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Novel sensory preconditioning procedures identify a specific role for the hippocampus in pattern completion

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ABSTRACT

Successful retrieval of a memory for an entire pattern of stimulation by the presentation of a fragment of that pattern is a critical facet of memory function. We examined processes of pattern completion using novel sensory preconditioning procedures in rats that had either received sham lesions (group Sham) or lesions of the hippocampus (group HPC). After exposure to two audio-visual patterns (AX and BY) rats received fear conditioning with X (but not Y). Subsequent tests assessed fear to stimulus compounds (e.g., AX versus BX; Experiment 1) or elements (A versus B; Experiment 2). There was more fear to AX than BX in group Sham but not group HPC, while there was more fear to A than B in group HPC, but not in group Sham. This double dissociation suggests that pattern completion can be based upon separable processes that differ in their reliance on the hippocampus.

1. Introduction

The hippocampus has been implicated in pattern completion, the process by which a stored representation for a pattern of stimulation can be retrieved by a subset of its elements (e.g., Leutgeb, Leutgeb, May-Britt Moser, & Moser, 2007; Rolls, 2013). The nature of this process can be investigated using sensory preconditioning (SPC): where exposure to a pattern (AX; e.g., an audio-visual compound) can allow fear conditioned to X to be evident during A (e.g., Brogden, 1939; Rescorla, 1980). SPC is disrupted by lesions of hippocampus made prior to behavioral testing in both rabbits (Port, Beggs, & Patterson, 1987) and rats (Talk, Gandhi, & Matzel, 2002; see also, Eichenbaum, Mathews, & Cohen, 1989; but see, Honey & Good, 2000). However, the pattern completion processes that underpin SPC is often characterized in two ways: A could elicit fear *at test* through the operation of an (elemental) A-X-footshock associative chain (e.g., Hebb, 1943), the component links being forged during exposure to A with X (A-X) and conditioning with X (X-footshock). Alternatively, A and X could become linked to a separate configural memory (AX), with the later presentation of A eliciting fear to the extent that it activates the configural memory (i.e., AX) linked to shock *during conditioning* with X (e.g., Honey, Iordanova, & Good, 2014). Here, the role of the hippocampus in pattern completion was investigated using SPC procedures where these specific contributions of elemental and configural processes can be determined on an *a priori* basis.

Recent research identifies a role for configural processes in some SPC procedures. In one study, rats received exposure to AX and BY (two audio-visual compounds), and then X and Y were paired with footshock. During the test, there was more fear during the exposed compounds (AX and BY) than nonexposed compounds (AY and BX; Lin, Dumigan, Recio, & Honey, 2016). These results suggest that when X and Y were presented during conditioning they evoked the configural memories of AX and BY which became linked to shock (cf. Iordanova, Burnett, Good, & Honey, 2011; see also, Holland, 1981). Additional support for this analysis can be derived from the simpler observation that after exposure to AX and BY, conditioning with X results in greater fear to AX than BX (see Lin, Dumigan, Dwyer, Good, & Honey, 2013; see also, Ward-Robinson, Coutureau, Honey, & Killcross, 2005; Ward-Robinson & Hall, 1996). The associative chain analysis does not predict this outcome because A is only held to provoke more fear than B by dint of its capacity to activate X, and the presence of X with A and B will now mean that both compounds will have this capacity. Experiment 1 used this procedure (see Table 1) to examine whether lesions to the hippocampus disrupt SPC when the contribution of simple elemental processes has been counteracted and configural processes should be most apparent: AX is more similar to the configuration created during exposure and linked to shock during conditioning than is A alone. Experiment 2 used an identical procedure with the exception that A and B were presented alone during the test. Under these conditions, the A-X-shock chain can contribute

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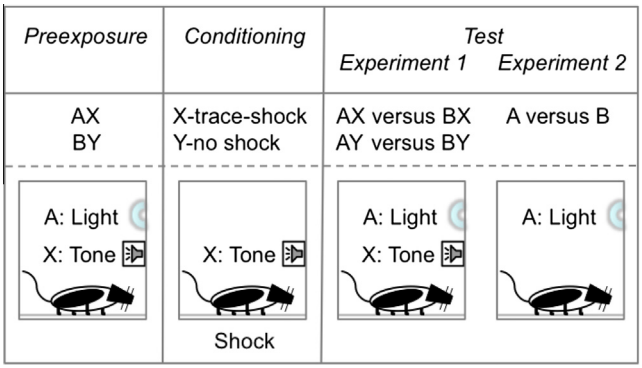
Table 1
Design of Experiments 1 and 2.



Note: Both Experiments 1 and 2 involved three stages: preexposure, conditioning and test. Rats in groups Sham and HPC received preexposure to two audio-visual compounds (AX and BY) before trace conditioning trials with X and nonreinforced presentations of Y. Rats in Experiment 1 then received tests with the compounds AX and BX (and AY and BY); while those in Experiment 2 received tests with A and B alone.

to the level of fear to A, but the contribution of configural processes will be constrained by the similarity of A to the configural AX representation.

