生物化学与生物物理进展 *Progress in Biochemistry and Biophysics*, 2006, 33(11): 1051~1060
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Activity of Single Granule Cell in the Dentate Gyrus of Guinea Pig During The Consolidation of Trace Eyeblink Conditioned Responses

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Abstract The hippocampus plays a critical role during the consolidation of trace eyeblink conditioned responses (CRs). However, the role of its related structure such as dentate gyrus (DG) remains unclear. The present study was aimed at monitoring the activity of single granule cell in the DG during the consolidation of trace eyeblink CRs, and elucidating the possible role of DG during this hippocampus-dependent task. Guinea pigs (n=8) were trained on a trace eyeblink conditioning paradigm using a 200-ms tone conditioned stimulus (CS), a 200-ms corneal airpuff unconditioned stimulus (US) and a 600-ms trace interval. Controls consisted of pseudo- conditioned guinea pigs (n=8). Extracellular single unit recordings *in vivo* were performed in the DG of learner animals during the consolidation of trace eyeblink CRs. The results revealed that all the trace-conditioned animals acquired the trace eyeblink CRs over 14 training days, however, none of the pseudo-conditioned animals did. Furthermore, 23 of 40 single granule cells in the DG of learner animals exhibited heterogeneous activity patterns during the consolidation of trace eyeblink CRs such as increases in activities to the tone CS, trace interval or airpuff US. The results suggested that the DG might participate in the neural circuit important for the consolidation of trace eyeblink CRs, and that the granule cells might encode different information during the consolidation of trace eyeblink CRs.

Key words dentate gyrus, granule cell, trace conditioning, eyeblink, single unit recording, guinea pig

Classical eyeblink conditioning is one of the best-characterized mammalian model systems for the study of associative learning, which involves paired presentation of a neutral conditioned stimulus (CS) such as a tone and a reinforcing unconditioned stimulus (US) such as a corneal airpuff in the eye [1]. There are two paradigms of eyeblink conditioning that differ in the temporal relationship between the CS and US. In the delay paradigm, the CS precedes, overlaps, and coterminates with the US. On the other hand, in the trace paradigm, the CS and the US are separated by a stimulus-free trace interval.

Studies of hippocampus indicated that the hippocampus was important for the acquisition of trace eyeblink conditioned responses (CRs) [2~5], and a number of *in vivo* electrophysiological studies were aimed at understanding how the hippocampal neurons encoded learning-related information during the acquisition of trace eyeblink CRs[6~8]. Moreover, recent study by Kim and colleagues found that the hippocampus was also essential for the consolidation but not retention of trace eyeblink CRs [9]. In their study, lesions placed in the rabbit hippocampus 1 day after asymptotic levels of CRs were reached disrupted the performance of CRs, whereas lesions placed 1 month after consolidation had no effect on the performance of CRs. Similar results were also observed in mice[10].

In mammals, the hippocampal formation differentiates into a recurrent network of densely interconnected pyramidal cells (CA3), a feed-forward network with almost no intrinsic excitatory connections (CA1) and a structure strongly connected with entorhnial cortex *via* the glutamatergic perforant path (DG) [11]. Within the hippocampal formation, neuronal information is communicated along a chain of neurons, beginning with the axons of granule cells

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Received: May 9, 2006 Accepted: June 30, 2006

of the DG. Their mossy fibers impinge on the neighboring dendrites of CA3 neurons, which project their axons to the CA1 region. The close anatomical relationship between the hippocampus and the DG arouses an interesting question whether the first synaptic region within the trisynaptic loop of the hippocampal formation involves in neural circuit important for the consolidation of trace eyeblink CRs. If it does, the granule cells in the DG should be activated when the learner animals were performing this hippocampus-dependent task. However, the definitive electrophysiological evidence for this issue was lacking.

In present study, long-trace interval eyeblink conditioning was established, which was generally considered to depend on the involvement of the hippocampus. The activities of granule cells in the DG of learner animals were recorded during the consolidation of trace eyeblink CRs, which allowed us to determine whether the DG involved in the neural circuit important for the consolidation of trace eyeblink CRs.

1 Materials and methods

1.1 Subjects

16 guinea pigs of 8～10 weeks old (350～480 g) were used as subjects and randomly assigned to 2 groups: trace-conditioned (n=8) and pseudo-conditioned (n=8). They were housed in clean cages on a 12 h light/dark cycle (with light onset at 08:00h) and given free access to food and water. Care of the animals conformed to the rules of Third Military Medical University for animal research during the entire course of present study. All efforts were made to minimize the use and the discomfort of animals.

1.2 Surgical preparation

Animals were allowed to remain undisturbed in their cages for ⩾1 week prior to surgery. Surgeries were performed under aseptic conditions, and the temperature of experimental environment was maintained at 20～25℃. Animals were anesthetized with sodium pentobarbital (30 mg/kg, i.p.), and the eyes were kept moist with a thin coat antibacterial ophthalmic ointment. The skull was firmly secured in a stereotaxic frame (Type: SR-6N, Narishige, Japan) and was exposed. A 2-mm diam hole was drilled into the left skull centering at 5.0 mm posterior and 3.0 mm lateral to bregma. In each animal, 5 other holes were drilled into the skull and were inserted with stainless steel bone screws. One of these screws, usually positioned in the anterior position over the right hemisphere, was used as ground. The other 4 stainless steel bone screws were inserted ～1.0 mm into the skull to anchor a head-restraining device. Dental cement then was used to reinforce the head-restraining device and close the remaining wound area. After surgery, animals were treated with antibiotics as needed and allowed at least 1 week of postoperative recovery before any handling.

