

## ARTICLE



# Rapid ejaculator rats are more susceptible to anxiety compared with normal ejaculator rats

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By observation of Sprague–Dawley male rats with different ejaculatory behaviors, we have identified distinct behavioral characteristics in rapid ejaculator rats. To validate these differential behaviors, we conducted multifaceted behavioral experiments on rapid ejaculator rats and normal rats. Through mating experiments, 42 male rats were categorized into 5 rapid ejaculator rats, 29 normal ejaculator rats, and 8 sluggish ejaculator rats according to their ejaculation frequency. We selected 5 rats exhibiting rapid ejaculation and 5 rats with normal ejaculation for participation in the Morris water maze, open-field test, and balance beam experiments. The open-field tests revealed that rapid ejaculator rats spent shorter time in the center region ( $1.23 \pm 1.21$  vs.  $6.56 \pm 2.40$  s,  $P = 0.0041$ ), less entered the center region ( $0.80 \pm 0.75$  vs.  $3.40 \pm 1.50$ , time,  $P = 0.0145$ ), traveled shorter distances ( $17,003.77 \pm 3339.42$  vs.  $25,037.90 \pm 5499.94$  mm,  $P = 0.0371$ ), and had a lower average speed compared with normal rats ( $66.09 \pm 62.36$  vs.  $195.56 \pm 83.41$  mm/s,  $P = 0.0377$ ). However, no significant differences were observed in the Morris water maze and balance beam experiments ( $0.25 \pm 0.05$  vs.  $0.26 \pm 0.07$ ,  $P = 0.7506$ ;  $16.40 \pm 3.77$  vs.  $16.25 \pm 2.05$ ,  $P = 0.9515$ ). These behavioral results indicated that the rapid ejaculator rats were more prone to anxiety. To further substantiate this claim, we examined Brain-derived neurotrophic factor expression levels in the hippocampus of rat brains using immunohistochemistry and western blotting. The results demonstrate lower Brain-derived neurotrophic factor expression in the hippocampus of rapid ejaculator rats compared with that in normal rats ( $P = 0.0093$ ). Thus, our experiments indicate that rapid ejaculator rats exhibit a higher propensity for anxiety, potentially linked to their abnormal neurophysiologic state. It is concluded that rapid ejaculator rats may be more susceptible to anxiety on a pathophysiological basis.

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## INTRODUCTION

Premature ejaculation (PE) is the most prevalent ejaculatory dysfunction in men, impacting the sexual quality of life for approximately 20%–30% of men worldwide [1]. Primary premature ejaculation (PPE) is typically characterized by consistent ejaculation that occurs before or within 1 min of vaginal penetration, along with the inability to delay ejaculation and subsequent negative personal consequences, such as frustration, annoyance, and avoidance of sexual activities [2]. Ejaculation involves a complex neurobiological process that encompasses both the central and peripheral nervous systems [3]. A growing body of research indicates that PPE is associated with physiological abnormalities in the nervous system, with sympathetic hyperexcitability considered to be a significant mechanism in its pathogenesis [4–6].

During the copulation test of screening rapid ejaculator rats, we observed some intriguing phenomena. Compared with normal rats, rapid ejaculator rats exhibit behaviors suggestive of anxiety, including reduced frequency of standing exploration, heightened alertness, increased mental tension and fear of external stimuli,

more frequent fights with other males, and a distinctive behavior of pronounced resistance when their tails are lifted, resembling a “motor rotating at high speed”. Moreover, during gastric gavage procedures, rapid ejaculator rats display greater resistance, characterized by intense struggling, louder screams, and increased difficulty during the procedure.

The abnormal neurophysiology of PPE may explain the observed behavioral differences, with the varied manifestations possibly linked to an overactivated sympathetic nervous system. Numerous depressive symptoms, such as anxiety, insomnia, and agitation, are thought to be associated with changes in the sympathetic nervous system [7]. Consequently, the hyperexcitability of the sympathetic nervous system in rapid ejaculator rats may contribute to a higher susceptibility to anxiety-like behaviors. To substantiate this hypothesis, we conducted multifaceted behavioral experiments on rapid ejaculator rats and normal rats, assessing their learning and memory abilities, anxiety and depression levels, and locomotor abilities. Moreover, we examined Brain-derived neurotrophic factor (BDNF) expression levels in the hippocampus of rat brains using immunohistochemistry and western blotting.

