Neurobiology of Learning and Memory 123 (2015) 110–116

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

The retrosplenial cortex is involved in the formation of memory for context and trace fear conditioning

Janine L. Kwapis [1], Timothy J. Jarome [2], Jonathan L. Lee, Fred J. Helmstetter *

Department of Psychology, University of Wisconsin–Milwaukee, 2441 E. Hartford Ave., Milwaukee, WI 53211, USA

ARTICLE INFO

Article history:
Received 11 April 2015
Revised 8 June 2015
Accepted 9 June 2015
Available online 14 June 2015

Keywords:
Retrosplenial cortex
Consolidation
Retrieval
Emotion
Trace fear
Context fear

ABSTRACT

The retrosplenial cortex (RSC) is known to play a role in the retrieval of context memory, but its involvement in memory formation and consolidation is unclear. To better characterize the role of the RSC, we tested its involvement in the formation and retrieval of memory for trace fear conditioning, a task that requires the association of two cues separated by an empty period of time. We have previously shown that trace fear extinction requires the RSC (Kwapis, Jarome, Lee, Gilmartin, & Helmstetter, 2014) and have hypothesized that trace memory may be stored in a distributed cortical network that includes prelimbic and retrosplenial cortices (Kwapis, Jarome, & Helmstetter, 2015). Whether the RSC participates in acquiring and storing cued trace fear, however, is currently unknown. Here, we demonstrate that blocking protein synthesis in the RSC before, but not after acquisition impairs rats' memory for trace CS and context fear without affecting memory for the CS in standard delay fear conditioning. We also show that NMDA receptor blockade in the RSC transiently impairs memory retrieval for trace, but not delay memory. The RSC therefore appears to critically contribute to formation of trace and context fear memory in addition to its previously recognized role in context memory retrieval.

1. Introduction

The retrosplenial cortex (RSC) is one of the largest cortical regions in the rat brain (Vogt & Peters, 1981), yet very little is known about its potentially important role in memory formation and storage (Todd & Bucci, in press). The RSC is well-positioned to coordinate information between higher-order brain regions, as it has direct reciprocal connections to the prefrontal cortex and hippocampus (Vann, Aggleton, & Maguire, 2009). Indeed, RSC functional activity usually correlates with autobiographical memory recall in humans (Svoboda, McKinnon, & Levine, 2006), suggesting involvement in explicit memory retrieval. The RSC is therefore particularly well-situated to support retrieval and storage of complex memory.

In rodents, the RSC participates in recent and remote context memory retrieval (Corcoran et al., 2011; Cowansage et al., 2014). Blocking NMDA receptors or damaging the RSC selectively impairs context-shock associations acquired during standard delay fear conditioning, in which an auditory conditional stimulus (CS) is paired with an aversive shock unconditional stimulus (UCS). Neither RSC manipulation prevents the successful acquisition of fear to the auditory CS, however (Corcoran et al., 2011; Keene & Bucci, 2008), suggesting that the RSC is selectively involved in retrieving more complex contextual memory.

The RSC has also been implicated in consolidation of context fear memory. Pre-training protein or mRNA synthesis inhibition in the RSC disrupts long-term memory formation for context-based inhibitory avoidance (Katche, Dorman, Slipczuk, Cammarota, & Medina, 2013). Further, immediate early genes such as Arc and c-Fos are increased in the RSC shortly after context fear conditioning (Robinson, Poorman, Marder, & Bucci, 2012), suggesting that RSC neurons are active during the consolidation of context fear memory. In contrast to these results, blocking NMDA receptors reportedly has no effect on the acquisition of context fear (Corcoran et al., 2011). As NMDA receptors are critically important for memory consolidation (Abel & Lattal, 2001), this suggests the RSC is either only involved in certain forms of context memory consolidation or requires NMDAR-independent molecular processes.

The RSC therefore appears to be important for context memory retrieval and possibly memory formation, but its precise role is unknown. The RSC may be selectively involved in context memory or, instead, may play a more general role in relational and composite memories that extends beyond contextual information per se. To

* Corresponding author at: Department of Psychology, University of Wisconsin–Milwaukee, 224 Garland Hal, 2441 E. Hartford Ave., Milwaukee, WI 53211, USA. Fax: +1 414 229 5219.
E-mail address: fjh@uwm.edu (F.J. Helmstetter).
[1] Present address: Department of Neurobiology and Behavior, Center for the Neurobiology of Learning and Memory, University of California, Irvine, Irvine, CA, USA.
[2] Present address: Department of Neurobiology, University of Alabama at Birmingham, Birmingham, AL, USA.

http://dx.doi.org/10.1016/j.nlm.2015.06.007
1074-7427/

better understand the function of the RSC, we tested its involvement in trace fear conditioning, a form of complex, non-contextual memory. In trace conditioning, the CS and UCS are separated by an empty period of time, making the two cues relatively difficult to associate. Trace fear conditioning requires cortical and hippocampal participation (Gilmartin, Balderston, & Helmstetter, 2014; Gilmartin & Helmstetter, 2010; Gilmartin, Miyawaki, Helmstetter, & Diba, 2013; Kwapis, Jarome, Schiff, & Helmstetter, 2011; Quinn, Oommen, Morrison, & Fanselow, 2002; Reis, Jarome, & Helmstetter, 2013; Runyan, Moore, & Dash, 2004) and contingency awareness (Knight, Nguyen, & Bandettini, 2006; Weike, Schupp, & Hamm, 2007) for successful acquisition, making it a good candidate for retrosplenial involvement. Recently, we demonstrated that the RSC is involved in trace fear extinction and retrieval (Kwapis, Jarome, Lee, Gilmartin, & Helmstetter, 2014), leading us to hypothesize that trace fear memory may be stored in a distributed cortical network that includes the prelimbic and retrosplenial cortices (Kwapis, Jarome, & Helmstetter, 2015). No one has yet tested whether the RSC participates in trace fear acquisition or consolidation, however.

