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Time course of the dependence of associative memory retrieval on the entorhinal cortex

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ABSTRACT

As the gateway between the hippocampal system and the neocortex, the entorhinal cortex (EC) is hypothesized to be the hub in which the transformation of recent memory to remote memory is processed. We explored the role of the EC on the retrieval of recent and remote associative fear memory. A within-subject approach was adopted to compare the freezing rates of rats in EC intact and EC inactivated conditions following trace fear conditioning. The EC was inactivated by infusing an AMPA antagonist. The fear conditioning used a combined visual and auditory conditioned stimulus with a foot shock. On week 1 following the conditioning, the rats in the EC intact condition exhibited a freezing rate of 92.4 ± 9.5% in response to the light stimulus compared with a 6.3 ± 7.9% freezing rate in the EC inactivated condition. The freezing rates were 87.0 ± 17.8% and 4.7 ± 6.5% on week 2 in the EC intact and inactivated conditions, respectively. These results indicate that the EC participates in the retrieval of associative memory. Extinction of the fear memory was observed in the EC intact condition, as the mean freezing rate decreased to 62.7 ± 23.0% on week 4 and 41.2 ± 26.4% on week 5. However, the freezing rate increased to 26.8 ± 14.2% on week 4 and 22.3 ± 14.4% on week 5 in the EC inactivated condition. The normalized dependence of fear memory retrieval on the EC was 93.2 ± 8.3% on week 1, and significantly decreased on weeks 4 and 5. In summary, the retrieval of associative memory depends on the EC, but this dependence decreases over time.

1. Introduction

Patients with damage to the medial temporal lobe, which includes the hippocampus and its adjacent areas, exhibit impaired memory for recent events but retain the ability to recall past memories (Scoville & Milner, 1957; Squire, 1992; Wiltgen, Brown, Talton, & Silva, 2004). The hippocampus acquires information quickly and functions as a transient storage for recent memory. Information is processed by the hippocampus before it is sent back to the neocortex for permanent storage. A remote memory is then formed in the neocortex (Graham & Hodges, 1997; Norman & O'Reilly, 2003). The entorhinal cortex (EC) serves as the gateway between the hippocampus and neocortex by forwarding the cortical information to the hippocampus and the hippocampal information back to the neocortex (Alvarez & Squire, 1994; Wiltgen et al., 2004). The EC processes multimodal sensory information (Lavenex & Amaral, 2000; Witter et al., 2000). Patients with Alzheimer's disease exhibit neuronal loss in layer II of the EC and patients with schizophrenia exhibit decreased volume in the EC, suggesting that the EC is related to human declarative memory deficits (Gomez-Isla et al., 1996; Baiano et al., 2008).

Fear conditioning provides a tractable behavioral paradigm to dissect the role of the medial temporal lobe (Sanders, Wiltgen, & Fanselow, 2003). In typical associative learning, a relationship is established between the conditioned stimulus (CS) and the unconditioned stimulus (US) (Sanders et al., 2003). Under normal conditions, auditory stimuli evoke responses in the visual cortex (McIntosh, Cabeza, & Lobaugh, 1998; McIntosh & Gonzalez-Lima, 1998) and visual stimuli modulate responses in the auditory cortex (Bizley, Nodal, Bajo, Nelken, & King, 2007; Kayser, Petkov, & Logothetis, 2008). Audiovisual interactions and representations enable the cross-modal processing of the visual modulation of neurons in the auditory cortex (Romanski, 2007; Kayser et al., 2008).

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In one of our recent studies (Chen et al., 2013), we conducted an experiment with electrodes implanted in the auditory cortex and a drug cannula in the EC of behaving rats. Reversible inactivation of the EC impaired not only the encoding of an artificial associative memory between a visual stimulus and bilateral auditory cortex stimulation but also the retrieval of the memory within a week after the establishment of the memory. These results confirmed earlier reports that the retrieval of recent memory depends on the medial temporal lobe (Alvarez & Squire, 1994; Wiltgen et al., 2004). However, this study failed to examine the dependence of the retrieval of remote memory on the medial temporal lobe due to the extinction of the artificial visuoauditory associative memory. In the present study, we have designed an experiment to examine the time course of the dependence of the medial temporal lobe on the EC to retrieve associative fear memory for a time range of 5 weeks.

2. Materials and methods

2.1. Subjects

The experiment was conducted on 22 Sprague–Dawley rats. Each rat was approximately 2 months old at the start of the experiment and weighed between 280 and 350 g. The rats were housed under a 12:12 light/dark cycle, kept in individual plastic cages and allowed free access to water and food. All experimental protocols were approved by the Animal Subjects Ethics Sub-Committees of City University of Hong Kong and The Hong Kong Polytechnic University.