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## MATERIALS AND METHODS

### Animals

50 male rats and 25 female rats were recruited. Adult Sprague–Dawley rats (males, 250–350 g; females, 200–300 g) were given food pellets and water ad libitum and kept in a 12:12-h light/dark cycle. Female rats were ovariectomized bilaterally under anesthesia with pentobarbital (30 mg/kg i.p.) and recovered for at least 1 week. Then they were brought into artificial estrus by a subcutaneous injections of 10 µg oestradiol benzoate (Hefei Xinkexin Animal Medicine Factory, Hefei, China) and 50 µg progesterone (Shanghai Quanyu Animal Medicine Factory, Shanghai, China) 48 h and 4 h prior to the copulatory test respectively [8]. Both steroids were delivered in 0.1 ml of sesame oil (Shandong Luhua Group, Yantai, China). Stimulus females were used once a week, and females that did not show receptive and proceptive behavior during testing were replaced by others.

This study was approved for all husbandry and experimental procedures by the Ethics Committee of Nanjing First Hospital.

### Selections of male rats with different ejaculatory behaviors

Primary rapid and normal ejaculator rats were screened following Patti's rat model screening method, which is considered to be the closest animal model to human PPE [8]. Between 19:00 and 21:00, under dim lighting conditions, male rats were initially placed in an observation cage (60 \* 40 \* 30 cm) to acclimate, after which female rats were introduced after 5 min to record mating videos for 30 min. Each male rat was recorded mating once per week at the same time, resulting in a total of six recordings. As male rat mating behavior tends to stabilize, the last three mating videos were considered valid for analysis. According to mating test results, the rats were categorized into different groups according to their ejaculation frequency (EF) [8]. Rats with EF differences greater than 2 times in the last three mating videos were excluded from the analysis.

### Behavioral assessments

**Morris water maze test.** The Morris water maze test consisted of acquisition training and probing experiments. A circular pool was used (150 cm in diameter and 26–28 °C water temperature) with an escape platform (12 cm in diameter) placed 1 cm below the water surface. In acquisition training, rats were trained to find the escape platform, and if they failed to do so within 60 s, they were gently guided to the platform and allowed to remain on it for 15 s. Acquisition training was conducted four times a day for five consecutive days. After the acquisition training, the platform was removed, and a probe experiment was performed to test their learning memory capacity, recording the time spent in the target quadrant, the distance traveled in the target quadrant, and the number of times the platform was crossed [9].

**Open-field test.** The open-field test involved placing rats in an open-field box (50 \* 50 \* 50 cm) to assess their spontaneous activity and anxiety-like behavior. Each rat's activity was recorded for 5 min. Parameters such as the total distance traveled, time spent in the center area, average speed, and number of entries into the center were calculated and analyzed [10].

**Balance beam test.** The balance beam used in this experiment was 1.3 m long, 0.02 m wide, and 0.5 m high. The locomotor ability of rats was assessed by measuring the time it took for them to reach a safe area by crossing the balance beam. The test was conducted on consecutive days, with training on the first two days and a timed trial on the third day [11].

Data collection and statistical analysis for the above behavioral experiments were performed using KEMaze animal analysis software (Nanjing Calvin Biotechnology Co., Ltd., China) and GraphPad Prism 6 software (GraphPad Software, 64-bit, USA), respectively.

### Immunohistochemistry for BDNF Expression in the hippocampus

The rats were deeply anesthetized and perfused transcardially with 100 mL of 0.01 M phosphate-buffered saline (PBS), pH 7.3, followed by 500 mL 4% paraformaldehyde in 0.1 M PBS, pH 7.2. The brains were then removed and fixed overnight for 48 h in the same fixative, followed by dehydration in 70% alcohol for 50 min, 80% alcohol for 1 h, and 95% alcohol for 1.1 h. The samples then underwent three rounds of anhydrous ethanol treatment for 50 min each, followed by three rounds of xylene treatment for 30/30/40 min before they were immersed in paraffin three times, each time for 1.3 h. The brain coronal sections of the hippocampus were embedded in

paraffin sections, dewaxed, hydrated, subjected to antigen retrieval, blocked, and then incubated with rabbit anti-rat BDNF antibody (AF300575, Aifang Biotechnology Co., Ltd, Changsha, China; 1:100) at 4 °C overnight. The sections were then incubated with goat anti-rabbit secondary antibody (PV-6001, Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China), subjected to DAB color development, hematoxylin restaining, followed by routine dehydration, transparency, and sealing. Images were captured using a Panoramic MIDI slide scanner (3D HISTECN, Budapest, Hungary) to obtain pathological image information of the entire section. Digital histomorphometric analysis was performed using ImageJ, version 1.8.0.112, software and the average optical density of the positive expression region was calculated.