1.3 Behavioral procedures

All animals received 2 consecutive daily sessions of acclimation to the testing chamber followed by 14 consecutive daily sessions of either trace- or pseudo-eyeblink conditioning. During the acclimation and the training sessions, animal was placed in a soundproof chamber (Type: Cl-15A, Eckel, Canada). The head of animal was secured by head-restraining device, and the left eye was held open in a comfortable position with the upper eyelid attached to a small clamp. Nylon strings were used to limit the excessive actions of animal's trunk and limbs. A speaker was placed in the 10 cm front of the animal to deliver the tone CS (2 kHz, 85 dB, 200 ms, 5 ms rise/fall time). An airpuff tube was placed ～1 cm from the animal's left eyeball and served to deliver the corneal airpuff US (4.0 N/cm^2 source pressure, 200 ms). The airpuff was supplied by compressed air, and its intensity was calibrated to evoke an eyeblink. A tensional potentiometer was used to detect the activity of left upper eyelid, and the signals were collected by a PowerLab acquisition system (Type: PowerLab/4sp, ADInstruments, Australia) with a sample rate of 20 kHz. For trace- and pseudo-conditioned animals, a CR was defined as an increase in the activity of left upper eyelid that was greater than the baseline amplitude plus four times the SD of the baseline activity. The CR also needed to have a minimal duration of ⩾15 ms and occurred at the 800-ms period after the onset of tone CS. Animals were considered learners if the percentage of CRs exceeded 50% on any day of training, and were considered nonlearners if the daily CR percentage failed to exceed 25% after 14 days of training.

Each acclimation session lasted ～1 h during which no stimuli were presented. One day following the acclimation, trace-conditioned animals (n=8) received daily sessions consisting of 80 trails presented at an intertrial interval 20～40 s (mean, 30 s). Each

trail consisted of the CS, followed by a 600 ms stimulus-free trace period, then the US (Figure 1). Pseudo-conditioned animals (n = 8) also received acclimation followed by daily sessions consisting random presentation of 80 CS-alone and 80 US-alone trials (intertrial interval 10 ～20 s). Trace- and pseudo-conditioning sessions lasted ~1h and consisted of the same number of CSs and USs.

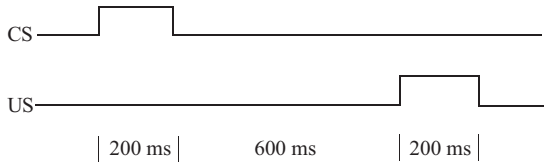

Fig. 1 Schematic diagram of trace conditioning procedures In trace conditioning, there is a temporal gap (600 ms trace interval) between the CS (200 ms tone) offset and US (200 ms corneal airpuff) onset.

1.4 Single unit recording techniques

Daily recording sessions began on the second day when the daily percentage of CRs exceeded 50%, and ended on the 14th day of training. In each recording session, the behavioral procedures were identical to which were performed during foregoing training sessions, except that a Teflon-coated tungsten microelectrode (1 ～3 μm diam, tip, 2 ～5 MΩ impedance at 100 Hz) was stereotaxically lowed into the left DG (5.0 mm posterior and 3.0 mm lateral to Bregma, 4.0～5.0 mm ventral to dura) by a microdrive machine (Type: PC-5N, Narishige, Japan) until the spikes with granule cell or interneuron firing characteristics were recorded [12,13]. Neuronal signals were amplified (×20 000), filtered (band pass, 500 and 5 kHz), and collected with PowerLab acquisition system (Type: PowerLab/4sp, ADInstruments, Australia), which sampled at 20 kHz. Digital analogue signals then were transferred to a computer (Lenovo, Beijing, China) for storage. The off-line analysis was carried out using custom software written in the Matlab (MathWorks). Putative single neurons were separated using measurements of spike height and width. To ensure that units (putative single neurons) were well isolated, interspike interval histograms (ISIHs) were generated for all units that were recorded simultaneously. Given that hippocampal units could rarely fire at instantaneous rates of 500 Hz primarily because of their absolute refractory period, ISIHs should not show a significant frequency of counts < 2 ms. A significant percentage of counts with such short interspike intervals were indicative of false-positive errors, i.e., inclusion of spikes arising from another unit close by. Only the data, in which < 5% of the interspike intervals were < 2 ms were included in the our analysis.

After the spike separation, the classification of DG units was made. Here, we characterized the waveform shape of each unit by computing the width of the waveform defined as the mean absolute value of the time from the peak to the trough of the units' spike. We then used the resulting distributions of widths, combined with the average firing rates of the units, to classify each DG unit as either a granule cell (GC) or an interneuron (IN). The detailed criteria were described in our previous study [14]. Briefly, a linear cutoff in firing rate-spike waveform width space was used to classify the two subtype units. Cells with average firing rates r (in Hertz) and spike widths w (in microseconds) such that $r < 0.025 \cdot w - 3.0$ were classified as GCs. Cells with average firing rates r (in Hertz) and spike widths w (in microseconds) such that $r > 0.025 \cdot w - 3.0$ were classified as INs.

