

# Impact of Cellphone Radiation on Sexual Behavior and Serum Concentration of Testosterone and LH in Male Mice

## Zhi-Jun Zang<sup>1</sup>, Su-Yun Ji<sup>2</sup>, Shi-Zong Huang<sup>1</sup>, Mei-Hua Jiang<sup>3</sup>, You-Qiang Fang<sup>4\*</sup>

<sup>1</sup>Department of Infertility and Sexual Medicine, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

<sup>2</sup>Department of Dermatology, Guangdong Provincial Dermatology Hospital, Guangzhou, China
 <sup>3</sup>Department of Anatomy, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China
 <sup>4</sup>Department of Urology, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, China Email: <sup>1</sup>fangyouqiangzssy@163.com

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## Abstract

Background: Cellphone radiation (CR) has been reported to be related to higher risk of many health problems, but if CR can impair sexual behavior and testosterone synthesis has seldom been studied. Objective: To evaluate the effects of CR on testosterone and luteinizing hormone (LH) levels and sexual behaviors of male mice. Methods: Forty 3-month-old male mice, 22 - 25 g, were randomly allocated into four equal groups (n = 10 per group): the control group and three CR exposure groups including 8-hour group, 16-hour group and 24-hour group. Each mouse received different dose of CR exposure for 30 consecutive days. Sexual behaviors and testosterone and LH levels in serum were measured at the end of experiment. Furthermore, we also observed the weights of reproductive organs of each group, including testis, epididymis and seminal vesicle. Results: The mount latency and intromission latency in 24-hour group were significant higher than the control (both P < 0.01), while no obvious changes were seen in 8-hour group and 16-hour group (all P > 0.05). No difference in ejaculation latency existed among each group after the experiment (all P > 0.05). The frequency of mount and intromission in 24-hour group was statistically significantly lower than that of the control group (P < 0.05 and P < 0.01, respectively). No obvious change in the frequency of mount and intromission of the 8-hour group and 16-hour group was seen (all P > 0.05). Only the copulatory efficacy in the 24-hour group was statistically lower than the control group (P < 0.05). The serum levels of testosterone and LH in the 24-hour group were obviously higher than the control group (testosterone level: P < 0.05; LH level: P < 0.01). No significant differences were seen among the other two experimental groups and the control group (all P > 0.05). After the exposure of CR, the changes in the weights of sexual organs in the 24-hour

\*Corresponding author.

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group were significant compared with the control (testis weights, relative testis weight, epididymis weight, the weight of seminal vesicle, and the relative weight of seminal vesicle, all P < 0.01; the relative epididymis weight, P < 0.05). Conclusions: High dose exposure of CR can decline the testosterone and LH levels in mice and inhibit their sexual behaviors.

## **Keywords**

Cellphone Radiation, Testosterone, Luteinizing Hormone, Sexual Behavior

## 1. Introduction

Cell communication is essentially ruling our daily lives nowadays through superior connectivity and intelligent cell phones. So, we are always living in an environment filled with cellphone radiation (CR). There has been a tremendous growth in communication industry along with growing concerns regarding health effects of CR exposure. It has been reported that CR is related to higher risk of many health problems, such as carcinoma [1], neurodegenerative diseases [2], immunosuppression [3], and tinnitus [4], etc. A recent systemic review has suggested potential harmful effects of cell phone use on semen parameters [5]. However, to the best of our knowledge, few researches have reported if CR can affect sexual function. The present study has investigated the influence of CR on the sexual behavior and the serum levels of testosterone and luteinizing hormone (LH) in male mice.

## 2. Materials and Methods

## 2.1. Animals

Fifty C57BL/6 mice (including forty males and ten females) were purchased from the Animal Center of Sun Yat-sen University (Guangzhou, China) and were kept on a 12 h-day/12 h-night schedule (lights on from 19:00 to 07:00 h) at constant temperature ( $22^{\circ}C \pm 1^{\circ}C$ ) and humidity (60%). These animals were approximately 3 months of age and weighted about 25 g. Animals and experiment protocols in the present research were approved and supervised by the Sun Yat-sen University Institutional Animal Care and Use Committee. All animals received humane care, and all efforts were taken to minimize the anguish of animals.

## 2.2. Treatments

All of the male mice were randomly arranged into four equal groups (10/group): one control group and three experimental groups receiving different dosage of CR exposure (8-hour group, 16-hourgroup, and 24-hour group). According to the method mentioned in former articles [6] [7], cellphones were put in the bottom of the cages and kept in a call stage. The radiofrequency of these cellphones is 900 MHz of global system for cell (GSM). Each experimental group received different dose of GSM exposure for 30 consecutive days (8 hours, 16 hours, or 24 hours per day, respectively).

