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—Research Note—

Exposure of C57BL/6J Male Mice to an Electric Field Improves Copulation Rates with Superovulated Females

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Abstract. It is well-known that there are considerable strain differences in the relative copulation rates between male and superovulated female mice. In particular, the C57BL/6J strain of mice has a lower rate of successful copulation. We examined the effect of exposure to an electric field on sexual behavior in C57BL/6J male mice. When C57BL/6J males were exposed to a 50 Hz, 45 kV/m electric field for 30 min per day for 11 days and placed in a cage with a superovulated female of the same strain, the successful copulation rates of males was significantly improved compared with unexposed males ($P<0.05$). These results suggest that the exposure of C57BL/6J male mice to an electric field improves their sub-fertility activity in mating with superovulated females.

Key words: Mice, Male, Electric field, Copulation, Superovulation, Sexual behavior

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For studies of embryology, developmental biology and transgenic technology in mice, oocytes or embryos are often obtained from superovulated females in mating. However, copulation is not always achieved with all of the superovulated females after overnight mating attempts. There are considerable strain differences in the copulation rates between superovulated females and males [1, 2]. For example, the copulation rates in the C57BL/6J and FvB strains, both of which have proven essential for the establishment of gene knockout or transgenic mice, are 50% and 80–90%, respectively [2]. A lower incidence of successful copulation presents certain time-constraint difficulties for scheduled experiments. In addition, the phenomenon in and of itself is an interesting subject in the field of reproductive physiology, however, neither the causal factor/s nor the precise pattern of this phenomenon have been elucidated as yet.

It has been suggested that exposure to an exogenous electric field changes the intracellular calcium ion concentration and protein synthesis *in vitro* [3, 4] and results in the modulation of certain biological functions such as the endocrine system, immune system, and cell signaling *in vivo* [5]. Furthermore, we have reported that exposure of restrained rats to an electric field suppressed increases in plasma ACTH, glucose and lactate

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levels induced by the restraint stress [6]. Although the biological effects of electric field exposure on reproductive function have not been fully identified yet, it has been reported that exposure of mice to magnetic fields had no adverse effects on fertility and reproduction [7, 8]. Thus, we examined the effects of electric field exposure on C57BL/6J male mice in terms of copulation rates with superovulated females.

Materials and Methods

Animals

C57BL/6J males and females 7 weeks of age, and ICR males also 7 weeks of age, were purchased from a commercial supplier (CLEA Japan, Tokyo, Japan). All animals were housed in polycarbonate cages, and maintained in a specific pathogen-free environment in light-controlled (lights-on from 07:00 to 19:00), and air-conditioned rooms (temperature: 24 ± 1 C, humidity: 50 ± 10%). They had free access to standard laboratory chow (CE-2; CLEA Japan, Tokyo) and water *ad libitum* except for the period of time of the electric field exposure. The animals used in this study were cared for and used under the Guiding Principles for the Care and Use of Research Animals promulgated by the Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan.

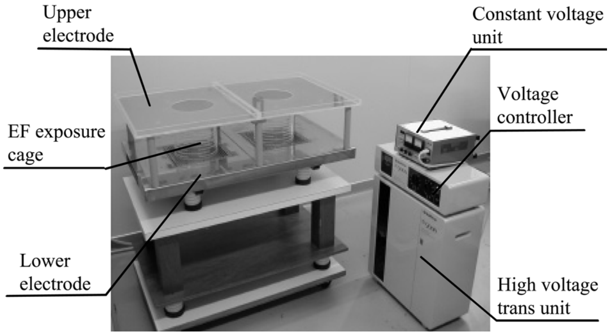

Fig. 1. The electric field exposure system. The electric field exposure system consists of electric exposure cages (also see Fig. 2), a constant voltage unit, high voltage transformer unit and voltage controller.

