Behavioural Brain Research 212 (2010) 35–40

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Neuronal firing activity of hippocampal pyramidal cells during an auditory discrimination task in conscious guinea pigs

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ARTICLE INFO

Article history:
Received 4 September 2009
Received in revised form 15 March 2010
Accepted 19 March 2010
Available online 25 March 2010

Keywords:
Guinea pig
Discrimination
Cognition
Complexity
Hippocampus
Pyramidal cell

ABSTRACT

This study sought to determine whether the dynamic feature of hippocampal neurons is related to the degree of complexity in complex auditory cognition. We examined the firing activity and complexity of hippocampal pyramidal cells on conscious guinea pigs during passive and active auditory discrimination tasks. The firing rate and the complexity in each sampled interspike interval (ISI) series were also computed. A larger value of complexity indicated a higher difficulty of the task or a less regularity of the discharge series. In spontaneous state, the complexity of the pyramidal cells was lower than that in low tone and low-high tone state. The complexity was increased in active discrimination state compared with passive state. The present results demonstrate that the difficulty in the auditory discriminatory task is marked by a progressive increase of complexity degree in the spike trains of pyramidal cells and cannot completely be described via mean firing rate. Moreover, the response of pyramidal cells to auditory information in different cognition states may be related to the nonlinear dynamics features, which can be quantitatively characterized by the degree of complexity. Taken together, we suggest that the degree of complexity provides an excellent experimental tool for the analysis of spikes in neural trains.

1. Introduction

The hippocampus plays an essential role in receiving and integrating information from multisystem to form senses [22,33]. In particular, the hippocampus has been implicated in a wide variety of learning and memory experimental paradigms and clinical conditions [26]. Processing of sensory input requires at least two stages, viz. vigilance stage and discrimination stage. The former determines whether a stimulus is present in the environment and the latter identifies the stimulus [1,29]. These processes need an associative learning to establish link between environmental stimulus and behavioral circumstances. The hippocampus plays a key role in discriminating auditory stimulus during the auditory discrimination, which is of great importance to prevent sensory inputs from overwhelming the brain's limited information discrimination capacity [1,22,32]. In experimental situations, neutral stimulus such as tone, light or smell signal are reinforced by reward or punished delivery, subsequently becoming predictors of the occurrence of the reward or potential threat through classical conditioning reflex. The acquisitions of auditory discrimination are impaired in humans and experimental animals with bilateral hippocampal lesions [1,11,12,17,21]. However, there is no difference between the subjects with temporal lobectomy and healthy controls with regard to simple two-tone discrimination learning [6,27]. In addition, some studies suggested that the impairment of rat hippocampus by scopolamine and methyl scopolamine was not associated with auditory negative patterning discrimination performance [30]. Accordingly, other researchers indicated that rats could combine information from auditory and visual cues to execute the correct place navigation, suggesting that the hippocampus processes the auditory information associated with the visual one [31]. Moreover, interneurons rather than pyramidal cells perform the two-tone discrimination [3,5]. Taken together, the results of detailed response of pyramidal cells to auditory stimuli may be debating. In an attempt to confirm the interpretation of results with relation to the brain function, it is important to elucidate the mechanism underlying auditory discrimination.

Measures of neuronal cell behavior are stochastic firing with some central tendency, such as mean firing rate appraised by statistical average. Moreover, burst and single-spike firing modes interweave to produce irregular ISI patterns. With the development of nonlinear theory, the complexity of neuronal activity has been

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doi:10.1016/j.bbr.2010.03.031

widely studied at different levels from a single cell to a large scale of neuronal ensembles as synonymous with nonlinearity or chaotic behavior [23,36]. In dopamine neuron, the complexity degree of action potential reduces with aging [7] and the response of the nociceptive discharge to analgesics recorded from lumbar dorsal horn may be related to nonlinear dynamics feature of neuronal activity complexity [37]. Although several studies have described the diverse features of electrophysiological characteristics of pyramidal cells, the complexity of neurons in discrimination task is poorly known.