#### 2.3. Tests of Sexual Behaviors

As depicted previously, in order to reach a state of estrous, the female mice were resected with double ovaries in advance [8]. One month later, each female mouse was injected intraperitoneally with estradiol benzoate (50  $\mu$ g, 48 h ahead of testing, dissolved in 50  $\mu$ l of peanut oil) and progesterone (500  $\mu$ g, 5 h ahead of testing, dissolved in 50  $\mu$ l of peanut oil).

The following items were recorded during the sexual behavior tests: mount latency, intromission latency, ejaculation latency, mount frequency, intromission frequency, and copulatory efficacy (calculated as intromission frequency divided by mount frequency + intromission frequency). In a quiet circumstance, the assays of sexual behavior were conducted in a testing cage ( $40 \text{ cm} \times 26 \text{ cm} \times 21 \text{ cm}$ ). At the beginning of the tests, each male mouse was placed individually into the testing cage. A pretreated female mouse was placed into this cage 15 minutes later. If no intromission occurred in 10 minutes, another pretreated female mouse was put into this

cage to take the place of the first one. If still no intromission occurred in the next 5 minutes, a third pretreated female mouse was introduced for a final 15 minutes period. Failure to achieve intromission within 15 minutes or ejaculation within 45 minutes from the beginning of the test would lead to termination of the test, and the maximum latency value of 45 minutes was assigned for that behavior.

## 2.4. Sexual Organs Weight

After the test of sexual behaviors, all of the male mice were weighed, and then anesthetized with ether. The blood samples were obtained from the vena cava for the assay of serum testosterone and LH. Immediately after blood sampled, bilateral testes, epididymides, and seminal vesicles were obtained and weighed.

#### **2.5. Hormone Examination**

Serum testosterone and LH were assayed by the testosterone enzyme-linked immunosorbent assay (ELISA) kit (Novusbio, KA2332) and Luteinizing Hormone ELISA Kit (Novusbio, KA2332) respectively, following the manufacturer's instructions.

## 2.6. Statistical Analysis

All statistical analyses were done using SPSS, version 21.0 (SPSS Inc., Chicago, IL, USA). Outcomes were presented as mean  $\pm$  s.e.m. and subjected to one-way ANOVA followed by Student's t test. P < 0.05 was thought to be statistically significant. All analytic results were performed using the GraphPad Software package (GraphPad Software 6.0, La Jolla, CA, USA).

## **3. Results**

## 3.1. Results of Sexual Behaviors Tests

According to the results shown in **Figure 1**, the latency of mount and intromission was lengthened in 24-hour group compared with the control (mount latency:  $11.82 \pm 2.40$  min vs.  $8.87 \pm 1.34$  min, P < 0.01; intromission latency:  $20.62 \pm 2.73$  min vs.  $16.88 \pm 4.30$  min, P < 0.01), and no obvious changes were seen in the other two groups. No statistical differences of ejaculation latency were observed between the control group and experimental groups (P > 0.05). Although all of the three experimental groups had a downward trend in mount frequency and intromission frequency, only the change in the 24-hour group was statistically lower than the control (mount frequency:  $0.55 \pm 0.14$  numbers/min vs.  $0.67 \pm 0.11$  numbers/min, P < 0.05; intromission frequency:  $0.30 \pm 0.10$  numbers/min vs.  $0.50 \pm 0.17$  numbers/min, P < 0.01). Similarly, there was a decrease of copulatory efficacy only in the 24-hour group compared with the control group (copulatory efficacy:  $0.35 \pm 0.07$  vs.  $0.41 \pm 0.06$ ; both P < 0.05).

## 3.2. Effects of BPA on the Weight of Sexual Organs

There were no differences in body weight between the control group and CR-exposure groups (data not show). As shown in **Figure 2**, the testis weight and relative testis weight in 24-hour group were significant lower than the control group (testis weight:  $0.186 \pm 0.015$  g vs.  $0.238 \pm 0.023$  g, P < 0.01; relative testis weight:  $0.663 \pm 0.055$  vs.  $0.812 \pm 0.097$ , P < 0.01). No obvious differences in testis weight and relative testis weight were seen among the control group and the other two experimental groups. The epididymis weight and relative epididymis weight were also down regulated by CR exposure in the 24-hour group compared with the control (epididymis weight:  $0.069 \pm 0.009$  g vs.  $0.083 \pm 0.008$  g, P < 0.01; relative epididymis weight:  $0.246 \pm 0.037$  vs.  $0.284 \pm 0.030$ , P < 0.05), while no noticeable change was seen in the other two experimental groups). Similarly, only the differences in the weight and relative weight of seminal vesicle:  $0.210 \pm 0.034$  g vs.  $0.268 \pm 0.055$  g, P < 0.01; relative weight of seminal vesicle:  $0.210 \pm 0.034$  g vs.  $0.268 \pm 0.055$  g, P < 0.01; relative weight of seminal vesicle:  $0.210 \pm 0.034$  g vs.  $0.268 \pm 0.055$  g, P < 0.01; relative weight of seminal vesicle:  $0.210 \pm 0.034$  g vs.  $0.268 \pm 0.055$  g, P < 0.01; relative weight of seminal vesicle:  $0.210 \pm 0.034$  g vs.  $0.268 \pm 0.055$  g, P < 0.01; relative weight of seminal vesicle:  $0.210 \pm 0.034$  g vs.  $0.268 \pm 0.055$  g, P < 0.01; relative weight of seminal vesicle:  $0.746 \pm 0.106$  g vs.  $0.914 \pm 0.184$ , P < 0.01), and no obvious differences exist among other groups.

