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PHENCYCLIDINE AFFECTS FIRING ACTIVITY OF VENTRAL TEGMENTAL AREA NEURONS THAT ARE RELATED TO REWARD AND SOCIAL BEHAVIORS IN RATS

T. KATAYAMA,[a] M. OKAMOTO,[a] Y. SUZUKI,[a] K.-Y. HOSHINO[b] AND E. JODO[a]*

[a] *Department of Neurophysiology, School of Medicine, Fukushima Medical University, Fukushima City, Fukushima 960-1295, Japan*

[b] *Department of Neuropsychiatry, School of Medicine, Fukushima Medical University, Fukushima City, Fukushima 960-1295, Japan*

Abstract—Patients with schizophrenia exhibit deficits in motivation and affect, which suggests an impairment in the reward system. The psychotomimetic drug, phencyclidine (PCP), also induces schizophrenia-like negative symptoms, such as reduced motivation, blunted affect, and social withdrawal in both humans and animals. Previous studies have indicated that the dopaminergic neurons in the ventral tegmental area (VTA) play a pivotal role in the development of reward-associated learning and motivation. However, how PCP affects the activity of VTA neurons during performance of a reward-related task and social interaction with others in unanesthetized animals remains unclear. Here, we recorded the unit activity of VTA neurons in freely moving rats before and after systemic administration of PCP in a classical conditioning paradigm, and during social interaction with an unfamiliar partner. In the classical conditioning task, two different tones were sequentially presented, one of which accompanied electrical stimulation of the medial forebrain bundle as an unconditioned stimulus. After identifying the response properties of recorded neurons in the classical conditioning task and social interaction, animals received an intraperitoneal injection of PCP. Our study demonstrated that most VTA neurons responsive to reward-associated stimuli were also activated during social interaction. Such activation of neurons was considerably suppressed by systemic administration of PCP, thus, PCP may affect the firing activity of VTA neurons that are involved in motivation, learning, and social interaction. Disruption of the response of VTA neurons to reward stimuli and socially interactive situations may be involved in PCP-induced impairments similar to the negative symptoms of schizophrenia.

Key words: schizophrenia, phencyclidine, ventral tegmental area, dopamine, reward, social interaction.

INTRODUCTION

Phencyclidine (PCP) is a psychotomimetic drug that induces schizophrenia-like symptoms in healthy individuals (Javitt and Zukin, 1991). PCP-induced psychosis is associated with both positive and negative schizophrenia-like symptoms (Javitt and Zukin, 1991), and administration of PCP to stabilized schizophrenic patients leads to exacerbation of preexisting symptoms (Luby et al., 1959; Domino and Luby, 1981). In animals, PCP also induces cognitive and behavioral abnormalities that partially correspond to the positive and negative symptoms of schizophrenia (Castellani and Adams, 1981; Jentsch and Roth, 1999). Therefore, the neural mechanisms by which PCP modulates behavior are of great interest, and PCP-treated animals are now considered to be a reliable pharmacological model of schizophrenia.

Dysfunction of the dopaminergic system has long been suggested to play a major role in the pathogenesis of schizophrenic psychosis (Carlsson, 1988; Laruelle et al., 1996; Breier et al., 1997). Large amounts of data have been obtained suggesting that the behavioral effects of PCP are also mediated via interaction with central dopaminergic neurons (Domino and Luby, 1981). The ventral tegmental area (VTA) is a major source of forebrain dopamine (Schultz, 2002), and plays a pivotal role in motivation and learning (Schultz et al., 1997). Because the VTA is a key structure for reward processing (Schultz, 2000), dysfunction of VTA neurons may be an essential factor for the development of social withdrawal, which is a typical negative symptom, in patients with schizophrenia. PCP-treated animals also exhibit behavioral abnormalities in socially interactive situations (Sams-Dodd, 1996). Although several studies have examined the effects of PCP on the basal activity of VTA neurons in anesthetized rats (Freeman and Bunney, 1984; French, 1986) and *in vitro* (Trulson and Arasteh, 1987), how VTA neurons behave in a socially interactive situation, and how PCP affects discharge

*Corresponding author. Address: Department of Neurophysiology, School of Medicine, Fukushima Medical University, One Hikarigaoka, Fukushima City, Fukushima Prefecture 960-1295, Japan. Tel: +81-24-547-1135; fax: +81-24-548-2571.

E-mail addresses: katayama@fmu.ac.jp (T. Katayama), mokamoto@fmu.ac.jp (M. Okamoto), ys-mayu@fmu.ac.jp (Y. Suzuki), kenyo-h@fmu.ac.jp (K.-Y. Hoshino), jodo1019@fmu.ac.jp (E. Jodo).

Abbreviations: ANOVA, analysis of variance; AP, action potentials; BNST, bed nucleus of the stria terminalis; CS, conditioned stimuli; ICS, intracranial electrical stimulation; MANOVA, multivariate analysis of variance; mPFC, medial prefrontal cortex; NMDA, *N*-methyl-D-aspartate; PBS, phosphate-buffered saline; PCP, phencyclidine; PSTH, peristimulus time histograms; P-task, passive reward task; R/B, response–baseline; SD, standard deviation; SEM, standard error of the mean; SI test, social interaction test; VTA, ventral tegmental area.

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activity of VTA neurons during social interactions, have not been elucidated.

We report here for the first time how VTA neurons that are active during a reward task respond to a socially interactive situation, and how those neurons are affected by systemically administered PCP in freely moving rats.