1.5 Data analysis

After the acquisition of trace eyeblink CRs, the activity of single GC during the consolidation of trace eyeblink CRs was recorded and analyzed. Computer-identified individual spikes were counted and summed into consecutive 10 ms bins for each trial. Peri- stimulus histograms of summed unit activity for 40 trials were constructed over a 2.8 s epoch, commencing 1 s prior to and 1.8 s following the CS onset. The activity pattern of each GC then was classified using those peri-stimulus histograms. Furthermore, means and standard deviations of firing rates in 1 pretone and 5 consecutive posttone 200 ms intervals were calculated, and were used to quantitatively analyze the changes in activities of the same pattern GCs.

To further determine the behavioral stages in which the GCs were recorded, the behavioral and neural data from each learner animal were analyzed in relation to the day of training when the initial increase of CRs occurred and the day of training when CRs became asymptotic. This strategy was designed by McEchron and Disterhoft to reduce the error variance associated with the heterogeneity in the rate and pattern of eyeblink CRs between animals [7]. In brief, the change in daily percent CRs was calculated for each conditioned animal [(percentage of CRs on day X)−(percentage of CRs on day X−1)] across the days

of training to determine the SD of change in daily percent CRs. The day of the initial increase in CRs was defined for each learner animal as the first day of training when the change in daily percent CRs was >1 SD of the mean. The asymptotic day of training were defined as the day of training after the maximum change in daily percent CRs occurred. The daily neural and behavioral data for each learner animal then were grouped into the following stages: the days before an animal showed an initial increase in CRs (day before initial CR increase); the day of training when an animal showed an initial increase in CRs (day of initial CR increase); the days after an animal showed an maximum increase in CRs (day of asymptotic CR); the left days between the day of initial CR increase and asymptotic day (day after initial CR increase).

1.6 Statistical analysis

All data were presented as $\bar{x} \pm s$. Statistical significance was determined by one-way ANOVA, or independent sample *t*-test using statistical software SPSS 10.0. All tests were used two-tailed and an error probability of < 5% was considered significant.

1.7 Histology

After all the recording experiment, a DC current (0.25 mA, 30 s) was passed to the last recording site. The locations of the microelectrode tips within the hippocampal formation were histologically verified (Figure 2). The animals were deeply anesthetized with sodium pentobarbital, and then were perfused with saline and 10% buffered paraformaldehyde solution. Brain were removed and stored for 72 h at 4℃ in a 20% sucrose solution in 0.1 mol/L phosphate buffer. Consecutive coronal frozen sections (20 μm thick) then were made through the length of the hippocampus, and all the sections were stained with Toluidne Blue and coverslipped. The recognizable microelectrode tip tracks were examined carefully using a light microscope. If the location of microelectrode could not be determined with a high degree of confidence, data from that animal were not used.

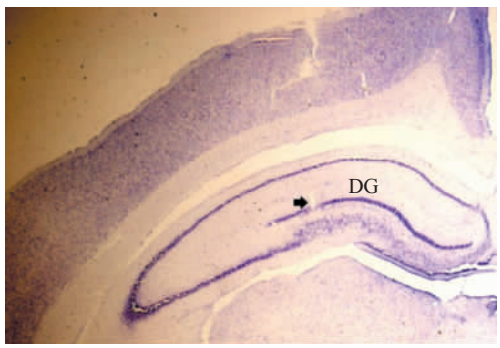

Fig. 2 The tip position of the tungsten microelectrode (black arrow)

Nissle-stained slices, ×15.

2 Results

2.1 Acquisition of trace eyeblink CRs

All animals received trace- or pseudo- eyeblink conditioning, and the conditioning was performed successively for 14 days. The behavioral results revealed that the trace-conditioned animals exhibited a significant increase in the percentage of trials with CRs over 14 daily sessions of trace eyeblink conditioning, while the pseudo- conditioned animals did not exhibit a significant increase (Figure 3). These results were based upon one-way ANOVA of each group by training day for the percentage of trials with CRs. The analysis revealed significant effects of training day ($F_{13, 98} = 50.978$, $P < 0.001$) in the trace- conditioned group. These results indicated that the trace-conditioned animals could acquire the eyeblink CRs with a long trace interval of 600 ms.

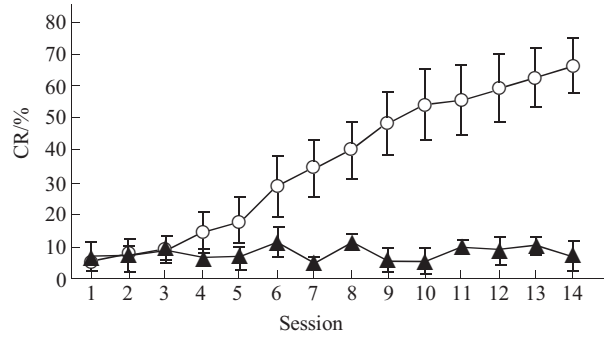

Fig. 3 Acquisition of trace eyeblink conditioned responses

Average CR rates of the trace-conditioned ($n = 8$, open circle, ○—○) and pseudo-conditioned guinea pigs ($n = 8$, filled triangle, ▲—▲) over 14 daily sessions were displayed. Error bar indicated the standard deviation of mean. The trace-conditioned group exhibited a significant increase in the CR rate over the 14 daily sessions of eyeblink conditioning with a trace interval of 600 ms, while the pseudo-conditioned group did not exhibit a significant increase.