Here, we show that protein synthesis in the RSC is required for acquisition or early consolidation of contextual and trace CS fear, but is not necessary for delay CS fear. Further, we found that NMDA receptors in the RSC are required to retrieve trace, but not delay fear memory. The RSC therefore participates in trace fear retrieval and consolidation in addition to its known role in context memory retrieval. This is consistent with our hypothesis that a distributed cortical network may participate in the consolidation of trace fear memory.

2. Materials and methods

2.1. Subjects and surgery

The subjects were 89 male Long-Evans rats (300–375 g) obtained from Harlan (Madison, WI). Rats were individually housed, given free access to food and water, and maintained on a 14:10-h light/dark cycle. All procedures were in accordance with the National Institutes of Health Guidelines and approved by the Institutional Animal Care and Use Committee at the University of Wisconsin–Milwaukee.

All animals were adapted to handling for 3 days before surgery. During surgery, rats were anesthetized with isoflurane (induction, 4%; maintenance, 2%) and placed in a stereotaxic frame. Animals were prepared with bilateral stainless steel 26-gauge cannulae aimed at the retrosplenial cortex (RSC) as previously described (Kwapis et al., 2014). The coordinates used were: 3.5 mm posterior, ±0.5 mm lateral, and 1.8 mm ventral relative to bregma (Paxinos & Watson, 2007).

2.2. Apparatus

Fear conditioning acquisition was conducted in a set of 4 identical chambers housed within sound-dampening boxes (Context A). The floor of each chamber was composed of stainless steel rods through which footshocks were delivered. Each chamber was illuminated by an overhead 7.5 W bulb and ventilation fans provided background noise (~60–62 dB). During training, the white noise CS was delivered through a speaker housed in the side of each chamber. Context A was cleaned with a solution of 5% ammonium hydroxide between animals.

A second set of chambers (Context B) was used to measure freezing to the auditory CS independent of the training context. Context B differed from Context A in a number of ways, including infrared illumination, a solid and opaque textured floor panel, and a different cleaning solution (5% acetic acid). Ventilation fans in Context B provided approximately 58–60 dB of background noise.

2.3. Infusion procedure and drugs

All rats received bilateral infusions of 0.5 μl/side into the RSC over a 60s period. After each infusion was complete, the injectors (33-gauge, extending 0.8 mm beyond the guide) were kept in place for an additional 90s to ensure proper diffusion. The protein synthesis inhibitor ANI (Tocris; 10 mg) was fully dissolved in 36 μl of HCl and diluted to its final concentration of 125 μg/μl with 44 μl of ACSF. The NMDA receptor antagonist D-APV (Tocris, 10 mg) was diluted with 1000 μl of ACSF to a final concentration of 10 μg/μl (Kwapis et al., 2014, 2015).

2.4. Behavior

All rats were exposed to the restraint procedure for three days before training. Each rat was transported to the laboratory, wrapped in a towel, and gently restrained by hand for several minutes while the infusion pump was activated to allow animals to acclimate to its noise.

Fear conditioning and context tests were conducted in Context A while CS tests were conducted in Context B. Animals were trained on day 1 with strength-matched delay ($n = 42$) or trace ($n = 45$) conditioning. Previous work from our lab (Kwapis et al., 2011, 2014, 2015) has demonstrated that a 6-trial trace fear conditioning protocol with a variable intertrial interval (ITI) of 240 ± 20 s produces approximately the same level of freezing as 4 trials of delay fear conditioning with a shorter ITI of 110 ± 20 s. For both conditioning types, the CS was a white noise cue (10 s; 72 dB) and the UCS was a footshock (1 s; 1 mA). For delay fear conditioning, the UCS was presented at the moment of CS offset. For trace fear conditioning, the CS and UCS were separated by an empty 20 s trace interval. Both protocols began with a 6-min baseline period and finished with a 4-min postshock period.

On day 2, animals were tested to both the CS and context in a counterbalanced manner, with at least 4 h between tests. For the context test, animals were returned to the conditioning chamber for 12 min. For the CS test, animals were placed in Context B, given a 1-min baseline period, and then given 8 discrete CS presentations (30 s; 72 dB) with a 60 s ITI.

Experiment 2 was a direct follow-up to the first experiment. After completion of the initial CS and context tests, the animals from Experiment 1 were regrouped and, 4 days later, given 2 additional CS tests separated by 24 h. These tests were identical to the CS test described above.

2.5. Histology

After behavioral testing was complete, animals were killed with an overdose of isoflurane and transcardially perfused. For detailed procedures, see Kwapis, Jarome, Lonergan, and Helmstetter (2009). Briefly, the brains were cryoprotected, frozen, and sectioned into 40 μm slices, which were mounted and stained with cresyl violet. Only rats with acceptable cannulae placements in the RSC were included in the analyses.

In order to better visualize the region targeted by our infusions, two untrained animals were implanted with RSC cannulae and injected with a fluorescent antibody (anti-rabbit Alexa 594) at the same volume as our drugs (0.5 μl/side). Approximately 10 min after infusion, these animals were perfused and the brains were placed in sucrose formalin for 3 days in a dark container. The brains were sliced at 40 μm in the dark, mounted on slides, and imaged with a fluorescence microscope (Nikon Eclipse) running NIS-Elements software.