In view of these observations, we hypothesize that the dynamics features of hippocampal neurons may be related to the degree of complexity in complex auditory cognition. We thus recorded the firing activity of hippocampal pyramidal cells and examined its complexity in conscious guinea pigs. The results obtained from these studies will improve the understanding of the role of hippocampus in processing and coding information in auditory discrimination cognition. This is important for further identifying the neural substrates for vocal communication in mammals and studying the development of speech and language in humans.

2. Materials and methods

2.1. Subjects

Twelve healthy male and female Dunkin–Hartley guinea pigs (3–4 weeks old), weighing about 150–250 g, were obtained from the Experimental Animal Center of Third Military Medical University, China. The animals were kept at 23–25 °C with a 12:12 light–dark cycle and allowed ad libitum access to food and water. The local Animal Experimentation Ethics Committee approved all surgical procedures performed in this study, which followed established principles of laboratory animal care (NIH publication no. 85-23, revised 1985). All efforts were made to minimize the number of animals used and suffering.

2.2. Auditory discrimination training

The guinea pigs were randomly assigned into active and passive groups. During the training sessions, the animals were placed in a cloth restraining hammock and then put in a sound-attenuating chamber. The ends of rubber tubes (1 cm diameter) were placed near each ear and served to deliver the auditory tones from the earphones. An airpuff tube was placed 1 cm away from the tested eyeball and served to deliver airpuff as unconditioned stimulus (US: 100 ms, 200 g/cm^2). After restraining, tones (85 dB and 100 ms duration, 2 ms rise/fall time) of 1 kHz (CS+, high tone) or 500 Hz (CS−, low tone) were delivered randomly at a ratio of approximately 1:6 and with a mean interval of 2 s [3]. The auditory conditioned stimulus (CS+) trials were conducted using a corneal airpuff US (paired) with free period. The auditory CS− trials were never reinforced with a US. The guinea pigs of active group received daily sessions (20 sessions total) consisting of 200 CS+_US trials. The animals of passive group were trained unpaired 200 CS+ or CS− tones and 200 US. The training before the surgery continued for one month until the guinea pigs in the active group learned eye blink when hearing the CS+ tone without the airpuff-stimulus (building the CS+ tone and eyeblink conditioning). The animals were selected when their correct-response rate reached more than 60 percent of correct-response rate [10,31,33].

2.3. Animal treatment and electrophysiological recording

The animals were anesthetized with pentobarbital (35 mg/kg, i.p.) after the training. A mini headstage was fixed on the head to keep the subject conscious in approximate physiological conditions without using muscle relaxant [9]. The dura mater over the hippocampus was kept intact, covered with a protective antibiotic cream, and given aseptic care daily. Finally, a tiny loop of thin thread was fixed on the left upper eyelid.

Approximately 7 days after the surgery, the heads were secured with blunt ear bars pressing on the headstage. The loop sutured into the tested eyelid was linked to the swivel arm of a spring-return potentiometer, which provided an output signal proportional to the movement of the eyelid. The animals were first given a 10-min acclimation session without auditory stimuli, and subsequently the extra cellular neuronal firing was recorded stereotaxically using a glass micropipette filled with 2 mol/L sodium acetate (10–20 mΩ). The microelectrode was manipulated to the CA1 (*P*: 3.0, *L*: 2.5, *H*: 5 mm) or CA3 (*P*: 4, *L*: 4.5, *H*: 5–6 mm) region with a hydraulic microdriver. The single neural activities of pyramidal cell were recorded when the animals were in the no-tone stimulus states including the single low tone state (CS− tone only) and double low-high tone states (CS+ and CS− tones) for discriminative eyeblink. We considered the firing with no-tone stimulus as a spontaneous state, the firing during CS− tone state as simple discrimination and the CS+–CS− tone discrimination as complex performation. The single-neuron analog signals were amplified (10,000 times) and the voltage changes from potentiometer and applied auditory stimuli maker were monitored by PowerLab/4sp workstation. An application software of LabChart 7.0 was employed to digitize all the data in the sampling frequency of 20 kHz and then converted the neuron signals into pulses using window discrimination for single unit isolation. The spike trains were recorded during 300–1000 s in a steady-state condition without any other external stimulus, which also referred to as spontaneous activity.