To evaluate the effects of trace-conditioned treatments and pseudo-conditioned treatments on the CR rate across 14 daily training sessions, a repeated independent sample *t*-test analysis was made. Parts of the statistical results were displayed in Table 1. The statistical analysis did not reveal a significant difference of CR rate between two groups during the first 3 days of training. In contrast, the analysis showed that the trace-conditioned group exhibited a greater CR

rate than the pseudo-conditioned group during the subsequent 11 days of training (Table 1). These results indicated the type of behavioral treatments had significant effects on the percentage of trials with CRs.

It should be noted that the day of CR rate exceeded 50% for each trace-conditioned animal was significantly different, whereas all the trace-conditioned animals showed consistent increases of CR rates during 14 daily training sessions. In the trace-conditioned group, the day of CR rate exceeded 50% was the 8th day of training for one animal, the 9th day for three animals, the 10th day for two animals, and the 13th day for two animals. Moreover, the beginning days of asymptotic CRs were also calculated. For the trace-conditioned animals, the beginning day of asymptotic CRs was the 7th day for three animals, the 9th day for three animals, the 10th day for one animal, and the 12th day for one animal.

Table 1 Results of repeated independent sample *t*- test across 14 daily training sessions

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14
CR1/%	5.2	7.5	8.8	14.2	17.6	28.6	34.2	39.8	48.2	54.0	55.5	59.2	62.5	66.1
CR2/%	6.5	7.2	9.3	6.2	6.6	11.2	4.5	11.2	5.7	5.2	9.8	8.6	10.0	6.8
T value	−0.700	0.155	−0.243	3.130	3.349	4.387	8.475	8.009	11.018	10.987	11.507	12.501	15.333	16.928
P value	>0.05	>0.05	>0.05	<0.05	<0.05	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

CR1 represented the CR rates of trace-conditioned animals on each day of training. CR2 represented the CR rates of pseudo-conditioned animals on each day of training. Because of the limitation of the table size, the values of CR1 and CR2 were only presented as means.

2.2 Simultaneous recording during trace eyeblink conditioning

One day following the day when the CR rate exceeded 50%, extracellular single unit recordings *in vivo* began to be performed in the DG of learner animals, and ended on the 14th day of training. The signals of neural discharge, tone CS and eyeblink activity were simultaneously recorded during trace

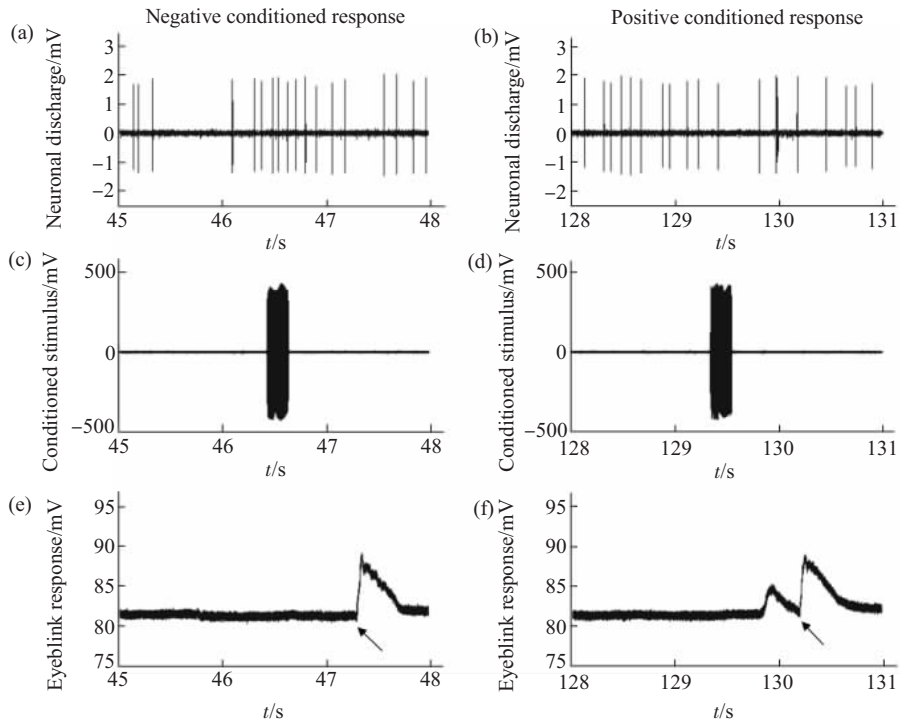

Fig. 4 Simultaneous recording of the neural discharge, tone CS and eyeblink activity during trace eyeblink conditioning

(a) The activity of DG unit when the negative conditioned response occurred. (b) The activity of the same DG unit in panel (a) when the positive conditioned response occurred. (c) and (d) The signals of tone CS. (e) Negative eyeblink response. No significant eyeblink occurred in the 800 ms period after CS onset. (f) Positive eyeblink response. A significant eyeblink occurred in the 800 ms period after CS onset, which also occurred in the ~300 ms period before airpuff US onset. The arrows, which were displayed in the panel (e) and (f), highlighted the onsets of the unconditioned eyeblink responses.

eyeblink conditioning (Figure 4). In present study, a total of 52 units were stably recorded and isolated. The recoding time were in the range of 20～30 min (mean, 25 min), which allowed us to investigate the activity across 40 CS-US trials of each unit. According to the classification of behavioral stages, all the DG units were recorded within the asymptotic CRs period (Figure 5).

