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# Prolonged deficits of associative motor learning in cynomolgus monkeys after long-term administration of phencyclidine

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ABSTRACT

Phencyclidine (PCP) is a potent drug of abuse that induces sustained schizophrenia-like symptoms in humans by blocking neurotransmission at *N*-methyl-D-aspartate (NMDA)-type glutamate receptors. Alterations in NMDA receptor function have been linked to numerous behavioral deficits and cognitive dysfunction. Classical eye-blink conditioning (EBC), including delay (dEBC) and trace (tEBC) paradigms, provides an effective means to study the neurobiology of associative motor learning in rodents, mammals and primates. To assess whether administration of low-dosage PCP for extended periods has prolonged effect to alter associative motor learning, in this study 19 adult cynomolgus monkeys were administered PCP (0.3 mg/kg, intramuscularly) or saline twice a day for 14 days. Twelve–fifteen months after PCP or saline injection, monkeys received dEBC, tEBC, or pseudo-paired training for 6 or 12 successive daily sessions, respectively. The results of this study show that percentage of conditioned response (CR) in dEBC increased as a function of training sessions in both PCP-treated and control monkeys and there was no significant CR% difference between the two groups. However, the CR timing in dEBC of PCP-treated monkeys was significantly impaired, as manifested by shorter CR peak latencies than those of the control group. PCP-treated animals showed significantly lower percentage of CR in tEBC compared to controls. PCP-treated animals were also more sensitive to outside stimuli in tEBC because the UR peak latency of PCP-treated group was significantly lower than the control group. These results indicated that cynomolgus monkeys manifested prolonged deficits in associative motor learning after long-term administration of phencyclidine.

## 1. Introduction

Phencyclidine (PCP) is a psychotomimetic drug and a noncompetitive antagonist of the *N*-methyl-D-aspartate (NMDA) glutamate receptor. PCP-induced psychosis is associated with both positive and negative schizophrenia-like symptoms in humans [1]. Repeated administration can result in a long-lasting syndrome marked by neuropsychological deficits, social withdrawal, and affective blunting as well as hallucinations, paranoia, and delusions [2,3]. PCP also induces cognitive and behavioral dysfunctions that partially correspond to the positive and negative symptoms of schizophrenia [4–6]. Abnormalities in working memory, behavioral inhibition, and social interactions have been observed in schizophrenic individuals and in PCP-abusing humans [7–9]. Because the long-term abuse of PCP in humans may represent a pharmacological model of learning and memory deficits that are associated with schizophrenia, we explored the prolonged associative learning effects of long-term exposure to PCP in the cynomolgus monkey (*Macaca fascicularis*).

Classical eye-blink conditioning (EBC) has been extensively used to study the neurobiology of associative motor learning in rodents, mammals and primates [10,11]. EBC involves paired presentations of a tone or light as a conditioned stimulus (CS) and a periorbital shock or air puff as an unconditioned stimulus (US). Initially, the CS produces no obvious eye-blink responses while the US elicits reliable eye-blink responses before learning, named the unconditioned response (UR). After repeated pairings of the CS and US, the CS comes to elicit an eye-blink called the conditioned response (CR). The two widely employed paradigms of EBC are delay EBC (dEBC) and trace EBC (tEBC). In the dEBC, CS is presented before the US, and the two stimuli are co-terminated. In tEBC, CS and US are presented separately in time so that a complete stimulus-free period exists between the two stimuli. EBC has been studied for more than half a century in humans [12–14] as well as

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in animals [11,15,16]. These studies led to a consensus that dEBC is dependent upon the cerebellum-brainstem circuits and that tEBC involves the hippocampus, prefrontal cortex and other brain areas in addition to cerebellum-brainstem circuits. While dEBC provides a well-validated method to investigate cerebellar timing function, tEBC seems to be a sensitive task related to functions of the prefrontal cortex and hippocampus [17,18]. As an ideal model for associative motor learning studies, changes of EBC in schizophrenia has received increasing attention in recent years [19–21].

Repeated exposure to PCP reportedly produces a sustained decrease in dopamine turnover within the prefrontal cortex, which is accompanied by a deficit in working memory and prefrontal cortex-dependent tasks in both rats and non-human primates [22–24]. In this study, we treated cynomolgus monkeys with PCP (0.3 mg/kg twice a day, intramuscularly) for 14 days, and 12–15 months later, we estimated the effect of PCP on establishment of both dEBC and tEBC. The goals of this study were (1) to assess whether administration of low-dosage PCP for extended periods has prolonged effect to alter associative learning ability in cynomolgus monkeys, and (2) to explore whether, in addition to damage to the frontal lobe and prefrontal cortex, extended PCP administration leads to impaired cerebellar function, which is involved in behavioral timing.

