Neuroscience 137 (2006) 685–698

UNAMBIGUOUS REPRESENTATION OF OVERLAPPING SERIAL EVENTS IN THE RAT HIPPOCAMPAL FORMATION

A. A. FURUSAWA,[a,b] E. HORI,[a,b] K. UMENO,[a,b] E. TABUCHI,[a,b] T. ONO[a,b] AND H. NISHIJO[a,b]*

[a]*System Emotional Science, Graduate School of Medicine, University of Toyama, Sugitani 2630, Toyama 930-0194, Japan*

[b]*CREST, JST, 4-1-8 Honcho Kawaguchi 332-0012, Japan*

Abstract—The hippocampal formation is suggested to be crucial in unambiguous representation of overlapping temporal sequences in episodic memory. We hypothesized that, if this was true, the hippocampal formation neurons would differentially respond to the same elements even in different temporal sequences. The present study was designed to investigate hippocampal formation CA1 neuronal activity of rats during performance of a conditional delayed stimulus-response association task in which three stimuli were conditionally and serially presented with a delay. In the task, the pairs of the second and third stimuli were overlapped across the trials, but separated by the preceding first stimuli. Conditioned tones coming from one of three possible directions were followed, after a short delay, by one of three pairs of reinforcement series. The pairs consisted of air puff (aversive sensory stimuli) and tube protrusion (which allowed licking sucrose behavior) in the following combinations: air puff–tube protrusion, tube protrusion–tube protrusion and tube protrusion–air puff. The pairs were interposed by a 2 s delay. The three conditioned tone directions were associated with these three pairs in a one-to-one correspondence, and its association was conditional to three possible conditioned tone frequencies (300, 530, and 1200 Hz). The responses of 107 neurons to the air puff and tube protrusion were analyzed by two-way ANOVA (task condition×reinforcement situation). Of 42 air puff-responsive and 64 tube protrusion-responsive neurons, 36 and 53 displayed significant main effects and/or significant interaction, respectively. Furthermore, neural responses during the delay periods were dependent on the task conditions. The results indicated that the majority of the hippocampal formation neurons showed task condition- and/or reinforcement situation-dependent responses, suggesting a crucial role of the hippocampal formation in representation of overlapping serial events in episodic memory. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: hippocampus, CA1, pyramidal neuron, context, episodic memory.

*Correspondence to: H. Nishijo, System Emotional Science, Graduate School of Medicine, Toyama Medical and Pharmaceutical University, Sugitani 2630, Toyama 930-0194, Japan. Tel: +81-76-434-7215; fax: +81-76-434-5012.
E-mail address: nishijo@toyama-mpu.ac.jp (H. Nishijo).

Abbreviations: ANOVA, analysis of variance; AP, air puff; CA1, Cornu Ammonis 1; CDSRT, conditional delayed stimulus-response association task; CT, conditioned tone; EMG, electromyogram; HF, hippocampal formation; LTD, long-term depression; LTP, long-term potentiation; PETHs, peri-event time histograms; TP, tube protrusion.

0306-4522/06$30.00+0.00 © 2005 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2005.09.002

Since Scoville and Milner (1957) first reported hippocampal formation (HF) relation to amnesia, extensive researches have described the role in memory; the HF is important in formation of declarative or episodic memory (Squire et al., 1993; Schacter et al., 1996; Vargha-Khadem et al., 1997) and working memory (Olton et al., 1979), important in the processing of cognitive mapping (O'Keefe and Nadel, 1978), or relational association (Eichenbaum et al., 1990; Bunsey and Eichenbaum, 1996). Although the theoretical bases of those studies were different, these theories all postulated the role of the HF in association or relation of multiple factors. Anatomical studies support this idea in that the HF receives highly-integrated diverse information from all association cortices to form such memory or association (Amaral and Insausti, 1990; Witter et al., 2000a,b).

One way to enable this postulated function of the HF (i.e. encoding of association or relation of multiple factors) is for the HF to represent sequences of multiple events or factors that compose the spatial and temporal context of an episode. Recent lesion studies using rats suggest that the HF is not important in encoding of new sensory inputs themselves, but in the encoding of novel temporal or spatial arrangements of each sensory stimulus (Honey et al., 1998; Wan et al., 1999; Eichenbaum, 2000). Furthermore, HF lesions induced deficits for memory of temporal serial information in an eight-arm radial maze in rats (Kesner and Novak, 1982; Chiba et al., 1994). In humans, amnesic patients with bilateral temporal lobe resection displayed deficits in relative recency memory (Sager et al., 1990). Furthermore, a neurophysiological study reported that the human HF was crucial in encoding and retrieval of novel (unfamiliar) arrangements of serial sensory inputs, but not encoding and retrieval of novelty of individual sensory inputs (Takakura et al., 2003). Recent computational studies also suggested a HF involvement in memory of temporal sequence (Granger et al., 1994; Levy, 1996; Wallenstein et al., 1998; Borisyuk et al., 1999; Lisman, 1999; Jensen and Lisman, 2005). Taken together, the HF is one of the brain regions critical for sequence information processing and memory.

