Corresponding author.
E-mail address: ajasnow@kent.edu (A.M. Jasnow).

http://dx.doi.org/10.1016/j.nlm.2015.07.001
1074-7427/© 2015 Published by Elsevier Inc.

(Gisquet-Verrier & Alexinsky, 1986; McAllister & McAllister, 1963; Metzger & Riccio, 2009; Perkins & Weyant, 1958; Richardson, Williams, & Riccio, 1984; Zhou & Riccio, 1996). A similar, yet distinct process occurs when animals are trained to discrete cues, or undergo discrimination training (eg., Jasnow et al., 2012; Thomas & Burr, 1969; Thomas & Lopez, 1962; Thomas et al., 1985). As with generalization to contextual cues, generalization gradients to discrete cues flatten as the retention interval increases. To account for the increase in contextual fear generalization, Winocur, Moscovitch, and Sekeres (2007) proposed that as the memory trace is transferred from the hippocampus to the cortex over time, it is transformed into a schematic-like memory. Thus, the memory becomes stored as a "gist-like" memory trace that lacks context-specificity. According to this hypothesis, the expression of a generalized fear memory results from activation of the general or schematic-like cortical fear memory by the novel contextual cues. Several studies using post-training inactivation or hippocampal lesions have supported this hypothesis demonstrating that the retrieval of generalized context memories do not involve activity of the hippocampus (Wiltgen et al., 2010; Winocur, Frankland, Sekeres, Fogel, & Moscovitch, 2009). As a result, it is generally concluded that at remote time points, animals retrieve a hippocampus-independent memory that lacks context specificity.

Although a few studies have implicated a more prolonged role of the hippocampus in the expression of contextually precise memories (Biedenkapp & Rudy, 2007; Ruediger et al., 2011), several studies suggest that the hippocampus plays a time-limited role in the consolidation and expression of a contextually precise memory. Once the memory becomes independent of the hippocampus, the precision or specificity of that memory is lost resulting in increased rates of fear generalization. However, other than the hippocampus, it remains unclear what neural structures are involved in the expression of a generalized fear memory. Further, most existing literature on consolidation focus solely on the dorsal hippocampus, overlooking the potential role of the ventral hippocampus. Here we directly investigate the involvement of the ventral hippocampus and prefrontal cortex in the loss of context specificity that occurs at remote time points.

2. Materials and methods

2.1. Subjects

All experiments were conducted on male C57BL/6J mice generated from a breeding colony in the Department of Psychological Sciences at Kent State University. Animals were 6 weeks of age or older before they were used for experimentation/surgical cannulation and were group housed (3–4 animals per cage) with free access to food and water in a room maintained on a 12:12 light/dark cycle. All procedures were conducted in a facility accredited by the Association for Assessment and Accreditation and Laboratory Animal Care. All animal procedures were carried out in accordance with the National Institutes of Health guidelines and were approved by Kent State University Institutional Animal Care and Use (IACUC) Guidelines.

2.2. Fluorescent in situ hybridization

Fluorescent in situ hybridization was performed on 16 μm sections sliced on a cryostat and mounted on Superfrost Plus slides (Fisher Scientific). cDNA clones containing the coding sequence for mouse Arc were linearized with appropriate restriction enzymes and fluorescently labeled riboprobes were generated with T3 RNA polymerase and digoxigenin (DIG) labeling. Following our prehybridization procedure, the sections were hybridized with DIG-labeled Arc probes at 55 °C for 16 h and then underwent a series of rigorous washes. Sections were then incubated with anti-DIG-POD, Fab fragments, followed by fluorescent amplification using TSA Plus Cyanine 5 Fluorescent System (Perkin Elmer). Sections were then incubated with Hoechst solution (Sigma) for nuclear staining followed by another series of washes and finally coverslipped with MOWIOL mounting medium.

Imaging was conducted on an Olympus 70X inverted microscope. The software program ImageJ (NIH) was used to quantify the hybridization signal intensities of brain regions of interest. Background was subtracted from the images using a rolling bar radius of 5.0. Each image was despeckled in order to filter image noise. The auto threshold feature was used to set the upper and lower signal threshold for each image and then converted the image to a binary image. Binary images were then further adjusted using watershed segmentation to automatically separate particles that were touching. The brain regions of interest, with coordinates in relation to bregma that were analyzed, include the infralimbic and prelimbic (+1.78 mm A/P) regions of the medial prefrontal cortex, the anterior cingulate cortex (+0.6 mm A/P), the CA1, CA3, and dentate gyrus regions of the ventral hippocampus (−3.16 mm A/P), and the CA1, CA3, and dentate gyrus regions of the dorsal hippocampus (−2.18 mm A/P).

Analysis of Arc mRNA expression involved calculating a percent change score compared to home cage control animals. A homecage control activity score was calculated by averaging activation levels for each area of interest across all animals. For each region of interest, a "percent homecage" was calculated for each animal in each group (i.e. 1 Day Training, 1 Day Novel, 14 Day Training, 14 Day Novel). We used 3 bilateral consecutive sections for each region and averaged the scores together, giving each animal one score per region of interest.

2.3. Surgery and cannulation

Animals were anesthetized with an intraperitoneal injection of a Ketamine (75 mg/kg) + Dexdomitor (dexmedetomidine) (1.5 mg/kg) cocktail. Following administration of anesthesia, mice were mounted on a stereotaxic apparatus (Kopf Instruments). A single guide cannula (Plastics One, Inc) was inserted into the skull above the anterior cingulate cortex for midline infusions using a 14° angle. Two guide cannulae were surgically implanted above the CA1 region of either the ventral hippocampus or dorsal hippocampus for bilateral infusions. Cannulae were positioned in the following coordinates using a 14° angle with respect to bregma including a 1 mm protrusion for the infusion needle: +0.8 mm A/P, 0.7 mm M/L, 1.75 mm D/V for ACC; −3.16 mm A/P, 4.2 mm M/L, 3.6 mm D/V for CA1 region of the ventral hippocampus (vCA1); −2.5 mm A/P, 2.5 mm M/L, 1.6 mm D/V for CA1 region of the dorsal hippocampus (dCA1).

Following the completion of behavioral experiments, we verified guide cannula placement by injecting 0.2 μL of India ink through guide cannulae. Brains were removed and flash frozen using dry ice for storage in −80 °C freezer. Brains were sectioned on a cryostat at a thickness of 25 μm. If the dye was not observed in the proper place, behavioral data from that mouse was excluded from analyses.

2.4. Drug infusions

The sodium channel blocker lidocaine HCl (Sigma) was used to suppress neuronal firing, thereby temporarily inactivating brain regions of interest. For all infusion experiments, lidocaine HCl will was dissolved in phosphate-buffered saline (PBS) to a concentration of 4% (w/v) lidocaine and adjusted to a pH of ~7.0 (Frankland et al., 2004). Mice received midline or bilateral

Please cite this article in press as: Cullen, P. K., et al. Activity of the anterior cingulate cortex and ventral hippocampus underlie increases in contextual fear generalization. *Neurobiology of Learning and Memory* (2015), http://dx.doi.org/10.1016/j.nlm.2015.07.001