EXPERIMENTAL PROCEDURES

We used adult male Sprague–Dawley rats (300–400 g, $n = 27$), and housed them in the Fukushima Medical University Animal Facility under conditions of constant temperature and humidity, with food and water available *ad libitum*. The treatment of these animals was in accordance with the National Institutes of Health guidelines for animal research in the USA, with all efforts made to minimize both animal suffering and the number of animals used. All procedures adopted in this study were approved by, and conducted under the control of, the Fukushima Medical University Animal Care and Use Committee.

Preparation of freely moving rats and single-unit recording

Each rat was anesthetized with pentobarbital (50 mg/kg, i.p.) for implantation of stimulating and recording electrodes for chronic experimentation. A parallel bipolar stimulating electrode made of insulated stainless-steel wires glued together with uninsulated tips was inserted stereotaxically into the left medial forebrain bundle, 2.3 mm posterior to the bregma, 1.64 mm lateral to the midline, and 7.95 mm below the cortical surface, according to the atlas of Paxinos and Watson (1997). A commercial micromanipulator (250 μm/turn) with a stainless-steel microelectrode (Unique Medical, Tokyo, Japan) was used to record single-unit activity in the VTA. The recording electrode was lowered unilaterally into the right VTA (action potentials (AP): −5.3 mm, L: 0.7 mm, DV: 7.7 mm), and the micromanipulator was then fixed onto the cranium using dental cement. As a reference, a miniature stainless-steel bolt was screwed to the skull near the point of insertion of the needle electrode to contact the dura mater. All lead wires from the electrodes were soldered onto a miniature connector, which was anchored to the cranium using dental cement. Rats received antibiotics after surgery. Recording began after at least 14 days of recovery from surgery.

Unit recording

The signal from the recording electrode was fed into a bioelectric amplifier (gain = ×54,000; Nihon Kohden, Tokyo, Japan) with a bandpass filter of 500–10,000 Hz via a miniature preamplifier (gain = ×1, JB220 J; Nihon Kohden, Tokyo, Japan) directly attached to the socket on the animal's head. All signals were digitized and stored on a personal computer hard disk for off-line analysis. Unit AP were separated from background noise using laboratory interface and spike sorting software (Microced, Spike 2 ver. 5, Cambridge Electronic Design, Cambridge, UK).

Passive reward task (P-task)

Conditioning sessions. Conditioning sessions were started at least 2 weeks after surgery, and were performed in a dimly lit, sound-attenuated room. Two auditory stimuli (1000 and 2000 Hz pure tones, 70 dB SPL) were sequentially delivered as conditioned stimuli (CS) into an electrically shielded transparent plastic box (W 200 mm, L 300 mm, H 300 mm) containing the rats for 1.5 s through a loudspeaker, at variable intervals of 12–15 s. One of the two tones was followed by intracranial electrical stimulation (ICS; 100–190 mA, 0.4 s, 100 Hz) of the medial forebrain bundle. The tone assigned to the ICS-paired tone (target) was distributed equally across animals. The target tone (CS+) was presented with a probability of 30% in all trials, and the total number of tone presentations (trials) was 400 in one conditioning session. A schematic diagram of the P-task is depicted in Fig. 1B.

Recording sessions. Recording sessions were performed the day after conditioning sessions. After the first recording session in the P-task, animals received an intraperitoneal injection of a subanesthetic dose of 10 mg/kg PCP or 1 ml/kg physiological saline. The second and third sessions were started at 15 and 180 min, respectively, after injection of PCP or saline. The total number of tone presentations (trials) was 200 in one recording session. A schematic diagram of the experimental protocol is depicted in Fig. 1A.

Data analysis of the P-task

To analyze the firing activity of recorded neurons for conditioned auditory stimuli, we generated cumulative peristimulus time histograms (PSTHs, 10-ms bin width) for each neuron sampled from 2 s before to 3 s after each tone presentation. To quantitatively examine the effects of systemic PCP on responsiveness of VTA neurons to the CS, we calculated the response–baseline (R/B) ratio during a period of 200 ms after the start of CS presentation. The R/B ratio was obtained as the spike count ratio in each bin to the average count of spikes during the baseline period, which was defined as the 2-s period preceding the tone presentation. The spontaneous firing rate of neurons was obtained as an averaged firing rate for a 30-s period just before the start of the P-task. Multivariate analyses of variance (MANOVA) were performed on the R/B ratio of the firing rate for the factors of group (PCP, saline) and time.

Social interaction test (SI test)

The general design of the SI test was adapted from our previous studies (Katayama et al., 2009; Jodo et al., 2010). The test was performed in an open arena ($56 \times 56 \times 40$ cm^3), on the floor of which intersecting lines were drawn at even intervals (4 cm), and a digital camera that was placed above the arena was used to record the behavior of the rats. After acclimation to the novel setting for at least 1 h, the first recording session was started. First, the baseline firing activity of VTA neurons was recorded for 10 min, and then a weight-matched unfamiliar male rat was introduced into the arena. This test lasted 10 min. The introduced (partner) rat was not paired again with the recorded rat in this experiment. A schematic diagram of the experimental protocol is depicted in Fig. 1A.

Data analysis of the SI test

Behavioral data were analyzed off-line in the form of digitized motion images during social interaction. Every 5 s, we measured the time during which the recorded rat had been actively engaged in socially interactive behaviors (contact/non-contact sniffing, grooming, following, crawling over/under, or boxing/wrestling) with the partner rat. Contact sniffing was counted when the recorded rat sniffed at the partner rat with direct contact, and non-contact sniffing occurred within a distance of 4 cm. Grooming was counted when the recorded rat directly licked its partner. Following was counted when the recorded rat chased the partner without contact. Crawling and boxing/wrestling were counted when the recorded rat performed these behaviors against the partner. Because some