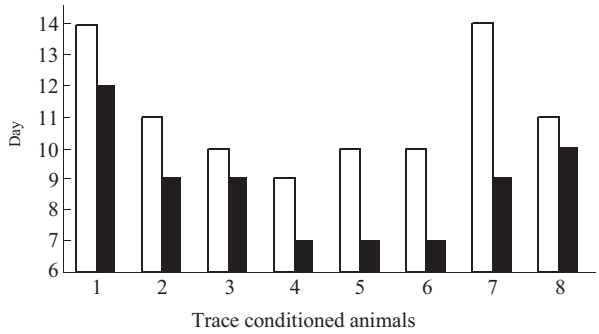

Fig. 5 Comparison between the beginning days of asymptotic CRs and the beginning days of single unit recording

For each trace-conditioned animal, the beginning day of recording was later than that of asymptotic CRs, which indicated all the DG units were recorded within the asymptotic CRs period. □ : Begining day of recording; ■: Begining day of asymptotic CRs.

2.3 Unit classification

According to the criteria of classification described in **Materials and methods**, 12 units were classified as having firing rates (19.843 ± 4.937) Hz and spike widths (0.346 ± 0.037) ms resembling interneurons. The remaining 40 units were classified as having firing rates (2.640 ± 2.158) Hz and spike widths (0.556 ± 0.105) ms within the range of granule cells. The firing rate and spike width of each unit was showed in Figure 6. In addition, the ISIH of each DG unit was also generated. The results revealed that the ISIHs of GCs exhibited obviously positive skewness with a long sharp decline; however, the ISIHs of INs exhibited an approximately symmetrical distribution (Figure 6).

Thompson and Best had described 'silent cell' in the hippocampus with very low firing rate [15]. In present study, a total of 7 GCs had very low firing rates (0.327 ± 0.262) Hz and showed no changes in activity during the entire course of recording. Those neurons, together with the 12 INs, were not included in our analysis of single neuron activity during the consolidation of trace eyeblink CRs.

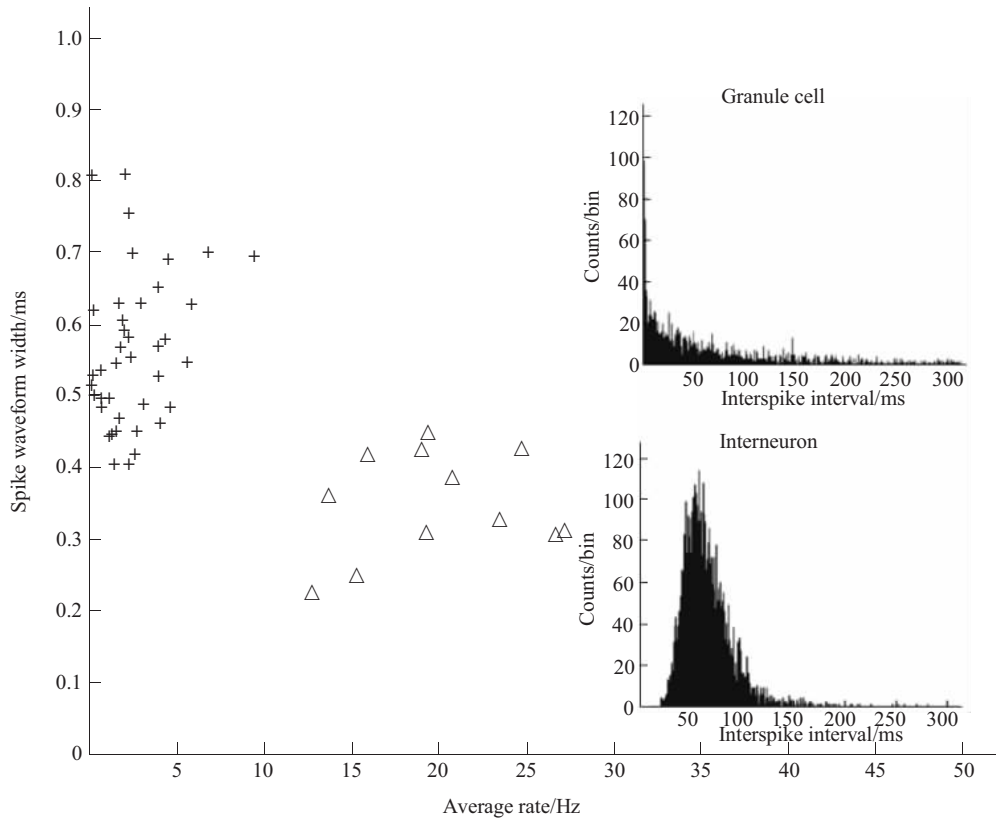

Fig. 6 Classification of DG units into granule cells (+) and interneurons (Δ)

Scatter plots of the relationship between spike waveform width and average rate for DG units exhibited an approximately bipolar distribution. Generally, the interneurons exhibited high firing rates and had narrow spike widths. In contrast, the granule cells discharged with low firing rates and possessed wide spike widths. Two exemplar ISIHs, respectively, were generated for a granule cell and an interneuron. The ISIH of the granule cell exhibited obviously positive skewness with a long sharp decline, while the ISIH of the interneuron exhibited an approximately symmetrical distribution.