## 3.3. Changes in Serum Testosterone and LH

As shown in Figure 3, the serum testosterone levels of these mice in 24-hour group decreased obviously after

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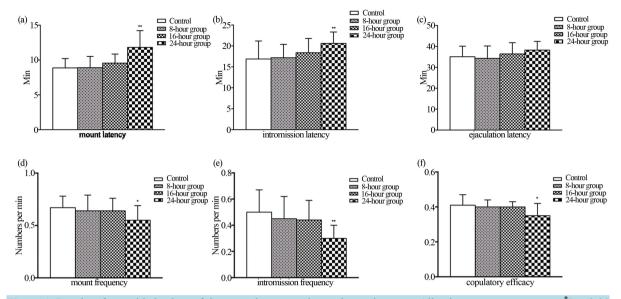


Figure 1. Results of sexual behaviors of the control group and experimental groups. All values are mean  $\pm$  s.e.m.,  $^*P < 0.05$  and  $^{**}P < 0.01$ , compared with control group. (a) Mean latency of mount; (b) Mean latency of intromission; (c) Mean latency of ejaculation; (d) Mean frequency per minute of mount; (e) Mean frequency per minute of intromission; (f) mean copulatory efficacy.

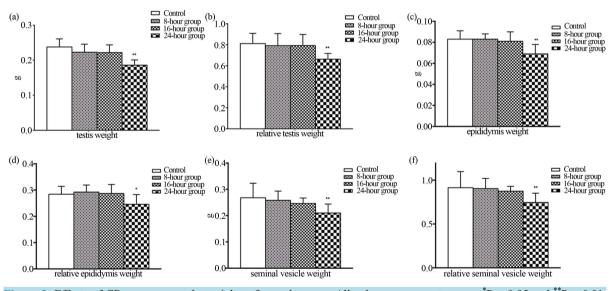


Figure 2. Effects of CR exposure on the weights of sexual organs. All values are mean  $\pm$  s.e.m., \*P < 0.05 and \*\*P < 0.01, compared with control group. (a) Testis weight; (b) Relative testis weight; (c) epididymis weight; (d) Relative epididymis weight; (e) Seminal vesicle weight; (f) Relative seminal vesicle weight.

CR exposure  $(7.81 \pm 1.36 \text{ ng/ml vs. } 9.04 \pm 1.18 \text{ ng/ml}, P < 0.05)$ . Accordingly, the concentration of LH in 24-hour group was statistically higher than the control group  $(31.00 \pm 3.62 \text{ ng/ml vs. } 22.90 \pm 4.07 \text{ ng/ml}, P < 0.01)$ . No changes were observed in other experimental groups compared with the control.

#### **4. Discussions**

In the present study, we selected adult male C57BL/6 mice as an animal model to test the effects of CR on serum testosterone concentration and sexual behavior of male mice. Our results demonstrated that long time exposure of CR could decrease the serum testosterone level and suppress sexual functions of male mice.

Nowadays, a rapid increase in the use of cell phones and other wireless devices is emerging all over the world.

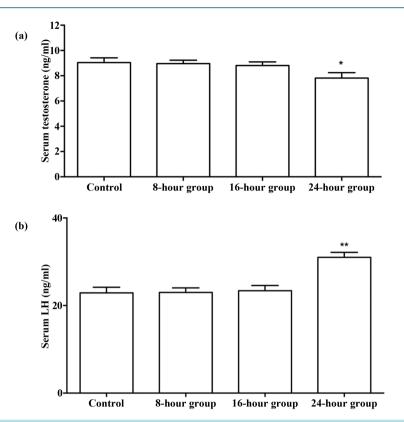


Figure 3. Effects of CR exposure on the levels of serum testosterone (a) and LH (b). All values are mean  $\pm$  s.e.m., \*P < 0.05, \*\*P < 0.01, compared with control group.