### Western Blot analysis for BDNF expression in the hippocampus

After the rats were euthanized, the brains were removed and hippocampuses were isolated, quickly frozen in liquid nitrogen, and stored in –80 °C. The punched hippocampus tissues were homogenized in cell lysis buffer (KGP250, Kaiji Biotechnology Co., Ltd., Jiangsu, China) to extract protein for Western Blot assay. Equal amounts of protein in each lane were separated by SDS-PAGE (10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis), then transferred to nitrocellulose membranes which subsequently were blocked with QuickBlock Blocking Buffer for Western Blot (Beyotime, China) for 15 min, first incubated overnight with rabbit anti-rat BDNF antibody (AF300575, Aifang Biotechnology Co., Ltd, Changsha, China; 1:1500) at 4 °C and then 2 h with goat anti-rabbit secondary antibody (PV-6001, Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China) at room temperature. Proteins were detected with ECL Plus (EMD Millipore, Billerica, MA, USA).

### Statistical analysis

SPSS 26.0 software was used for statistical analysis. Normal distribution test was performed on all measurement data. Mean ± standard deviation was used to represent measurement data conforming to normal distribution. Independent sample t-test was used for comparison of two groups, and one-way analysis of variance was used for comparison of three groups. Measurement data that did not conform to normal distribution were represented by median (lower quartile, upper quartile). Mann-Whitney test was used for comparison between two groups, and Kruskal–Wallis test was used for comparison between multiple groups. Chi-square test was used to compare the counting data of the two groups.  $P < 0.05$  was considered to be statistically significant.

## RESULTS

### Copulation test

Out of the initial 50 male rats, 8 were culled from the study due to either the absence of ejaculatory behavior or fluctuations in the number of ejaculations exceeding two in the last three mating experiments. Based on the average EF obtained from the last three mating experiments, the remaining 42 male rats were categorized into 5 rapid ejaculator rats, 29 normal ejaculator rats, and 8 sluggish ejaculator rats. The copulatory parameters were listed in Table 1.

### Behavior Characteristics

Behavioral experiments were conducted on 5 rapid ejaculator rats and 5 randomly selected normal rats out of the available group. In the Morris water maze test, no significant differences were observed between rapid ejaculator rats and normal rats in terms of the ratio of the distance traveled in the quadrant where the platform was located and the total distance and the ratio of their movement time and the total time ( $0.25 \pm 0.05$  vs.  $0.26 \pm 0.07$ ,  $P = 0.7506$ ;  $0.24 \pm 0.07$  vs.  $0.24 \pm 0.06$ ,  $P = 0.9293$ ). Similarly, the balance beam test did not show any significant difference in the time taken by the two groups of rats to cross the balance beam ( $16.40 \pm 3.77$  vs.  $16.25 \pm 2.05$ ,  $P = 0.9515$ ). However, in the open-field experiments, the rapid ejaculator rats exhibited distinct behaviors compared with normal rats. The rapid ejaculator rats spent a shorter time in the center region ( $1.23 \pm 1.21$  vs.  $6.56 \pm 2.40$  s,  $P = 0.0041$ ), less entered the center region