2.4 Activity of single granule cell during the consolidation of trace eyeblink CRs

The major aim of present study was to examine single GC activity during the consolidation of trace eyeblink CRs. Therefore the activity of the nonsilient GCs (n=33) was examined during the days of asymptotic CRs.

First, GCs were divided into categories with the most consistent activity patterns: cells with changes in activities during the first 200 ms period after the CS-onset (tone excitatory / inhibitory); cells with changes in activities during 201～800 ms period after CS-onset but no changes during the first 200 ms period after the CS onset (trace excitatory / inhibitory); cells with changes in activities during the 200 ms period after the US-onset but no change during the 800 ms period after the CS onset (airpuff excitatory / inhibitory); cells with no changes in activities during the entire trace conditioning period.

The results revealed that 23/33 (69.7%) GCs showed obvious increases in activity, and exhibited three different activity patterns during the consolidation of trace eyeblink CRs. In present study, 5 tone excitatory cells, 6 trace excitatory cells, 12 airpuff excitatory cells and 10 no change cells were observed. Peri-stimulus histogram of each GC was generated, and histograms of exemplar single GCs were shown in Figure 7.

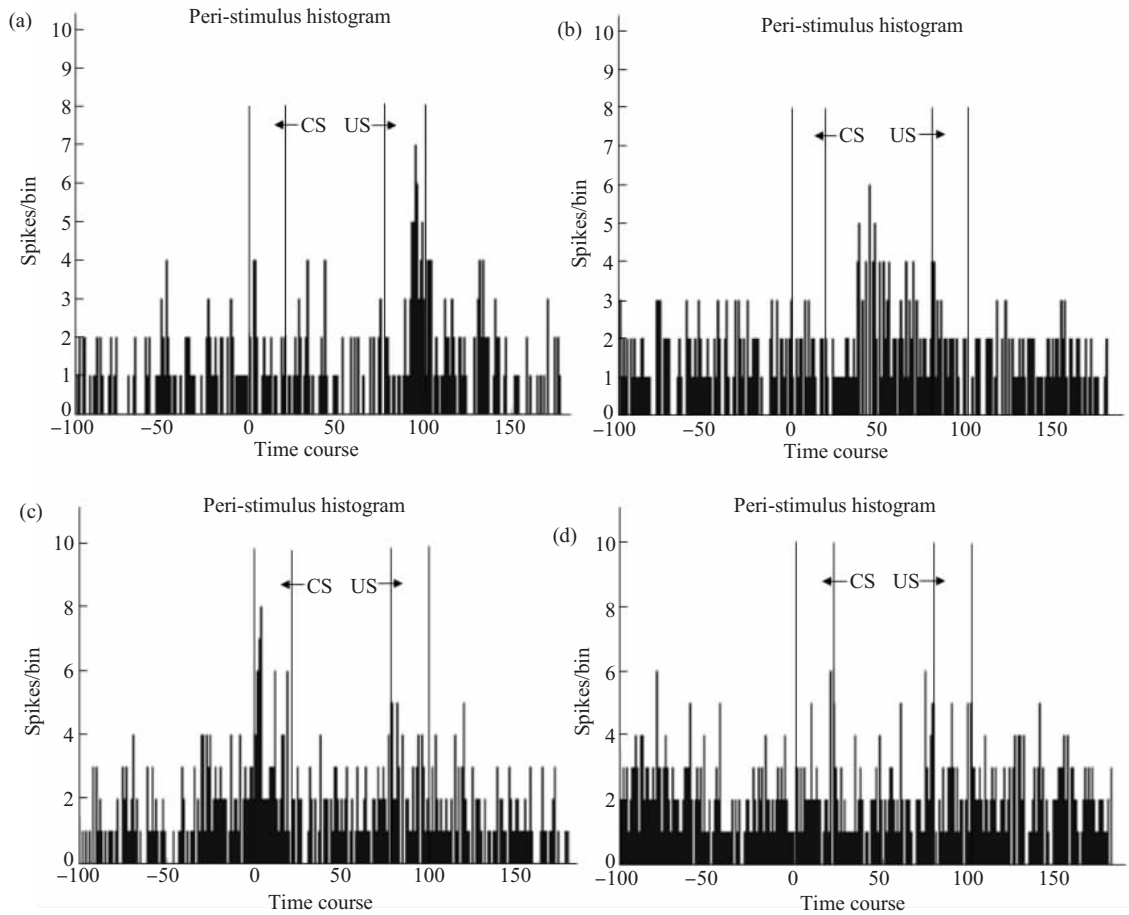

Fig. 7 Peri-stimulus histogram (10 ms bins) of spike discharge activity across 40 CS-US trials of putative single granule cell recorded from the DG of learner animal

The duration of the histogram is 2 800 ms, and the duration of the pre-stimulus period prior to the onset of the CS is 1 000 ms. Time 0 represented the onset of CSs. CS represented the conditioned stimuli. US represented the unconditioned stimuli. (a) Increase in activity of single granule cell to the airpuff US. (b) Increase in activity of single granule cell to the long trace interval. (c) Increase in activity of single granule cell to the tone CS. (d) No change in activity of single granule cell during trace eyeblink conditioning.