Accompanying with this increase in cell phone ownership, there is a concern over the potential effects of cell phone exposure on human health. It has been proved that cell phones can emit electromagnetic radiation at a frequency of between 800 and 2200 MHz which can be absorbed by the human body [9]. People are giving more concern about whether there are human health hazards associated with communication radiofrequency. Oftedal *et al.* have reported that headaches often began during or within half an hour after using cell phone and usually lasted for up to 2 hours [10]. Braune *et al.* showed that resting blood pressure increase during exposure to a radiofrequency electromagnetic field [11]. The results of Huber *et al.* demonstrated that exposure of CR during waking modifies the electroencephalographic (EEG) activity during sleep [12]. It has also been suggested that cell phones, and other electromagnetic devices which can emit radiation, are detrimental to human fertility [13]. Animal researches have dedicated that exposure to a radiofrequency electromagnetic field could affect the cell cycle of sperm [14], produce histological changes in the testes [15], and increase sperm cell death [16]. However, to the best of our knowledge, seldom articles reported the effects of CR on male sexual functions.

The present experiment investigated the effects of CR exposure with different dosage on the sexual behaviors of adult male mice. As far as we know, this research is the first time for topic conducted on adult male animal mode. We selected 3-month-old male mice as an animal model for examining the effects of CR on sexual functions and serum levels of testosterone and LH. In order to investigate whether the administration of CR can have negative effects on the sexual behaviors of male mice, we tested several relevant items about sexual behaviors. The results showed that there are no statistically difference in these items among 8-hour group, 16-hour group and the control group. Only the items in 24-hour group are quite different from the control group. This phenomenon indicated that low and middle dosage exposure of CR did not affect sexual behaviors of these adult male mice, but high dosage exposure to CR can impair sexual functions of them.

It has been demonstrated that the synthesis of testosterone is modulated by the HPG axis and is primary to the maintenance of sexual behaviors [17]. Clark J.T. *et al.* showed that the latencies to initial mount, intromission and ejaculation were significantly decreased in castrated male rats [18]. In order to explore the mechanism through which CR exposure affects sexual behaviors, we also observed the effects of CR exposure on the serum

concentrations of testosterone and LH. The results displayed that serum testosterone and LH levels did not change much after 8 hours' or 16 hours' exposure of CR per day for 30 consecutive days. This phenomenon indicated that CR exposure at low and middle dose might not affect testosterone synthesis function of testis. But serum testosterone level in 24-hour group is quite lower than that in the control group, while serum LH level is higher than that in the control. This result indicated that long time exposure of CR can impair testis function but don't affect the function of pituitarium. After long term CR exposure, the function of testis was suppressed, the synthesis of testosterone was decreased, and the serum testosterone declined, which weakened the negative feedback of testosterone to pituitarium function, followed by the increase of the serum LH concentration.

In addition, the weights of reproductive organs were measured. We found that the sexual organs' weights and relative weights only decreased after the exposure of CR at high dose. Low and middle dose exposure of CR didn't affect the weight of reproductive organs. This phenomenon indicated that the function of testis might have been weakened by high dose CR exposure.

According to our results, the effect at the dose of 24 h exposure of CR seemed most noticeable, and no obvious effects were seen in 8-h and 16-h group. This phenomenon indicated that CR might affect sexual behavior only at high dosage, and people might avoid it by reducing the utility time of cellphone. However, there are several limitations in our study. Firstly, previous researches have documented that the synthesis of testosterone is modulated by the hypothalamic-pituitary-gonadal axis (HPG axis). Further studies are required to investigate whether CR exposure has an effect on the release of gonadotropin-releasing hormone (GnRH) and follicle-stimulating hormone (FSH). Secondly, the synthesis of testosterone is modulated by some enzymes in testis, especially three rate-limiting enzymes including steroidogenic acute regulatory protein (StAR), cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc), and  $3\beta$ -hydroxysteroid dehydrogenase ( $3\beta$ -HSD). Thus, further efforts should be made to elucidate CR's mechanism of action in this process. Thirdly, the number of animals in the present research is relatively small, which may affect the power of statistical tests.

## **5.** Conclusion

The results of this research indicated that only high dose CR exposure suppressed the sexual behavior of adult male C57BL/6 mice, which might be attributed to the impairment of Leydig cells and the decrease of testosterone levels *in vivo*. Besides, according to the outcomes of sexual hormone assay, CR exposure might depress the function of testis, while not affect the function of pituitarium. Since CR exists everywhere in the environment, it is impossible to avoid contacting with it completely, but reducing CR exposure is also enough to limit the injury of it to male sexual health.

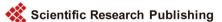
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