Atropine sulfate (0.05 mg/kg) was administered subcutaneously 15 min before anesthesia and at regular intervals during the operation to inhibit tracheal secretion. The rats were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg, Ceva Santé Animale Co., France) prior to the surgery. Supplemental doses of anesthesia (25 mg/kg/h) were administered regularly to maintain the anesthetized state throughout the surgery. The rat's body temperature was maintained at 37–38 °C during the surgery.

Following the induction of anesthesia, the rat was mounted on a stereotaxic instrument (Narishige Scientific Instrument Lab, Japan) and an incision was made along the mid-line of the head. The stereotaxic instrument was adjusted to place the bregma and lambda on a flat skull surface. Four to six small skull screws (1 mm) were positioned to anchor the dental cement (Mega Press NV, Germany) to the skull. The craniotomy was performed bilaterally 6.6 mm posterior to the bregma and 4.7 mm lateral to the mid-sagittal suture. The drug infusion site was identified in a pilot study conducted in anesthetized rats. In this study, activation of this region modulated neuronal responses in the auditory cortex in response to an auditory stimulus. The cannula (0.48 mm in diameter; RWD Life Science Co. Ltd, Shenzhen, China) was placed vertically at the target location in each hemisphere by a micromanipulator (Narishige, Japan). A layer of silicon elastomer (Kwik-Cast, World Precision Instrument, Inc. Florida, USA) was then used to cover the dura-removed space around the cannulae to protect the cortex from further damage. Dental cement was then applied to the skull to fix the cannulae. The rats were allowed to recover for 5 days before the behavioral experiments were conducted.

2.2. Behavioral apparatus

The fear conditioning and tests of associative memory were conducted in a homemade Plexiglass chamber (27 cm × 28 cm × 51 cm). The chamber was placed in a double-walled soundproof room (NAP, Clayton, Australia). The chamber had a stainless steel grid that was connected to a stimulator (GRASS s48 stimulator) to generate a scrambled foot shock. The voltage was set to a range between 32 and 42 V. A computer equipped with Tucker-Davis Technologies workstation controlled the stimulation. A dim light illuminated the chamber to enable videotape recordings of the behavioral responses of the rats.

2.3. Auditory and visual stimuli

The auditory stimuli were delivered through an open-field speaker (Tucker-Davis Technologies, Florida, USA). The sound pressure level (SPL) was calibrated with a condenser microphone (Center Technology, Taipei). A pure tone equal to 42 dB SPL was used for the experiments. The visual stimuli were generated by a homemade LED matrix. The illumination of the light was 26 lux as measured from the bottom of the chamber.

2.4. Fear conditioning training

A classical trace fear conditioning paradigm was adopted. The parameters of the paradigm were set based on a previous research (Chen et al., 2013). A combined stimulus of a 100-ms light and a 20-ms sound (200-ms delay from the onset of the light) was used as the conditioned stimulus (CS) and a 500-ms foot shock (500-ms delay from the onset of the light) was used

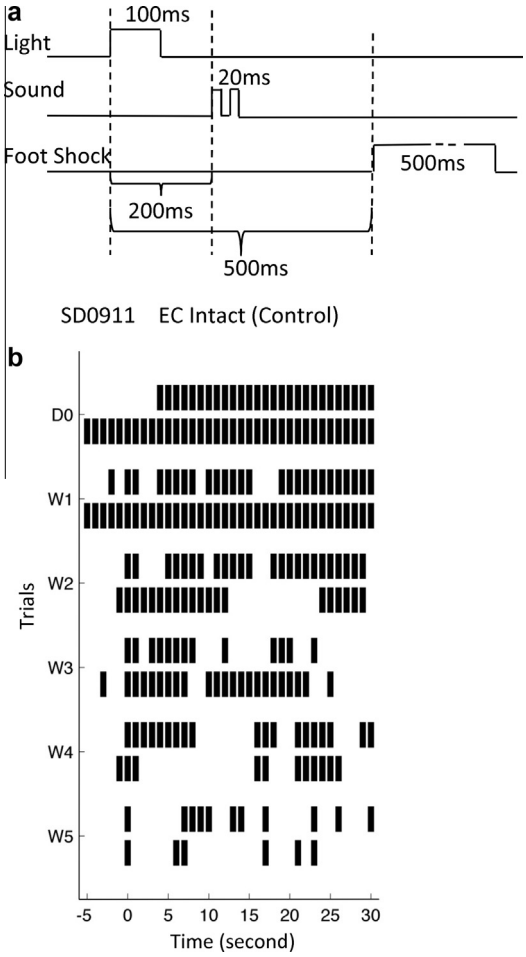

Fig. 1. Associative memory encoding and extinction. (a) The presentation of the stimuli and foot shock in the trace fear conditioning. (b) The raster display of the freezing time 5 s before and 30 s after the presentation of the light stimulus across different weeks following the fear conditioning; D0: immediately following the conditioning; W1–W5: week 1 through week 5 following the conditioning.