NOTE *Physiology*

Effect of Electric Field in Conditioned Aversion Response

Shinji HARAKAWA[1,2], Takaki NEDACHI[2], Takuya HORI[2], Kunihito TAKAHASHI[2], Koji TOCHIO[2] and Noboru INOUE[1]

[1]*National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080–8555 and* [2]*Hakuju Institute for Health Science, 1–37–5 Tomigaya Shibuya-ku, Tokyo 151–0063, Japan*

(Received 26 September 2007/Accepted 1 February 2008)

ABSTRACT. The aim of the present study was to estimate whether rat sense exogenous electric field (EF) including one used in our previous studies. Employing a conditioned place aversion response paradigm based on an aversive behavior against light environment, alteration in both voluntary behavior of Wistar rat to a 50 Hz sinusoidal EF was examined. Following conditioning without EF, the times spent in white place in rats was significantly shortened ($P<0.05$). While, such changes were not shown in rats conditioned with EF. Thus, it was considered that the aversion response to light environment was interfered by exposure to EF. An interference in recognition of brightness via EF induced effect to visual system or in learning system via direct effect to central nerve system was considerable as a factor for EF-induced effect. In addition, it was remained that rat possibly sense exposure to EF as preferable. In order to confirm which factor functioned, further studies are needed.

KEY WORDS: conditioned aversion response, electric fields, rat.

J. Vet. Med. Sci. 70(6): 611–613, 2008

It was previously reported that increment of plasma ACTH due to stress was down regulated by 50 Hz EF in rat or other species, and then hypothesized that exposure to EF has impact in stress response [1–4]. The mechanism(s) of the response to EF have been suggested that either the EF-induced electric current or the perception of the EF through the skin surface act as a trigger on cellular, hormonal or behavioral responses [3, 5–8]. It is essential to determine whether rat did percept the EF used in previous studies, even though it is easily expectable that strong intensity of EF is sensed by animals [9–11]. In order to clarify this question, the conditioned place aversion response paradigm was employed in the current study.

All experiments described here were conducted in accordance with the guiding principles for the care and use of research animals promulgated by Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan. Twenty two, six weeks old, Wistar rat weighing 160–220 g (from Charles River Japan) were housed in a temperature (21–27°C) and humidity (35–75%) controlled room. They were maintained on a 12 hr light/dark cycle (7:00 light on, 19:00 light off) with laboratory rat chow and water available *ad libitum*.

CPP apparatus was consisted of a shuttle box (300 × 600 × 300 mm: W × L × H) made of acrylic resin (Fig. 1a). Shuttle box was composed of 2 compartments of same size, which were divided with partition (Natsume Seisakusho, Tokyo, Japan). One compartment was colored white and the other was black [12].

The exposure system was composed of three major parts, a high voltage transformer, a constant voltage unit, and upper and lower electrode (Fig. 1a) [4]. The system was designed for a rat or a smaller animal. The intensity of EF, which was generated in shuttle box placed on lower electrode, was estimated at selected 50 points on floor of CPP shuttle box by electro-optic voltage sensor (Sumitomo Electric Industries, Co., Ltd., Osaka, Japan) (Fig. 1b).

When electric signal of 50 Hz 7,000 V was loaded to the upper electrode, EF intensity within shuttle box placed on lower electrode was approximately 16,000 V/m (rms), which was almost equal at the same point where the shuttle box was removed. Figure 1c shows the EF distribution.

Rats were divided into two groups according to body weight: EF group (n=11); sham group (n=11). To check the frequency of time spent, rats were confined within the shuttle box removed the partition for 15 min per day for a period of 3 days. Place aversion was evaluated in each rat as a time spent in white compartment and such time was defined by gross observation as the time from when the entire body of the rat was in the side of white compartment until completely out, with exception to the tail.

During the 6-day period of place aversion conditioning, rats were firstly confined daily to a white compartment separated with partition under EF for 30 min and then placed to in black one without EF for 30 min. Rats of sham group were similarly handled but in the absence of EF. Following conditioning period, the times spent in white compartment in non-separated shuttle box were measured immediately on the next day. Rats were placed in the center of shuttle box without partition in the absence of EF for a period of 900 seconds. The extent of aversion by rat was evaluated as time spent in white compartment by gross-observation, and animal behavior was concomitantly recorded by a video camera. Shuttle box was used once, thereafter washed up for the next session. All procedures were conducted from 10:00 to

* CORRESPONDENCE TO: HARAKAWA, S., National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080–8555, Japan.
e-mail: shinji@hakuju.co.jp