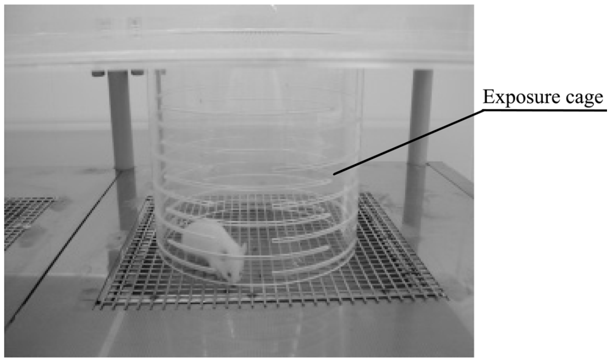

Fig. 2. The electric field exposure cage. A cylindrical plastic cage is placed between upper and lower stainless steel electrodes. The cylindrical cage has slits (length: 100 mm; width: 5 mm) at approximate intervals of 5 mm from each other to prevent smudges, due to feces and saliva, which might disturb the formation of a stable electric field.

Electric field exposure system

The electric field exposure system was composed of three major parts, namely, a high voltage transformer unit (Healthtron, maximum output voltage: 9000 V; HAKUJU Co. Ltd., Tokyo, Japan), a constant voltage unit (TOKYO SEIDEN, Tokyo, Japan), and electric field exposure cages (Fig. 1) [6]. The exposure cage was comprised of a cylindrical plastic cage (diameter; 200 mm, height; 200 mm) and two electrodes made of stainless steel (400 × 400 mm) were placed over and under the cylindrical cage (Fig. 2). In order to establish the electric field (50 Hz, 45 kV/m) in the cage, a stable alternating current (50 Hz, 9000 V) was applied to the upper electrode.

Experimental procedures

Male mice (n=30) were exposed to 50 Hz, 45 kV/m electric field in the electric field cage for 30 min per day for 11 days. After the final electric field exposure, each male mouse was placed in a cage with a superovulated female mouse. Superovulation was induced by intraperitoneal injections of 5 i. u. equine chorionic gonadotrophin (eCG; Serotrophin, the Teikoku Hormone Mfg. Co., Tokyo, Japan) and 5 i. u. human chorionic gonadotrophin (hCG; Puberogen, Sankyo Co., Tokyo, Japan) 48 hr apart. As a control, a similar male mouse was placed in the electric field cage for 30 min per day for 11 days without the electric field exposure and subsequently mated with a superovulated female mouse (n=30). Establishment of copulation was determined by detection of a vaginal plug the morning after overnight mating.

Statistical analysis

Data presented in this study were analyzed statistically by the chi-square test. In all statistical tests, the difference was considered significant when p was <0.05.

Results

In the absence of electric field exposure, the copulation rates of C57BL/6J male mice with superovualted females were significantly lower than those of ICR males. When ICR and C57BL/6J males were mated with C57BL/6J superovulated females, all of the C57BL/6J females mated with the ICR males had a vaginal plug while approximately half (51%) of the females mated with the C57BL/6J males exhibited a vaginal plug. These data are consistent with the results of our previous report [2]. These results clearly indicate that the cause of the poor copulation rates in C57BL/6J mice depends on male reproductive performance rather than on the female.

The effect of electric field exposure on C57BL/6J male mice copulation rates with the superovulated females was remarkable. As shown in Table 1, when male mice were exposed to the 50 Hz electric field for 11 days before being placed in a cage with a superovulated female, the rate of successful copulation was 90% (27/30), while only 63% (19/30) of the superovulated females placed with control C57BL/6J males exhibited a vaginal plug. This difference was statistically significant (*P*<0.05). The male mice used in this study did not show any clinical symptoms or abnormal behavior before, during or after electric field exposure. The fertilization rates of the oocytes recovered from the plug-positive females were not statistically different between the experimental groups (*P*>0.05).

To identify the essential periods of electric field exposure to improve copulation rates, C57BL/6J males were exposed to a 50 Hz, 45 kV/m electric field for 30 min per day for 1, 3 and 11 days and subsequently mated with superovulated C57BL/6J females. When C57BL/6J males were exposed to the electric field for 11 days, the copulation rates with the superovulated C57BL/6J female were significantly improved (Fig. 3, *P*<0.05), a finding similar to the previous experiment (Table 1). However, exposure of C57BL/6J males to the electric field for 1 or 3 days did not significantly increase the copulation rates with the C57BL/6J superovulated female compared with the unexposed control. These results suggest that cumulative electric field exposure is required for improvement of the copulation rates in C57BL/6J male mice mated with superovulated females.

