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Dissociations across the dorsal–ventral axis of CA3 and CA1 for encoding and retrieval of contextual and auditory-cued fear

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Abstract

The present study was designed to dissociate the roles of dorsal CA3, dorsal CA1, ventral CA3, and ventral CA1 in contextual and auditory-cued classical fear conditioning. Rats received excitotoxic lesions of dorsal CA3, dorsal CA1, ventral CA3, or ventral CA3 prior to acquisition of classical fear conditioning. Dorsal CA3 and dorsal CA1, but not ventral CA3 or ventral CA1, lesions caused a deficit for the acquisition of contextual fear. Dorsal CA1, ventral CA3, and ventral CA1, but not dorsal CA3, lesions caused deficits for the retrieval/expression of contextual fear when tested either 24 or 48 h after encoding. Ventral CA3, but not dorsal CA3, dorsal CA1, or ventral CA1, lesions caused a deficit for retrieval of auditory-cued fear when tested either 24 or 48 h after encoding. The data suggest that dorsal CA3 mediates encoding of contextual fear, whereas ventral CA3 mediates retrieval of contextual fear. The data also suggest that dorsal CA1 mediates encoding and retrieval of contextual fear, whereas ventral CA1 mediates only the retrieval of contextual fear.

Keywords: Learning and memory; Animal cognition; Context; Classical conditioning; Fear learning; CA3; CA1; Encoding and retrieval; Dorsal hippocampus; Ventral hippocampus

1. Introduction

Classical fear conditioning has been used to assess fear-related learning in the amygdala and hippocampus (Phillips & LeDoux, 1992). In recent years, there has been an increased interest in the role of the anterior and posterior hippocampus (in humans) or ventral and dorsal hippocampus (in rats) for encoding and retrieval processes (Knight, Smith, Cheng, Stein, & Helmstetter, 2004; Lepage, Habib, & Tulving, 1998; Maren & Holt, 2004; Rogers, Hunsaker, & Kesner, 2006; Rudy & Matus-Amat, 2005; Zeineh, Engel, Thompson, & Bookmeyer, 2003; cf. Greicius et al., 2003; Yoon & Otto, 2007). Lesions and inactivations within the ventral hippocampus result in deficits for retrieval of both auditory and contextually cued fear (Maren & Holt, 2004; Rogers et al., 2006; Rudy & Matus-Amat, 2005; Yoon & Otto, 2007), whereas lesions and inactivations within the dorsal hippocampus have been shown to attenuate contextual, but not auditory-cued, fear (Lee & Kesner, 2004; Phillips & LeDoux, 1992; Quinn & Fanselow, 2006; Quinn, Loya, Ma, & Fanselow, 2005; Quinn, Oomen, Morrison, & Fanselow, 2002; Rogers et al., 2006; Yoon & Otto, 2007).

In addition to overall hippocampal involvement in fear conditioning, subregional analyses are becoming increasingly prominent and have focused on the role of CA1 for the encoding and retrieval of trace conditioning (eye-blink (fear) conditioning; McEchron, Tseng, & Disterhoft, 2003; Weible, O'Reilly, Weiss, & Disterhoft, 2006). To date, studies have focused on dorsal hippocampal subregions (Lee & Kesner, 2004; McEchron et al., 2003), or on CA1 and CA3 as a whole via NMDAR1 receptor knockout (Huerta, Sun, Wilson, & Tonegawa, 2000; Kashimoto, Nakazawa, Tonegawa, Kirono, & Kano, 2006). Thus far, only a single study has assessed differential effects of ventral CA1 and dorsal CA1 lesions for fear conditioning (trace fear conditioning; Rogers et al., 2006). In addition to dor-

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sal CA1, Lee and Kesner (2004) have shown that all dorsal hippocampal subregions contribute to encoding of contextual information during delay fear conditioning. They also have shown that both dorsal CA1 and the dorsal dentate gyrus, but not dorsal CA3, participate in retrieval of contextual fear.

Lee and Kesner (2004) have shown that dorsal CA3 lesions result in an encoding deficit for delay fear conditioning without a concomitant retrieval deficit. It has also been shown that CA1 can be dissociated across the dorsal–ventral axis for retrieval of contextual and auditory-trace fear conditioning, with ventral CA1 lesions resulting in greater deficits than dorsal CA1 for retrieval of contextual and auditory-trace cued fear (Rogers et al., 2006). The present experiment was designed to evaluate the roles of dorsal CA3, dorsal CA1, ventral CA3, and ventral CA1 for encoding and retrieval of contextual and auditory-cued fear by replicating the experimental methods of Lee and Kesner (2004) using dorsal CA3, dorsal CA1, ventral CA3, and ventral CA1 lesioned animals. The data reveal a double dissociation of dorsal CA3 and ventral CA3 for contextual fear encoding and retrieval wherein dorsal CA3 subserves encoding and ventral CA3 subserves retrieval of contextual fear. The data reveal that dorsal CA1 is involved in both encoding and retrieval of contextual fear, whereas ventral CA1 is recruited during retention of contextual fear.