## 2. Materials and methods

### 2.1. Subjects

Nineteen healthy adult cynomolgus monkeys (9 females, 10 males) from the breeding colonies at the Hainan Jingang biological technology Co., Ltd. were served as subjects. The monkeys weighed between 3.5 and 6.5 kg at the beginning of behavioral tests, had no obvious eye diseases, and were sensitive to sounds stimuli. The monkeys were individually housed under standard conditions (a 12-h light/dark cycle with light on from 07:00 to 19:00, humidity 60%, 21 ± 2 °C, 3 times/day deliveries of food and 1 time/day delivery of fruit, water available ad libitum). The experimental procedures were approved by the Animal Care Committee of the Third Military Medical University and were performed in accordance with the principles outlined in the NIH Guide for the Care and Use of Laboratory Animals.

### 2.2. Primate chair

A special primate chair was designed according to the monkey's physical characteristics to ensure that the monkey can sit in the chair comfortably with its limbs easy to be fixed. The design of the chair prevented monkeys from touching their heads with their hands or feet. Once a monkey was secured in the chair, the circular bayonet on the top section of the chair can limit the up and down movement of its head and body, the head could only rotate around.

### 2.3. Eye-blink Detection Goggles

To detect monkeys' eye-blink behavior, a specially designed pair of goggles was constructed and worn by the monkey during behavior training. Eye-blinks were measured with an infrared sensor consisting of an infrared emitter (FBCB30, HengSheng, Shenzhen, China) and an infrared detector (TBBB30, HengSheng, Shenzhen, China) encased side by side and aligned with converging optical axes. The converging optical axes were aligned for maximum sensitivity to detect eye-blinks at a distance of 0.5-cm from the infrared sensor surface to the pupil surface. The infrared sensor and a plastic pipe with 0.2-cm diameter were established and secured together with dental acrylic on the goggle's right lens. The goggles were held in place by a rubber strap that attached to the lateral edges of the goggles and around the back of the subject's head (under the ears). The rubber strap looseness was adjusted to ensure that animals worn goggles comfortably and firmly (Fig. 1).

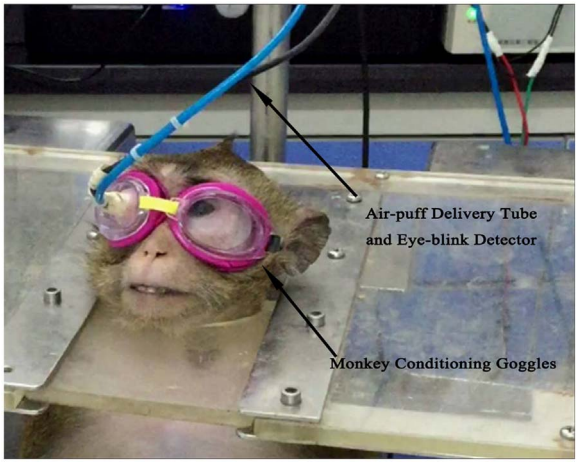


**Fig. 1.** Monkey conditioning goggles and primate chair. The goggles include the eye-blink detector and air-puff delivery pipe. A monkey wearing the conditioning goggles was sat in the primate chair with its legs and arms fixed. The right eye is monitored for eye-blinks, and the left eye is unobstructed.

### 2.4. Training and recording system

During behavioral training, the CS was a 1-kHz, 80-dB pure tone. All sound stimuli were delivered by two speakers placed 60 cm to the left and right ears of the animals, respectively. A sound-level meter (type 2240, Brüel & Kjær) was used to calibrate the intensity of the CS tone. The US was a 100-ms, 5-psi (measured at the tip of a plastic pipe attached to goggles) source pressure air-puff delivered to the right cornea through a plastic pipe. Presentations of the CS and US were controlled by a self-made computer system. All signals including markers of the applied stimuli and signals from the infrared sensor were digitized by a data-acquisition system (RM6240BDJ, Cheng Yi, Chengdu, China) and were acquired and stored by the system software (v. 4.7). Data analysis was carried out on a dedicated Windows PC.

### 2.5. Experimental procedures

#### 2.5.1. PCP or Saline treatment

A total of 19 adult cynomolgus monkeys were included in the experiment, including dEBC paired learning (n = 6); tEBC paired learning (n = 7); and pseudo-paired training for both dEBC and tEBC (n = 6). Monkeys were treated with daily intramuscularly injection of PCP (Chemsky [Shanghai] international Co., LTD.) at the dosage of 0.3 mg/kg (for PCP-treated group), or equal volume of saline (for control group), twice a day for 14 days [22]. Twelve–Fifteen months after injection of the PCP or Saline, monkeys in different groups received dEBC, tEBC, or pseudo-paired behavioral training, respectively.

#### 2.5.2. Habituation

All subjects were first habituated to sitting in the primate chair and wearing eye-blink detector goggles for 2 h each day for 6 days. No stimulation was applied to the monkeys during the habituation period.

#### 2.5.3. Behavioral training

Six monkeys (PCP-treated: n = 3; control: n = 3) included in dEBC paired learning underwent delay eyeb-link conditioned training for 6 successive daily sessions after habituation; 7 monkeys (PCP-treated: n = 3; control: n = 4) included in tEBC paired learning underwent trace eye-blink conditioned training for 12 successive daily sessions. In addition, in order to control the possible non-associative learning, 6 monkeys (PCP-treated: n = 3; control: n = 3) were included in pseudo-