Table 1. Effect of electric field exposure on the couplation rates in C57BL/6J males mated with superovulated females

Treatment	No. of plug detected/no of mating (%)
Exposed	27/30 (90)*
Unexposed	19/30 (63)

*: *P*<0.05 vs. Unexposed.

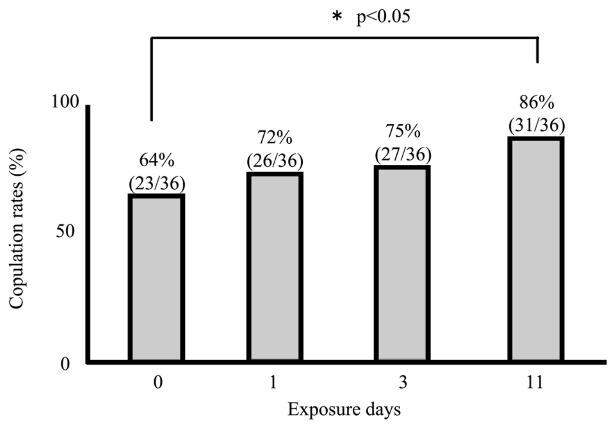

Fig. 3. Cumulative effect of electric field exposure of C57BL/6J males on the copulation rate with superovulated females.

Discussion

The reason for the lower copulation rate seen in C57BL/6J male mice placed with a superovulated female, but not with a naturally ovulated female, is still unclear. However, this lower rate is a major obstacle in the collection of sufficient numbers of oocytes or embryos for transgenesis, as well as impeding research advances in embryology and developmental biology. In the present study, we clearly showed that exposure of C57BL/6J male mice to an electric field improved the copulation rate with superovulated females (Table 1 and Fig. 3). It has been suggested that exposure to an electric field may modulate biological functions of the endocrine and immune systems, as well as cell signaling [5]. Since various medical instruments which harness an electric field are in wide use, it has also been suggested that percutaneous electric nerve stimulation might be beneficial to patients with chronic low back pain [9], and transcutaneous electric nerve stimulation, in fact, has been shown to support the repair of soft tissues [10]. Furthermore, it has been reported that mechanical vibration (5–50 Hz) of the skin surface activates neurons of the primary somatosensory cortex corresponding to the site of stimulation [11]. More recently, the ameliorative effects of electric field therapy on a variety of types of pain, such as headache, stiff shoulders low back pain and stomachache, have been reported in over a thousand clinical cases [12]. However, there is as yet no report on the effect of electric field exposure on reproductive function in either pre-clinical or clinical studies, except for certain toxicological studies [7, 8]. As shown in Table 1 and Fig. 3, however, the exposure of C57BL/6J male mice to an electric field clearly improved their sub-fertility in mating with superovulated females, with an apparent cumulative effect of the electric field exposure apparently requisite. The results of the present study appear to indicate that electric field exposure might have potential as a treatment for male sexual dysfunction, such as the erectile dysfunction which commonly comes with ageing, and is also a consequence of various chronic pathologies such as diabetes, etc.

Although sex hormones and other factors such as pheromones influence sexual behavior in both male and female animals, it is difficult to identify the etiology of sub-fertility in both males and females. It has been reported that sexual behavior of castrated mice rises with the administration of testosterone [13]. The exposure of C57BL/6J male mice to an electric field improved the copulation rate with superovulated females (Table 1 and Fig. 3); however, the plasma concentrations of both testosterone and luteinizing hormone in C57BL/6J males did not increase upon exposure to the electric field (data not shown). In addition, although there is a well-known involvement of nitric oxide in erectile dysfunction [14, 15], electric field exposure did not influence the plasma levels of nitric oxide in C57BL/6J males (data not shown). Although exposure to an electric field evidently exerts certain effects on male sub-fertility, further studies are required to clarify the mechanism involved in the improvement of sub-fertility that appeared in C57BL/6J males exposed to an electric field. Further studies across species will also importantly determine whether this is a conserved phenomenon or one peculiar to certain strains of mice; if conserved, there is potential for its application to human beings.

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