2.4. Analysis of pyramidal cell firing

Neuronal activity, CS+, CS− tone presentations and eyelid position were stored digitally on PowerLab system and converted to the computer with four-channel signals for off-line analysis. The recordings from active group were used for analysis only when the guinea pigs properly responded to the CS+ tone. All signals were converted to interspike interval (ISI) and then the firing rate and degree of complexity were analyzed with the software in Matlab programmed by our department. The neurons, which fired more than 1000 spikes in each state and throughout one entire session including spontaneous, single low tone and double-tone states, were selected for further analysis. The mean firing rate was calculated as the numbers of spikes within the time of discharge. Peri-stimulus time histogram (bin = 100 ms) was also analyzed with respect to the CS+ and CS− tone presentations using about 50 trial data. Only when the cells fire with statistically different activities in different states and the differential activities reflect neither stimulus-elicited nor eyeblink-related activities during a "no task condition", the firing is regarded as the related task [5,7,31,33]. To classify the rhythms in discharges and the characteristics of pyramidal cells, the histograms of interspike interval distribution and autocorrelation histogram were analyzed.

2.5. Arithmetic of ISI complexity

The complexity (c) is a useful quantity to characterize spatiotemporal patterns [25]. Lemple and Ziv consider for simplicity only strings which are composed of zeros and ones and the complexity of string can be calculated. The degree of complexity for periodic or regular sequence was taken as 0 and for the random sequence as 1. The calculation of c for pyramidal cell spike proceeds as follows: (1) the ISI (the time between two adjacent spikes) series have been reconstructed randomly as sequences $X(i) = [X_1, X_2, \ldots, X_n]$ (n as the number of the ISI and $n > 1000$).The program produced random numbers $S_i \in [0,1]$ and we took $S_i = 0$ if $X_i \leq$ mean ISI and $S_i = 1$ otherwise. (2) S_r was newly inserted into the string ($S_1, S_2, \ldots, S_n$). The string up to S_r was denoted by $S = S_1, S_2, \ldots, S_r$, where the dot indicated that S_r is newly inserted. (3) One takes $Q = S_{r+1}$ (Q can be simply obtained by copying a element of S), and asks whether this term is contained in the vocabulary of the string S until Q becomes so large that it can no longer be obtained by copying a term from S. (4) The complexity of string $X(i)$ can simply be mathematical formula trending to the same value: $\lim_{n\to\infty} c(n) = n/\log_2^n$, where n is the length of given string [20,25].

2.6. Histology

At the end of experiments, the recording site was labeled by an injection of pontamine sky blue dye infusing the electrode with a 20 μA current for 10 min. The brains were perfused, fixed with 10% formaldehyde for 2 days, and then sliced in 40 μm sections and stained for the precise localization of micropipette tips.

2.7. Data analysis and statistics

Values are expressed as mean ± SEM and analyzed by variance ANOVA, followed by Newman–Keuls post hoc test, if appropriate. A value of $P < 0.05$ was considered statistically significant.

3. Results

All of the animals used in this study had electrode tips placed directly in the pyramidal cell body layer of the dorsal hippocampus. Data from two animals were excluded in analysis because the electrode tips were inserted into the thalamus.

3.1. General properties of recorded cell

A total of 88 pyramidal cells were recorded in the dorsal hippocampus. Pyramidal cells were distinguished from interneurons and background by measuring the action-potential width and firing rate using the criteria as previously described [8,9,18,19,26,34]. The cells fired in simple spike mode and multiple-spike burst with mean firing rate r (Hertz) and spike widths w (in microseconds) so