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Changes in Male Rat Sexual Behavior and Brain Activity Revealed by Functional Magnetic Resonance Imaging in Response to Chronic Mild Stress

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ABSTRACT

Background: Non-organic erectile dysfunction (noED) at functional imaging has been related to abnormal brain activity and requires animal models for further research on the associated molecular mechanisms.

Aim: To develop a noED animal model based on chronic mild stress and investigate brain activity changes.

Methods: We used 6 weeks of chronic mild stress to induce depression. The sucrose consumption test was used to assess the hedonic state. The apomorphine test and sexual behavior test were used to select male rats with ED. Rats with depression and ED were considered to have noED. Blood oxygen level-dependent–based resting-state functional magnetic resonance imaging (fMRI) studies were conducted on these rats, and the amplitude of low-frequency fluctuations and functional connectivity were analyzed to determine brain activity changes.

Outcomes: The sexual behavior test and resting-state fMRI were used for outcome measures.

Results: The induction of depression was confirmed by the sucrose consumption test. A low intromission ratio and increased mount and intromission latencies were observed in male rats with depression. No erection was observed in male rats with depression during the apomorphine test. Male rats with depression and ED were considered to have noED. The possible central pathologic mechanism shown by fMRI involved the amygdaloid body, dorsal thalamus, hypothalamus, caudate-putamen, cingulate gyrus, insular cortex, visual cortex, sensory cortex, motor cortex, and cerebellum. Similar findings have been found in humans.

Clinical Translation: The present study provided a novel noED rat model for further research on the central mechanism of noED.

Strengths and Limitations: The present study developed a novel noED rat model and analyzed brain activity changes based at fMRI. The observed brain activity alterations might not extend to humans.

Conclusion: The present study developed a novel noED rat model with brain activity alterations related to sexual arousal and erection, which will be helpful for further research involving the central mechanism of noED. **Chen G, Yang B, Chen J, et al. Changes in Male Rat Sexual Behavior and Brain Activity Revealed by Functional Magnetic Resonance Imaging in Response to Chronic Mild Stress. J Sex Med 2017;XX:XXX–XXX.**

Key Words: Non-Organic Erectile Dysfunction; Psychogenic Erectile Dysfunction; Functional Magnetic Resonance Imaging; Depression; Male Rat Sexual Behavior; Brain Activity

INTRODUCTION

Erectile dysfunction (ED) is a medical condition characterized by the inability to achieve and/or maintain penile erection sufficient for sexual performance.[1] As a disorder with a high prevalence and incidence, ED negatively affects the quality of life and psychological health of patients and their partners.[2,3] As reported by the well-known Massachusetts Male Aging Study, 52% of men 40 to 69 years old are affected by ED.[4] Psychogenic ED is defined as ED caused predominantly or exclusively by interpersonal or psychological conditions such as depression and

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generalized anxiety disorder.[5] Etiologic studies of ED found that approximately 39% of patients with ED had psychogenic ED.[6,7]

In recent decades, numerous functional neuroimaging studies have focused on the central nerve control of male sexual function. These studies identified the involvement of different cortical and subcortical structures, such as the cingulate cortex, insular cortex, medial prefrontal cortices, amygdala, caudate nucleus, and hypothalamus.[8–11] The relation between decreased sexual function and brain function has been reported by some researchers. As the research on functional neuroimaging and neurobiology of psychological disorders proceeds, psychogenic ED, which is based on an obsolete view of mind-body distinctions, has caused confusion in diagnosis and treatment in clinical practice.[12,13] Therefore, we prefer to use the term *non-organic erectile dysfunction* (noED). To study the brain activity of patients with noED and healthy controls, Cera et al[14] used functional magnetic resonance imaging (fMRI) simultaneously with penile tumescence evoked by visual erotic stimulation. They found that the left superior parietal lobe played an important role in inhibiting sexual response. Bilateral gray matter atrophy of the nucleus accumbens was reported in patients with noED.[15] Zhao et al[16] reported disrupted global network topology in patients with noED. The disruption involved networks that mediate the cognitive, motivational, and inhibitory processes of male sexual arousal, which might account for the symptoms in patients with noED. To date, most studies that involved noED were functional neuroimaging studies. Because of ethical issues, human brain tissue is not accessible for research. The molecular mechanism related to this aberrant brain function remains unknown. Animal models are needed for further studies of the central molecular mechanism of noED.

Primary noED models were developed by researchers to investigate the effect of stress on male fertility and reproduction.[17] The most frequently used stress methods are immobilization, electric foot shocks, and immersion in cold water. Retana-Márquez et al[18] exposed male rats to different acute and chronic stressors and found inhibited sexual behavior and decreased testosterone. However, in castrated male rats, testosterone propionate treatment failed to block the effects of stress on sexual behavior. This result could be explained by the inhibition of central mechanisms, such as the secretion of corticotropin-releasing hormone from the hypothalamus, that remain to be further studied. Functional neuroimaging research has not been performed using noED animal models to determine whether noED animal models have the same central inhibiting mechanisms as human patients with noED. The aim of this study was to investigate whether a rat model of noED would show the same sexual function-related brain activity alterations as human patients with noED. To achieve this goal, we used chronic mild stress (CMS) to induce an anhedonic-like state, which is the core symptom of depression in humans.[19] After exposure to stressors, we used a sucrose consumption test (SCT), an apomorphine test, and a sexual behavior test to identify rats with depression and ED; these rats were considered to have noED. Resting-state fMRI studies were conducted and the amplitude of low-frequency fluctuations (ALFF) and functional connectivity (FC) were analyzed to determine how noED altered brain activity.

Table 1. Stress protocol

	Day	Evening
Monday	Intermittent illumination*	No stress
Tuesday	Water deprivation	Cage tilting‡
Wednesday	Strobe†	Wetting§
Thursday	No stress	Food and water deprivation
Friday	No noise test	Grouping‖
Saturday	Food deprivation	Cage tilting‡
Sunday	Cage tilting‡	Wetting§

*Light on and off every 2 hours.
†Stroboscopic lightning (5 Hz).
‡Tilting the cage to a 45° position.
§Wet bedding by pouring water into the cage.
‖Pairing rats with unfamiliar partners.

METHODS

Subjects

Male Wistar rats (age = 6–7 weeks, weight = 180–220 g) and female Wistar rats (age = 6–7 weeks, weight = 160–180 g; Nanjing Medical University, Nanjing, China) were housed in cages. The room temperature was adjusted to 22°C, and the relative humidity was set at 40% to 60%. The daily light-dark cycle was 12-12 hours. For the 1st week, male rats were housed in groups of 5 and subsequently as singles. Female rats were housed in groups of 5. All animal experiments were conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the experimental animal welfare and ethics committee of Nanjing Drum Tower Hospital (Nanjing, China; permit number 20161003).

Chronic Mild Stress

The SCT described by Wiborg[20] was used to assess the hedonic state. After 14 hours of food and water deprivation, rats were exposed for 1 hour to a bottle that contained 1.5% sucrose. The bottle with the sucrose solution was weighed at the beginning and end of the test to measure the SCT value. During the first 5 weeks of the training period, the rats were exposed to the SCT twice a week. The mean of the last 3 SCTs was used as the baseline value for each rat. Then, the rats were divided into 2 matched groups based on baseline sucrose intake and placed in different rooms. The CMS group (n = 16) was exposed to a series of mild stressors for 6 weeks. The control group (n = 16) was not exposed to stressors and remained undisturbed. The stress protocol (Table 1) was adapted from Wiborg.[20]