2. Materials and methods

2.1. Animals

Thirty-one male Long-Evans rats (Simonsen Laboratories, Inc., Gilroy, CA), approximately six months of age and weighing 350–400 g at the start of experimentation served as subjects. The rats were housed individually in plastic tubs located in a colony with a 12 h light–dark cycle. All testing was conducted during the light portion the cycle. All rats were free-fed and had ad libitum access to water. All experiments were conducted according to the University of Utah Institutional Animal Care and Use Committee (IACUC) guidelines and conformed to all AAALAC protocols. An IACUC veterinarian evaluated the health of animals weekly. All animals had previously been run on an unrelated temporal ordering paradigm prior to the present classical conditioning experiment.

2.2. Experimental apparatus

Two observation chambers were used during three consecutive days of testing. The first chamber was used for encoding of contextual and auditory-cued fear and for the contextual fear retention test. This chamber ($28 \times 21 \times 22$ cm; Coulbourn Instruments; Allentown, PA) consisted of two clear transparent Plexiglas walls (rear wall and front door) and two aluminum sidewalls. The chamber floor was made up of 18 steel rods connected to a precision-regulated shock delivery apparatus (Coulbourn Instruments) used to deliver an electric foot-shock stimulus. A speaker was inserted into one of the aluminum walls of the conditioning chamber and a commercial software package (Graphic State v1.013.00; Coulbourn Instruments) controlled the presentation of all stimuli. The chamber was located in an isolated room illuminated by fluorescent and halogen lamps. Numerous visual cues such as toys and posters were located around the conditioning chamber to provide contextual cues and remained undisturbed throughout experimentation. A video camera recorded the animal's behavior, which was viewed and scored by an experimenter in an adjacent room. The camera was camouflaged from the animal's view so as to not act as a retrieval cue during subsequent tests. The chamber was cleaned immediately before conditioning and testing using a weakened cleaning solution.

A second observation chamber tested retention of auditory-cued fear. This chamber ($32 \times 32 \times 32$ cm) was constructed entirely of transparent Plexiglas. A speaker was attached to an opening (2.5 cm diameter) made in one of the walls near the floor of the chamber to deliver the auditory cue. The chamber was located in the same room but was surrounded by completely different visual cues, as well as a white curtain to provide a novel visual environment. It was cleaned with the same cleaning solution as the conditioning box immediately after each test, but was cleaned again with unscented water immediately prior to testing to dilute olfactory cues.

2.3. Behavioral methods

2.3.1. Encoding—day 1

Rats were placed in the conditioning chamber for 2 m prior to the first auditory stimulus as a contextual pre-exposure period to allow animals to efficiently encode the context (cf. Quinn & Fanselow, 2006; Rudy & O'Reilly, 2001) prior to conditioning. After the pre-exposure period, rats received 10 auditory-shock pairings separated by 74 s. An auditory stimulus (10 s duration, 2 kHz, 85-dB) was presented through a speaker to initiate each trial. An electric foot-shock (2 s duration, 0.75 mA) was delivered through 18 floor-rods coterminal with the auditory stimulus (e.g., the last 2 s of the auditory stimulus were in fact the tone + shock pairing). A 74 s intertrial interval (ITI) separated each successive tone + shock pairing. After the ten tone + shock pairings and subsequent ITIs, rats remained in the chamber for an additional 2 m without auditory stimulus or shock. A freezing response (e.g., absence of movement except respiratory; cf. Blanchard & Blanchard, 1972, 1969) was measured by an observer who scored freezing behavior every 8 s during the pre-exposure period and ITI (during the ITI the 2 s immediately following the shock were discarded (due to the animal still reacting to the aversive shock) and freezing during the subsequent 72 s were recorded) and every 4 s during the tone stimulus; resulting in two auditory observations and nine ITI observations for each trial. ITI freezing was used to assess contextually cued fear. The first 9 trials of overall freezing during each trial (e.g., tone and ITI freezing together) were blocked into three 3-trial blocks for analysis of overall acquisition. Tone and ITI freezing were also analyzed individually in a single 10-trial block to evaluate whether any group differences in freezing were due to differential freezing to the tone or during the ITI.

2.3.2. Contextually cued fear retention test—day 2 or 3

Each rat was tested for retrieval of contextually cued fear either 24 or 48 h after acquisition (half received the contextual fear retention test on day 2 and half on day 3, counterbalanced with auditory-cued fear retention tests). The rat was placed in the encoding chamber for 8 m in the absence of the auditory-cue and aversive stimulus (shock) for eight continuous 1 m trials. Freezing behavior was measured every 8 s. Due to extinction in the control group, only the first 4 m of testing were used for statistical analysis.

2.3.3. Auditory-cued fear retention test—day 2 or 3

Each rat was tested for retrieval of auditory-cued fear either 24 or 48 h after acquisition (half received the auditory-cue retention test on day 2 and half on day 3, counterbalanced with contextual fear retention tests). The rat was placed in a different chamber from that used during encoding in the presence of the auditory stimulus for eight continuous 1 m trials. Freezing behavior was measured in 8 s intervals. Due to extinction in control animals, only the first 4 m of testing were used for statistical analysis.

2.4. Surgical methods

Prior to conditioning, animals were randomly separated into five groups, ventral CA3 ($n = 9$), ventral CA1 ($n = 5$), dorsal CA3 ($n = 5$), dorsal CA1 ($n = 6$), and vehicle control ($n = 6$). All animals were anesthetized