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Research report

Responses of rat medial prefrontal cortical neurons to Pavlovian conditioned stimuli and to delivery of appetitive reward

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HIGHLIGHTS

- Medial prefrontal cortical neurons were extracellularly recorded by means of tetrode electrodes.
- Neuronal activity changed during the presentation of CSs, approach behavior or consummation.
- Neurons discriminated among predicted rewards during approach behavior.
- Neurons discriminated also among rewards during consummation.
- Neuronal responses differed among the CSs and trials with or without consummation.

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ABSTRACT

In the present experiments, medial prefrontal cortical (mPFC) neurons were extracellularly recorded by means of tetrode electrodes to examine their possible role in the prediction of appetitive reward. Two different cue tones (CS) and sucrose solution or water reward (US) were associated in a Pavlovian conditioning paradigm. In order to test behavioral correlate of the CS–US association, the head acceleration before the first lick of licking cluster was measured. Neuronal activity changes in the mPFC were analyzed (i) during the CS presentations; (ii) before the first lick of licking clusters; (iii) during consummation; and (iv) we also examined whether consummation was represented in neurons responding to the CSs. There was a difference between the head accelerations to the different USs during early or late occurring first approaches, but there was no such a difference during intercluster approaches. A significant proportion of neurons changed their firing rate during the CS presentation, before the first lick of licking cluster or during licking of the reward. Both, excitatory and inhibitory responses were observed. A subpopulation of neurons responding to the CSs also responded during reward consumption. Differential population activities of excitatory neurons were recorded in response to the different CSs, CS evoked approach behaviors and consumption of different rewards. Neuronal responses also discriminated among the CSs and trials with or without consummation. These results provided evidence for the involvement of mPFC neurons in the prediction, representation and organization of conditioned behavioral actions, such as approaches to rewards and consummation.

1. Introduction

Recent theories of motivation and reinforcement learning suggest that reward prediction is important for setting the adequate behavioral actions [1–3]. Rewards are environmental incentive stimuli that possess biological value and take on motivational properties giving force, direction and goal for the ongoing behavior [4–6]. By means of the incentive motivation non-specific responses, such as enhanced arousal, orientation and approach are induced. These nonspecific responses direct the organism toward the goal and result in consummatory behavior [4,6,7]. Rewards are the reinforcers of the association between an action and a particular behavioral outcome or between neutral stimuli and reward, as it

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can be observed in the instrumental or in the Pavlovian situation, respectively. In Pavlovian conditioning the learned conditioned stimulus (CS) can be associated with a specific sensory feature, the motivational properties of reward, and also with unconditioned responses. Thus, the CS may act as a substitute for the predicted reward [6].

Neuronal activity changes in relation to the CS and the actual reward have been shown in several limbic structures during Pavlovian task. Dopamine neurons in the ventral tegmental area exhibit responses during CS in relation to the expected value [8] and during reward presentation, coding reward prediction error in monkeys [3,9,10] and in rodents [1,11,12]. Moreover, the stimulus-reward contingency, the predicted risk of failed reward or the unpredictability of reward delivery was already detected in the neuronal responses of the monkey basolateral and central amygdala [13–15]. In the rat, basolateral amygdala neurons responded to CSs representing sensory [16] and affective features of reward [17], and also to the unexpected omission or delivery of reward [18]. Neural correlates to expected reward have also been detected in the rat orbitofrontal cortex [19], cingulate cortex [20], and in the striatum [21] as well. There are relatively few single-neuron studies, however, in the medial prefrontal cortex (mPFC) of non primate mammals related to Pavlovian conditioning using food reward [22].

The medial prefrontal cortex of the rat (mPFC) receives strong afferentation from the dopaminergic neurons of the ventral tegmental area [23], and it is connected with all limbic structures known to be involved in processes related to the prediction or the availability of the actual reward [24–26].

Recent studies focused on the processes of decision making between a specific behavior over other behaviors due to expectation of the reward, and on the neuronal representation of response-reward contingency in the mPFC neurons [27,28]. The aim of the present study was to examine whether the mPFC contributes to representation of the predicted and actual reward in Pavlovian conditioning. Changes in neuronal activity were observed during the presentation of CS associated with appetitive reward in Pavlovian conditioning [22]. Moreover, in an instrumental situation, inactivation of the mPFC decreased Pavlovian instrumental transfer [29] and neurons amplified their activity changes to instrumental response when reward associated CS was presented in advance [30]. Changes of the neuronal activity in relation to consumption of the appetitive reward have been detected in our previous investigations [31]. In the recent study of Horst and Laubach, a clear evidence was found for that the mPFC monitors reward delivery and controls consumption, the licking behavior of fluid reward [32].

