ORIGINAL RESEARCH—BASIC SCIENCE

Chronic Stress Influences Sexual Motivation and Causes Damage to Testicular Cells in Male Rats

Gonglin Hou, PhD,* Wei Xiong, PhD,* Mingming Wang, MSc,* Xiangming Chen, PhD,† and Ti-Fei Yuan, PhD*‡

*Department of Psychology, Zhejiang Sci-Tech University, Hangzhou, Zhejiang, China; †Department of Anesthesiology, Zhejiang University College of Medicine, Hangzhou, Zhejiang, China; ‡Department of Psychology, Nanjing Normal University, Nanjing, Jiangsu, China

DOI: 10.1111/jsm.12416

ABSTRACT

Introduction. The suppressing effects of chronic stress on sexual desire have long been noted. Yet the biological mechanisms underlying such effects, especially at the level of cellular biology of testicular cells, have not been fully investigated.

Aim. In the present study, we used a chronic unpredictable mild stress model to examine the association between chronic stress and structural alterations in the male reproductive system.

Main Outcome Measures. The main outcome measures were the structural changes in sperm cells and Leydig cells of male rats. We used Agmo and Ellingsen's procedure to study partner preference behavior and observed the morphology of Leydig cells and germ cells in the control and stress groups.

Methods. Our methods included histology, electron microscopy, and animal behavior tests.

Results. The results showed that after 5 weeks of chronic stress exposure, partner preference behavior was impaired, the total surface area of Leydig cells and the number and diameter of seminiferous tubules decreased significantly, and the number and size of Leydig cells, as well as the number and the short-axis diameter of spermatogenic cells, also decreased. At the ultrastructural level, transmission electron microscopy revealed that the basement membranes of seminiferous tubules in stressed rats was far thinner, had a low density, and was uneven in thickness compared with the normal group, with enhanced apoptosis in germ cells.

Conclusion. We conclude that chronic stress can trigger organic damage to testicular cells in male rats. **Hou G, Xiong W, Wang M, Chen X, and Yuan T-F. Chronic stress influences sexual motivation and causes damage to testicular cells in male rats. J Sex Med 2014;11:653–663.**

Key Words. Testis; Chronic Stress; Preference Behavior; Sexual Motivation; Ultrastructure; Electron Microscopy; Testicular Cells

Introduction

Hypoactive sexual desire disorder is defined in the *Diagnostic and Statistical Manual of Mental Disorders*, *Fourth Edition* (American Psychiatric Association, 1994) as "persistently or recurrently deficient (or absent) sexual fantasies and desire for sexual activity." Male patients suffering from chronic stress exhibit decreased sexual interest and arousal difficulties [1–3]; In addition, chronic stress can negatively affect sexual behavior in animals [4–6]. The traditional theory is that chronic stress affects the central nervous system, thus decreasing sexual desire.

In recent years, it has been found that chronic stress can adversely affect testicular structure, sperm count, and reproductive function [7,8]. Stress and stress-related disorders are associated with reduced serum testosterone [9]. In addition, male patients with infertility caused by psychological stress showed improved spermatogenesis after a cycle of "conveyor of modulating radiance" therapy [10]. There is also evidence suggesting that chronic stress can result in organic damage in

male germ cells. For instance, after 32 days of exposure to variable chronic stress, rats showed a reduction in testis weight and a decrease in percentage of progressive epididymal spermatozoa [11]; in another study, 15 days of forced swimming stress did not impair fertility in male rats but significantly decreased spermatid production [12]. Also, 50 days of forced swimming stress reduced the number and motility of spermatozoa in male rats, as well as their fertilization capacity [13]. Chronic immobilization stress led to delayed testicular maturation in male rats [14], and when they were mated with female rats, the pregnant females exhibited a more than twofold increase in both preimplantation and postimplantation pregnancy loss [14].

Recent studies have demonstrated that chronic stress can cause loss of Leydig cells [15,16]. In the present study we aim to further investigate the potential organic injuries caused by chronic unpredictable mild stress in the male reproductive system in correlation with alterations in sexual preference behaviors. The results may provide novel directions for treatment of infertility caused by chronic stress in humans.

Materials and Methods

Animals

A total of 18 adult male Sprague Dawley rats weighing 150 ± 10 g and four female Sprague Dawley rats weighing 120 ± 10 g (45–60 days old) were obtained from Shanghai Laboratory Animal Center at the Chinese Academy of Sciences (Shanghai, China). All animals were housed in temperature- and humidity-controlled rooms (22 ± 2°C, 50–60% humidity). The room air change rate was 8–15 times per hour. Rats were placed in the room 7 days prior to testing in order to ensure adaptation to the environment. They were exposed to artificial lighting (12-hour/12-hour light/dark cycle, lights on from 9 AM to 9 PM) and had free access to food and water. Rats were handled for 7 days to ensure that they were habituated to handling.

All protocols were conducted in accordance with NIH guidelines for the care and use of laboratory animals and approved by the Animal Care and Use Committee for the Department of Psychology at Zhejiang Sci-Tech University.

Chronic Unpredictable Mild Stress

The experimental procedure was adapted from that described by Willner [17]. After habituation to handling, 16 male rats were randomly separated into two groups: the rats in the control group (n = 8) were housed together, remained undisturbed in their cage, and had free access to food and water, whereas the rats in the stress group (n = 8) were housed separately in a different room and were exposed to chronic unpredictable mild stress for 35 days. Rats in the stress group faced a variety of stressors—food deprivation (24 hours), water deprivation (24 hours), a tail clamp stimulus (1 minute), electric foot shock (1.0 mA at 36 V, with a shock duration of 10 seconds, 30 times with an interval of 60 seconds), cold water immersion (5 minutes, 4°C), wet bedding (24 hours, 100–300 mL water in cage bedding), and reversed light/dark schedule (12 hours/12 hours). These stressors were applied in a random order for 35 consecutive days during the light phase. One stressor per day was applied, and none were presented on two consecutive days. The remaining two male rats were left undisturbed in their room and served as the stimulus animals. The experiment was conducted in accordance with the National Animal Welfare Standards and codes of ethics.

Estrous Cycle Determination

The estrous cycle phases of female rats were determined as previously described [18]. Vaginal secretion was collected with a cotton swab moistened with normal saline (NaCl 0.9%) by inserting the tip into the rat vagina, but not deeply. Vaginal fluid was placed on glass slides. Unstained material was observed under a light microscope, without the use of the condenser lens, with 10× and 40× objective lenses. The vaginal secretions of the four female rats were collected and observed twice per day, at 8 AM and 10 PM. Determination of estrous cycle phase was based on the two complete estrous cycles observed before the test day. The criteria we utilized to determine the estrous cycle phase of female rats were the characteristics of the vaginal secretions of female rats observed on test day.

The Unconditioned Sexual Motivation Test

The apparatus for the test of unconditioned sexual motivation (a male's preference for a female to a male in an open arena) was identical to that employed in earlier studies [19,20] (Figure 1A): a rectangular arena (floor 100 × 50 cm, walls 45 cm high) with two openings (25 × 25 cm) diagonally opposite each other on the long walls. Cages (floor 15 × 25 cm, walls 25 cm high) containing male or female rats (sexual incentives) could be fitted to these two openings. The fronts of the cages were