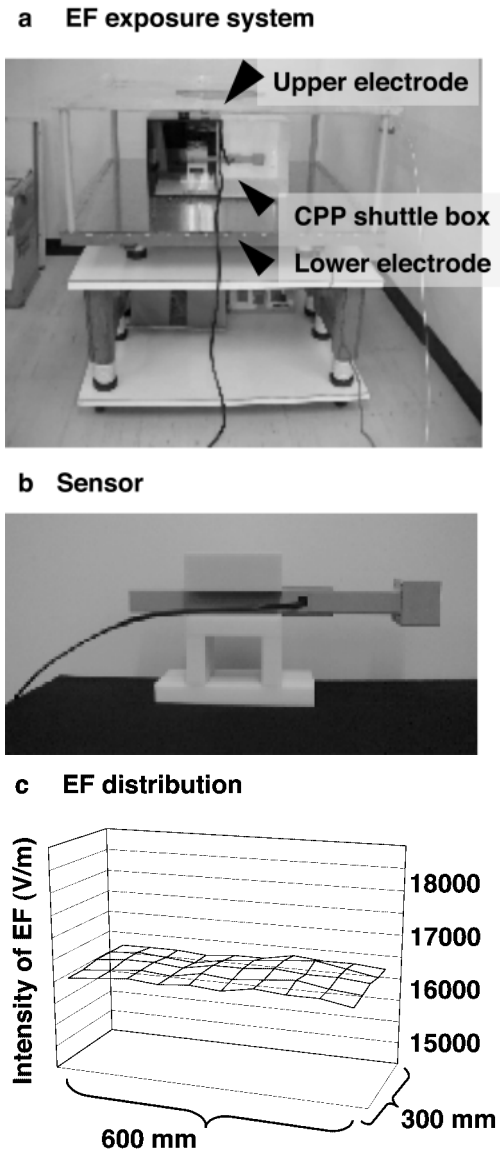

Fig. 1. Electric field (EF) exposure system (a). The shuttle box consisted of white and black color compartment were placed on the center of lower electrode of EF exposure system. Distribution in intensity of electric field (EF) on the bottom of shuttle box placed on center (c).

14:00 to avoid influence due to circadian rhythm. Each rat was conditioned and measured at an approximately same time.

The value of result was expressed as mean ± SD. The statistical significance of differences between two groups was calculated by two-way repeated-measures ANOVA and Bonferroni post-test. Significance level was defined as $P<0.05$. Statistical analyses were carried out in Prism Version 4.0c (GraphPad Software Inc., CA).

Time spent in white compartment in sham and EF group

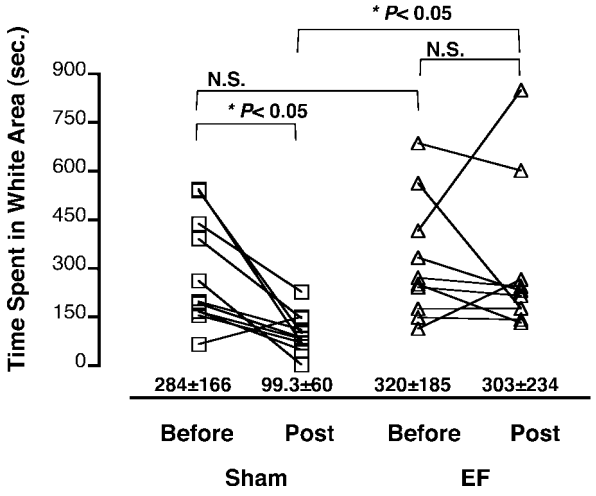

Fig. 2. Time spent in white compartment before and after conditioning period. Squares and triangles indicate values in sham and EF group, respectively. *: Time spent in white compartment in EF group was higher than those of sham group by Bonferroni post-test ($P<0.05$). N.S.: No significant.

before of conditioning sessions were 284 ± 166 and 320 ± 185 seconds, respectively (Fig. 2). Such time in sham group after a 6-day training period decreased to 99.3 ± 60 seconds and those in EF group was 303 ± 234 seconds. The difference of values before and after the conditioning session was significant by repeated-measures ANOVA ($P<0.05$). Comparing values after the conditioning session, time spent in white compartment in EF group was 3-fold higher than those of sham group by Bonferroni post-test ($P<0.05$). There were two exceeding values that were over 600 seconds in EF group. Except for such 2 data, the average shows 197 seconds, which is still 2-fold higher to 99.3 seconds in sham group by Weltch's *t*-test ($P=0.0014$).

Time spent in white place was affected by the conditioning with sinusoidal 50 Hz, 16,000 V/m EF, suggesting that aversion response to light environment was interfered due to exposure to EF. As a mechanism of such the effect, it was estimated an interference in recognition of brightness via EF induced effect to visual system, in learning system via direct effect to central nerve system [13, 14]. In addition, there remained another possibility that rat perceived EF as preferable. In order to confirm whether EF played aversively, or whether EF was sensible, further studies are needed. In addition, it is necessary to determine whether EF acts as reinforcer or dysphoric stimulation. To do that, using brightness controlled place conditioning box with some of clue e.g., stripe, and then it is needed confirm whether such the clue play as an unconditioned stimulus which induces aversion or preference in rat. Furthermore, to strictly decide that a reduction of aversive response is occurred due to exposure to EF, study considered effects of several factor either the noise or vibration by EF-generator must be conducted.

The authors wish to thank Dr. Thekisoe, National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine for his advice and constructive criticism during the preparation of this manuscript. In addition, this research was conducted by the grant from Hakuju Institute for Health Science co., ltd. Japan.

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