Therefore, to study the role of mPFC in reward prediction as a result of stimulus-reward association, we examined whether different rewarding USs are represented in the neuronal activity of mPFC during (i) the presence of CSs; (ii) approach behavior as a conditioned response; (iii) US elicited licking behavior (consummation); and finally, we wanted to elucidate whether consummation and CRs are represented in the activity of neurons responding to CSs.

To answer the above questions, we designed an experimental protocol complying with the requirements of the electrophysiological examinations. Activity changes of mPFC neurons were investigated in freely moving rats during appetitive Pavlovian trace-conditioning. Two different tones were associated with two different rewarding USs, water and 5% sugar solution, respectively. Approaches to the drinking tube at the beginning of US delivery were treated as CRs related CSs. Data were recorded only after acquisition of learning and the US was delivered during all trials to avoid extinction.

2. Materials and methods

2.1. Animals

Three 4 months old male Wistar rats were used in the experiments. Animals were handled daily and were caged individually with 12–12-h light/dark cycle (light on at 6 a.m.) in a temperature and humidity controlled ($24 \pm 2\,^{\circ}C$) vivarium. Standard laboratory food pellets (CRLT/N, Charles River Laboratories, Budapest, Hungary) and tap water were available ad lib. To motivate the water intake during the behavioral experiments, water was presented only in the operant cage and it was supplemented for 20 min in the home cage to provide constant thirst motivation between two sessions. Body weight was measured regularly and it was maintained at about 85–90% of their initial body weight. Rats were cared for in accordance with institutional, national and international standards (BA02/2000-8/2012; Law XXVIII, 1998; 40/2013 Government Decree, 2013, Hungary; European Community Council Directive 86/609/EEC, 1986; 2006; 2010). All efforts were made to minimize the number of animals and to reduce pain and suffering.

2.2. Electrode implantation

Prior to behavioral tasks rats were implanted with printed circuit board (PCB) based microdrives loaded with eight tetrodes [31,33]. For surgery, the rats were anesthetized with sodium-pentobarbital solution (60 mg/kg Nembutal, Phylaxia-Sanofi, Hungary) followed by atropine (2 mg/kg EGIS, Hungary). The skin was removed from the upper surface of the skull and anchor screws were inserted into the bone. One of them was used as ground and another one as reference electrode. Burr holes were made and the PCB-microdrives were positioned by means of a micromanipulator above the mPFC (AP: 2.7 mm from bregma and ML: 0.8 mm, according to the rat brain atlas of Paxinos and Watson [34]). In this position, the thick silica tubes serving as guiding tubes for the tetrodes, reached the brain surface above the medial prefrontal cortex. All tetrodes were brought approximately to the upper border of the prelimbic area.

2.3. Behavioral procedure and equipment

Recording of behavioral actions and multiple unit activity has been started after a recovery period of 2 weeks. The experiments were performed daily in a sound attenuated 40 cm × 40 cm operant box during the light period. A 1 cm broad, 3 cm high opening was made in the wall of the box. Two movable drinking bottles were used for delivery of sugar solution or water. During reward delivery, one of the drinking bottles was moved to the opening by a computer controlled solenoid allowing the rat to reach the spout.

Each time animals were placed into the experimental box after 23 h water deprivation. Rats were trained to learn access to (5%) sucrose solution or water signaled by differential tones. A 16 kHz tone was associated with the sugar solution delivery (CS1–US1) and an 8 kHz tone with the water delivery (CS2–US2). The fixed inter-stimulus interval lasted for 24 s. The 2 s presentation of the CS was terminated one second prior to US onset according to the trace condition. The liquid reward was available for 12 s. Trials of CS1–US1 or CS2–US2 were randomized and both reward types were presented equally within the 100 associations during the 40 min long sessions. If rats did not drink anything for at least 5 min the recording of data was stopped. Rats were trained for one week and data of 24 experimental sessions were analyzed.

Behavioral testing, presentation of cue tones and delivery of reward were controlled by the Labcommander software (Noted Bt; Hungary). Cue tones were generated by playing of audio files (.wav) using the sound card of the computer. Licking was detected