**Table 1.** Behavioral parameters monitored during mating tests.

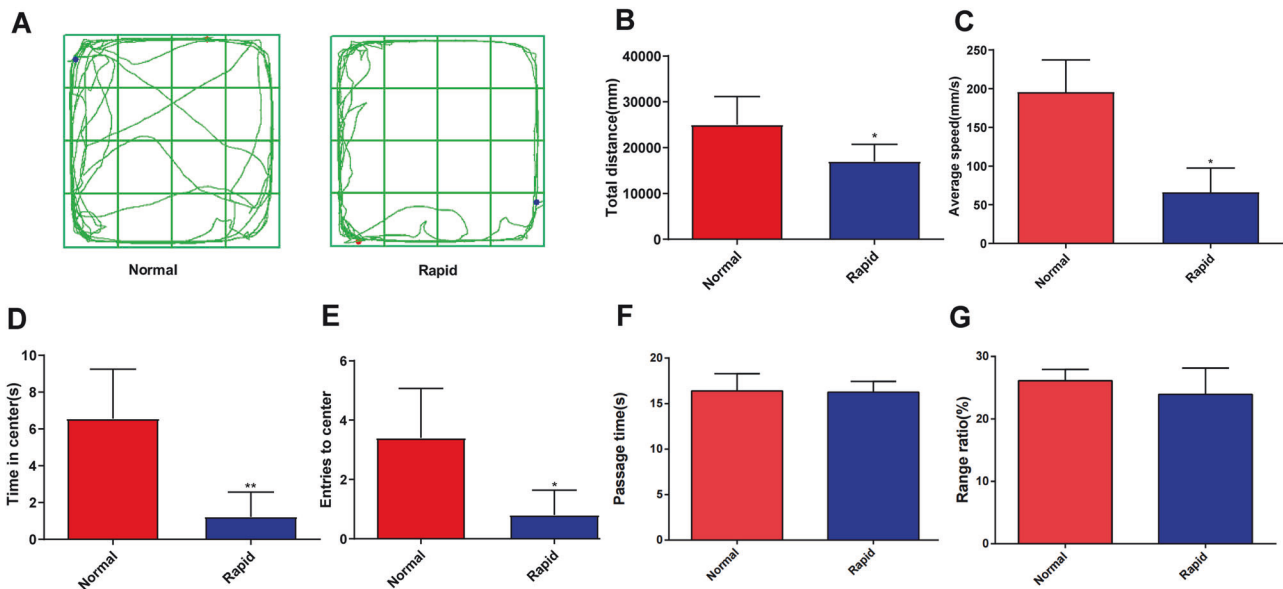
	Rapid	Normal	Sluggish	$\chi^2/F$	<i>P</i>
ML	16 (14, 19.5)	17 (12, 23.5)	34 (16.25, 37.5)	5.460	0.065
IL	18 (15.5, 29.5)	29 (19, 41)	45.5 (28.25, 56) <sup>a</sup>	8.252	0.016
EL	301 (239.5, 316)	666 (583.5, 678.5) <sup>a</sup>	1396.5 (1145.25, 1547) <sup>a,b</sup>	26.950	0.000
PEI	323 (315.5, 343)	398 (382.5, 454.5) <sup>a</sup>	501.5 (447, 599.5) <sup>a,b</sup>	19.223	0.000
MF	4.20 ± 1.64	19.10 ± 3.66 <sup>a</sup>	22.13 ± 4.16 <sup>a,b</sup>	43.256	0.000
IF	11.20 ± 2.77	15.97 ± 3.95 <sup>a</sup>	16.00 ± 2.20 <sup>a</sup>	3.900	0.029
EF	2.67 (2.67, 3)	2 (1.33, 2) <sup>a</sup>	0.84 (0.42, 1) <sup>a,b</sup>	28.545	0.000
IR	0.76 ± 0.04	0.45 ± 0.09 <sup>a</sup>	0.42 ± 0.04 <sup>a</sup>	32.583	0.000

$\chi^2$  = Kruskal–Wallis test; *F* = one-way analysis of variance.

ML mount latency, latency until the first mount, IL intromission latency, latency until the first intromission, MF mount frequency, IF intromission frequency, EL ejaculation latency, time from first intromission until ejaculation, PEI postejaculatory interval, time from ejaculation until the first mount/intromission event of the next cycle, IR intromission ratio, IF / (IF+MF).

<sup>a</sup>*P* < 0.05 compared with rapid ejaculators.

<sup>b</sup>*P* < 0.05 compared with normal ejaculators.



**Fig. 1** The rapid ejaculator rats exhibited anxiety-like behaviors. **A** Automated tracking in the open field. **B–E** The rapid ejaculator rats spent a shorter time in the center region, less entered the center region, traveled a shorter total distance, and had a lower average speed than the normal rats. **F, G** No significant differences were observed between rapid ejaculator rats and normal rats in terms of behaviors in the balance beam and Morris water maze test. Data were presented as Mean ± SD, *n* = 5 per group. \**P* < 0.05 compared with normal ejaculators, \*\**P* < 0.01 compared with normal ejaculators, *t*-test.