Second, the activities of three kinds of changing GCs during 1 pretone and 5 consecutive posttone 200 ms intervals were quantitatively analyzed. During the consolidation of trace eyeblink CRs, the activities of airpuff excitatory GCs (n=12) increased reliably within the 200 ms period after the US-onset

(Figure 8a). One-way ANOVA confirmed that the mean firing rates differed significantly ($F_{5,66}$=10.229, P < 0.001) across the six 200 ms intervals. The Tukey method of multiple comparisons established that the mean firing rate in the fifth posttone 200 ms interval was significantly increased over the mean firing rate in the pretone 200 ms interval (P < 0.001).

The activities of trace excitatory granule cells (n=6) were also analyzed. These neurons augmented their firing rates within the 401～600 ms period after the CS-onset (Figure 8b). One-way ANOVA revealed that the mean firing rates differed significantly ($F_{5,30}$= 7.779, P < 0.001) across the six 200 ms intervals. The Tukey method of multiple comparisons confirmed that the mean firing rates in the third posttone 200 ms interval were significantly increased over the mean firing rate in the pretone 200 ms interval (P < 0.01).

In addition, the activities of tone excitatory granule cells (n=5) were calculated. These neurons increased their firing rates within the first 200 ms period after the CS-onset (Figure 8c). One- way ANOVA revealed that the mean firing rates differed significantly ($F_{5,24}$=11.124, P < 0.01) across the six 200 ms intervals. The Tukey method of multiple comparisons confirmed that the mean firing rates in the first posttone 200 ms interval were significantly increased over the mean firing rate in the pretone 200 ms interval (P < 0.01). These results indicated that the GCs in the DG were activated when the learner animals were performing trace eyeblink conditioning task.

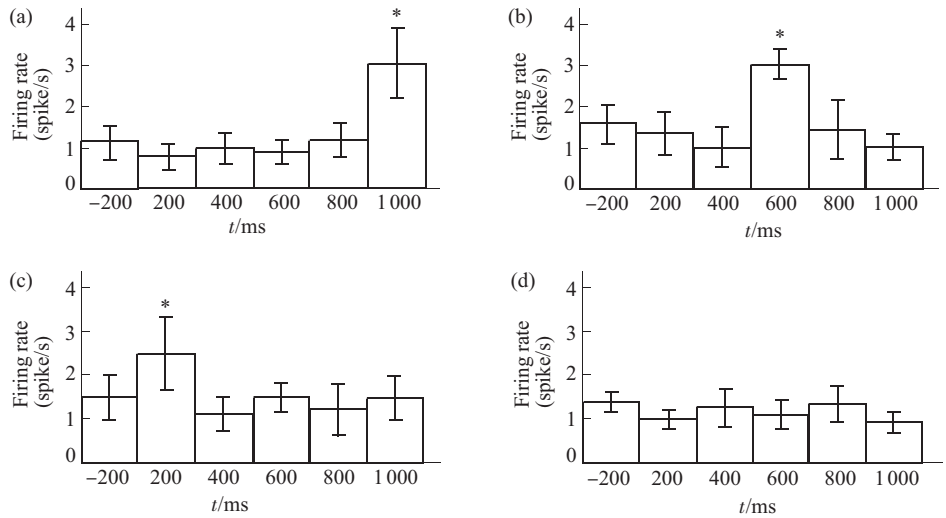

Fig. 8 Firing rates of 4 subtype granule cells during the consolidation of trace eyeblink conditioned responses
Mean firing rates (± standard deviation) were represented in 1 pretone and 5 consecutive posttone 200 ms interval in all panels. (a) Granule cells (n=12) exhibited significant increases in activities during the fifth posttone 200 ms interval (airpuff US period). (b) Granule cells (n=6) exhibited significant increases in activities during the third posttone 200 ms interval (trace interval period)). (c) Granule cells (n=5) exhibited significant increases in activities during the first posttone 200 ms interval (tone CS period). (d) No change in activity of single neuron during trace eyeblink conditioning. Single asterisk indicates significant differences from pretone mean firing rates at 0.01 level of significance.

3 Discussion

3.1 Acquisition of trace eyeblink CRs

In present study, we first established the long-trace eyeblink conditioning in guinea pigs. In this trace eyeblink-conditioning paradigm, there were a 600 ms period of no stimulation (trace interval) between CS offset and US onset. The behavioral data from present study revealed that all the conditioned animals acquired the trace eyeblink CRs across fourteen daily training sessions, while the pseudo-conditioned animals did not exhibit an increase in the frequency of CRs. It also should be noted that, because of the longer trace interval, the learning rate of our conditioned animals was slower than that of rabbits trained with a 500 ms trace paradigm[7].