Recent behavioral and computational studies suggest that the HF is particularly important in disambiguation of overlapping serial elements among element sequences based on preceding non-overlapping elements (Agster et al., 2002; Levy, 1996). It has been reported that HF neuronal activity was sensitive to temporal sequence in rats (Foster et al., 1987; Chiba et al., 1994; Skaggs et al., 1996). However, neural representation of overlapping tem-

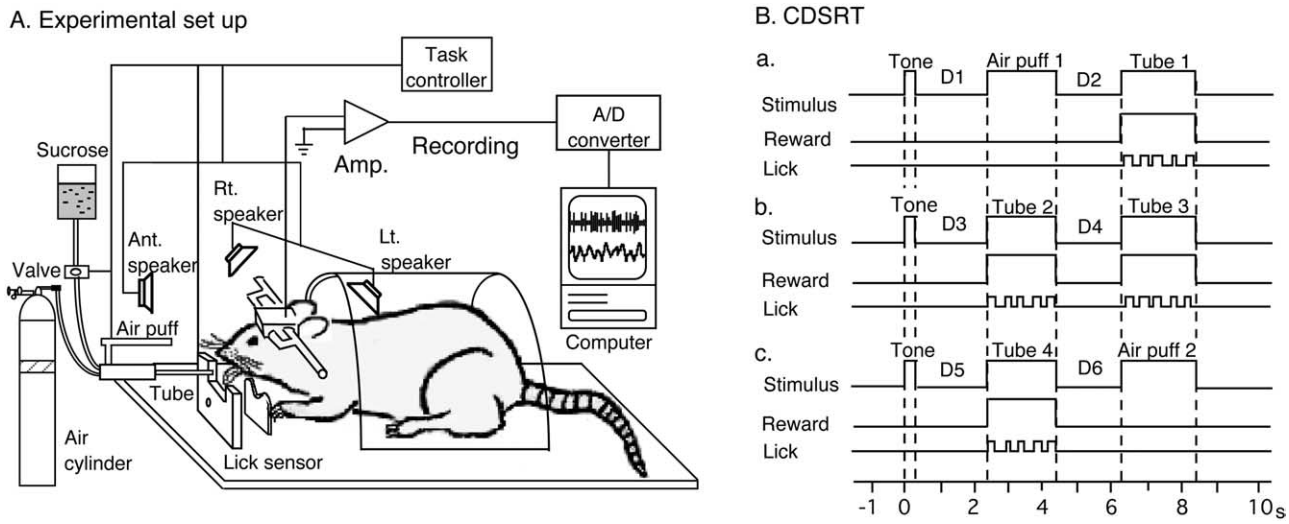

Fig. 1. Experimental diagram. (A) Experimental setup. Rats were prepared for chronic recording by forming receptacles of dental cement to accept fake earbars. The rat was trained to lick a small tube to obtain reward (0.3 M sucrose solution) when it was automatically placed close to its mouth. Licking was signaled by a photoelectric sensor triggered by its tongue. For conditional sensory stimulation, three speakers and one AP tube were located near a rat's head. (B) Paradigms of a CDSRT. A task was initiated by the CT presented from one of the three directions (right, left, and anteriorly), which was followed by one of the three pairs of the serial reinforcements [i.e. aversive sensory stimulation (AP)–TP near a rat's mouth to evoke licking sucrose solution (Ba), TP–TP (Bb), and TP–AP (Bc)] after a delay of 2.0 (or 1.5) s. The two serial reinforcements were separated by a delay period of 2.0 s.

poral sequences in the HF remains unclear. We hypothesized that, if the HF was crucial in disambiguation of overlapping serial elements, the HF neurons would differentially respond to the same elements in different sequences. The present study was designed to investigate HF neuronal activity of rats during performance of a conditional delayed stimulus (conditioned stimulus) -response (responses to unconditioned stimulus) association task (CDSRT), in which three stimuli (first conditioned stimulus, second unconditioned stimulus, third unconditioned stimulus) were conditionally and serially presented with a delay. In the CDSRT, the pairs of the second and third unconditioned stimuli were overlapped across the trials, but separated by the preceding first conditioned stimuli. Analyses of the response patterns to the second and third stimuli in reference to the first stimuli should disclose neural representation of overlapping sequences in the HF.

EXPERIMENTAL PROCEDURES

Subjects and task paradigm

Fifteen male Wistar rats weighing 230–300 g (SLC, Hamamatsu, Japan) were used. The housing area was temperature-controlled at 23 °C and maintained on a 12 h light/dark cycle. Before surgery, rats were housed individually in clean cages with free access to water and laboratory chow. To minimize the number of rats used and their suffering, all rats were treated in strict compliance with the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals and the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the Guideline for the Care and Use of Laboratory Animals in University of Toyama.

After recovery from the surgery (see *Surgery*) (10–14 days), a rat was painlessly placed in the special stereotaxic apparatus with a device for the CDSRT (Fig. 1A). Three mid-range speakers located anterior, right, and left to the rat's head delivered the conditioned tones (CTs) (Tone 1, 300 Hz; Tone 2, 530 Hz; Tone 3, 1200 Hz) for 0.3 s. The three directions of each tone frequency yielded a total of nine different trials (see below in detail; Table 1).

Table 1. Summary of conditional relations between the CTs and reinforcements

Task condition [CT]	Direction of CTs	Delay	Reinforcements
Task condition 1 [Tone 1 (300 Hz)]	Left CT	D1	AP1-D2-TP1
	Anterior CT	D3	TP2-D4-TP3
	Right CT	D5	TP4-D6-AP2
Task condition 2 [Tone 2 (530 Hz)]	Anterior CT	D1	AP1-D2-TP1
	Right CT	D3	TP2-D4-TP3
	Left CT	D5	TP4-D6-AP2
Task condition 3 [Tone 3 (1200 Hz)]	Right CT	D1	AP1-D2-TP1
	Left CT	D3	TP2-D4-TP3
	Anterior CT	D5	TP4-D6-AP2

D, AP and TP with the different numbers in each task condition indicate the different situations within the same task condition. D, delay.