2. Materials and methods

2.1. Animals

Thirty-two male Lister hooded rats (*Rattus norvegicus*; supplied by Harlan Olac Ltd, UK) were used in two experiments: Experiment 1 (group Sham: $N = 8$; group HPC: $N = 8$; mean *ad libitum* weight: 421 g, range: 394–492 g), and Experiment 2 (group Sham: $N = 8$; group HPC: $N = 8$; mean *ad libitum* weight: 352 g, range: 313–388 g). The rats were ≈4 months old at the start of the experiments, and were housed in pairs in the colony room that was illuminated between the hours of 08.00 and 20.00, with food and water available *ad libitum* in the home cage throughout the experiment. Following a minimum of 2 weeks of postoperative recovery, rats received behavioral training that began at ≈09.30 on each day. All experimental procedures and animal husbandry conformed to the "principles of laboratory animal care" (Guide for the Care and Use of Laboratory Animals, NIH publication No. 85-23, revised 1985) and the UK Animals (Scientific Procedures) Act (1986), and received local ethics committee approval at Cardiff University.

2.2. Surgery and histology

The surgical procedure and the coordinates of injection sites were closely modeled on those described in Marshall, McGregor, Good, and Honey (2004). To summarize, rats were first anaesthetized with Isoflurane and then placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). After scalp incision, the bone overlying the area of neocortex directly above the hippocampus was removed, and a 2-µl Hamilton syringe mounted on the stereotaxic frame was used to infuse ibotenic acid into the hippocampus. The ibotenic acid (supplied by Biosearch Technologies, San Rafael, CA) was dissolved in phosphate-buffered saline [pH 7.4] providing a solution with a concentration of 63 mM; and injections of 0.05–0.10 µl were made at 30 sites with a KD Scientific electronic pump (Model 5000; Boston, MA) at a rate of 0.05 µl/min (see Table 2). After each injection, the needle was left in position for 2 min to allow diffusion of the ibotenic acid and to limit the spread of the drug into overlying cortical areas. Sham-operated rats received an identical treatment with the exception that dura was perforated with a 25-gauge Microlance3 needle (Becton Dickinson, Drogheda, Ireland) and no fluid was infused.

After behavioral testing, rats received a lethal overdose of sodium pentobarbitone (Euthatal), and were then transcardially perfused, first with 0.9% saline and then with 10.0% formal-saline. Their brains were then extracted and postfixed for 24 h, and transferred to phosphate-buffered (0.1 M) 25.0% sucrose solution for 24 h. Subsequently, each brain was frozen, sectioned coronally using a −20 °C cryostat, and the 40 µm sections were collected on gelatine-coated slides. These slides were left to dry at room temperature for 24 h, and then stained with cresyl violet. The sections were examined using a microscope, and histological borders of hippocampal lesions were verified with reference to the boundaries defined by Paxinos and Watson (2005).

2.3. Apparatus

The apparatus used was that described in Lin et al. (2013) and consisted of 8 operant chambers (Test chamber 80004-D001; Campden Instruments Ltd., Loughborough, England; 30.5 cm × 26 cm × 20 cm; width × depth × height) arranged in 4 × 2 array. Each chamber was housed within a sound-attenuating shell, had two aluminum side walls, a transparent Perspex back wall and ceiling. The front wall was also Perspex, and served as the door to the chamber. The chambers were lit by a 3-W light bulb, with a white plastic cover, positioned centrally and 13.5 cm above the floor. Two additional visual stimuli served as A and B: illumination of covered 3-W jewel lights that were located on the left- and right-hand sides of the left aluminum wall that contained the food well. These lights were mounted 13.5 cm above the floor and were positioned 9.2 cm to the left and right of a central wall light that was unused and mounted at the same height above the floor but immediately above the food well. The left and right lights were both constantly illuminated throughout their 30-s durations. Two 30-s auditory stimuli served as X and Y: a 2-kHz tone and a 2-Hz clicker. These stimuli, presented at an intensity of ≈75 dB, were produced by an internal audio generator and delivered through a speaker located centrally and at 14.5 cm above the floor on the left aluminum wall. A grid constructed from 19 stainless steel bars (diameter 0.47 cm, spacing from bar center to bar center, 1.07 cm) served as the floor of the chamber. A 0.5-s 0.64 mA footshock could be delivered through the grid floor.

Table 2
Stereotaxic coordinates and volume of ibotenic acid for lesions of the hippocampus.

	AP	ML	DV	Volume (µl)
From bregma:	−5.5	±4.2	−7.6	0.10
			−3.9	0.10
		±5.5	−6.8	0.10
			−5.8	0.10
			−5.0	0.10
	−4.7	±4.0	−7.5	0.10
			−3.5	0.05
		±4.5	−8.0	0.10
	−3.9	±2.2	−3.7	0.10
			−3.0	0.10
		±3.5	−2.7	0.10
	−3.1	±1.4	−4.0	0.10
			−3.0	0.10
		±3.0	−2.7	0.10
	−2.4	±1.0	−3.8	0.05

Note: AP, ML and DV indicate the coordinates in relation to bregma from anterior to posterior (AP), from medial to lateral (ML) and from dorsal to ventral (DV).