3.2 Unit classification

Results from previous studies of rats suggested that the neurons in the DG could be divided into two different electrophysiological populations, granule cell (GC) and interneuron (IN)[12,13]. Similarly, the single DG neurons we recorded were classified into GCs and

INs according to the criteria described in the **Materials and methods**. The classification results revealed that the INs exhibited high firing rates and had narrow spike widths. In contrast, the GCs discharged with low firing rates and possessed wide spike widths. Our results confirmed the previous reports in rats and proved our classification criteria to be reasonable and appropriate for guinea pigs.

3.3 Activity of single granule cell during the consolidation of trace eyeblink CRs

Recent studies reported that the associative learning such as trace eyeblink conditioning enhanced the survival of new neurons in the DG[16,17], and those new neurons involved in the formation of trace memories [18]. These results implied the DG might participate in the neural circuit important for the acquisition of trace eyeblink CRs. However, there were only a few studies on whether the DG involved in the neural circuit for the consolidation of trace eyeblink CRs, and many questions remained as to the role that those neurons from DG played in this hippocampus-dependent task.

It was generally considered that the days when the asymptotic level of CRs was reached located in the behavioral stage of consolidation. By comparing the beginning time of single unit recording and asymptotic CRs, the behavioral stage in which the DG units were recorded was determined. In present study, all the DG units were recorded in the asymptotic days. The results revealed that the GCs in the DG of learner animals exhibited three heterogeneous activity patterns during the consolidation of trace eyeblink CRs such as increases in activities to the tone CS, trace interval and airpuff US, which indicated that the GCs in the DG were activated during this hippocampus-dependent task. Consequently, it was reasonable to assume that the DG might participate in the neural circuit important for the consolidation of trace eyeblink CRs.

In the brain, hippocampal neurons could use firing rate to encode information[19,20]. As such, place cells were hippocampal pyramidal cells that increased their firing rates in a particular portion of the environment, and they provided a coarse rate code for the characteristics animal's location. In present study, the GCs increased their activities during the different phases of the consolidation of trace eyeblink CRs, which indicated different GCs of the DG might form several subclasses, and encoded different information during the consolidation of trace eyeblink CRs. However, the detailed encoding mechanisms were still unclear.

Furthermore, it was reported that the CA1 hippocampal neurons showed significant increases in activity to the CS and US on the day of training when initial increase in CRs began to emerge, however, these learning-related increases in activity of CA1 single neuron diminished during the asymptotic days[7]. On the contrary, in present study, the increase in activity of granule cell was observed in the asymptotic period. An inverse phenomenon was discovered when comparing the activity characteristics of granule cells in the DG and pyramidal cells in the CA1 hippocampus during the consolidation of trace eyeblink CRs.

The inverse activity characteristics of DG and CA1 might be indirectly supported by a number of computational models of hippocampal functions. Several models suggested that the function of DG neurons might be to encode patterns of multiple sensory inputs during a learning episode and transmit this information to downstream areas of the hippocampus, such as CA3 and CA1[21]. Other models suggested that CA1 neurons might serve as comparator of inputs from entorhinal cortex and DG and CA3 areas, and this comparison might serve to distinguish familiar and novel patterns [22]. Our findings here, together with these models, might provide a meaningful interpretation of the results in present study.

Acknowledgements We thank the technical assistance of Mr. GAN Ping, Mr. XIAN Xiao-Dong and Mr. HUANG Yang-Fan of Chongqing University, who helped to design and build the airpuff regulator. The authors are also grateful for many useful suggestions of the anonymous reviewers.

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豚鼠齿状回颗粒细胞在追踪性眨眼条件反应巩固过程中的活动

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摘要 海马在追踪性眨眼条件反应的巩固过程中发挥重要作用，但解剖学上与其紧密联系的齿状回在此过程中的作用尚不清楚. 实验拟观察齿状回颗粒细胞在追踪性眨眼条件反应巩固过程中的放电活动，阐明齿状回在此海马依赖任务中所发挥的作用. 条件反射组动物 (n=8) 首先接受 200 ms 声音条件刺激，间隔 600 ms 后，再被给予 200 ms 吹气非条件刺激，多次重复配对，建立追踪性眨眼条件反应. 对照组动物 (n=8) 接受非配对出现的上述两种刺激. 采用在体单细胞外记录技术，研究习得条件反应豚鼠的齿状回颗粒细胞在条件反应巩固过程中的放电活动. 结果显示：a. 通过 14 天的训练，条件反射组动物均建立了追踪性眨眼条件反应，而非配对组动物则没有建立该条件反应；b. 齿状回颗粒细胞在追踪性眨眼条件反应的巩固过程中表现出不同的活动模式，如在声音条件刺激、间隔期或吹气非条件刺激出现后活动的增强. 这些结果提示：齿状回可能参与巩固追踪性眨眼条件反应所需的神经环路，其颗粒细胞在追踪性眨眼条件反应巩固过程中可能编码不同的信息.

关键词 齿状回，颗粒细胞，追踪性条件反射，眨眼，单电极记录，豚鼠
学科分类号 Q424，R338

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收稿日期：2006-05-09，接受日期：2006-06-30