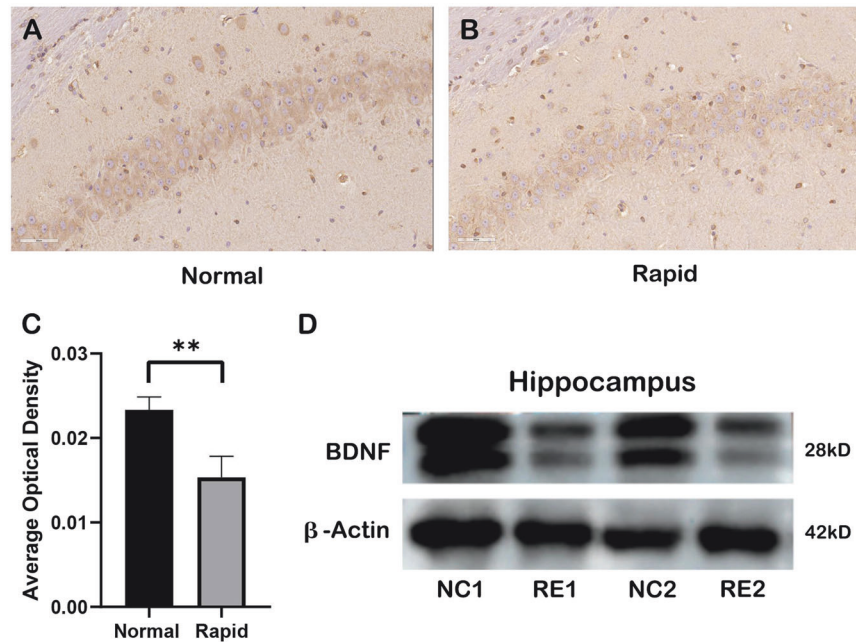
(0.80 ± 0.75 vs. 3.40 ± 1.50, time, *P* = 0.0145), traveled a shorter total distance (17,003.77 ± 3339.42 vs. 25,037.90 ± 5499.94 mm, *P* = 0.0371), and had a lower average speed (66.09 ± 62.36 vs. 195.56 ± 83.41, mm/s, *P* = 0.0377) than the normal rats (Fig. 1). These findings suggest that rapid ejaculator rats do not differ from normal rats in terms of learning memory ability and motor ability. However, they may display a higher tendency towards anxiety-like behaviors.

#### Expression of BDNF in the hippocampus of male rats

6 male rats (3 rapid ejaculator rats and 3 normal ejaculator rats) were used for Immunohistochemistry. 5 male rats (2 rapid ejaculator rats and 3 normal ejaculator rats) were used for Western blot. Qualitative and quantitative analyses revealed that lower BDNF expression in the hippocampus of rapid ejaculator rats compared with that in normal rats (Fig. 2).

#### DISCUSSION

Based on the 10% principle [8], male rats were categorized into rapid, normal, and sluggish ejaculator rats according to the number of ejaculations they had within 30 min. During our experiments involving rapid ejaculator rats, we observed that these rats displayed anxiety-like behaviors. Firstly, they exhibited a higher proportion of fights in the cage, indicating a propensity for anger. They also stood less frequently, displayed heightened alertness to the outside world, and exhibited increased nervousness and fear. Secondly, when we lifted their tails and suspended them in the air, the rapid ejaculator rats reacted more strongly, demonstrating a typical high-speed wagging and rotating behavior, which was rarely seen in normal rats. Additionally, they exhibited greater resistance during gastric gavage, often accompanied by louder screams. Furthermore, during mating, the rapid ejaculator rats appeared to have a higher frequency of



**Fig. 2 Expression of BDNF in the hippocampus of each group.** A, B, D showed the expression of BDNF in the hippocampus of normal rats (NC) and rapid ejaculator rats (RE). C showed lower average optical density in the hippocampus of RE compared with NC. \* $P < 0.05$  compared with normal ejaculators, \*\* $P < 0.01$  compared with normal ejaculators, *t*-test, Bar = 60  $\mu\text{m}$ .

movements, particularly faster running rates and movement transformations while chasing females to complete a mount, indicating greater locomotor ability. The open-field experiments supported these observations, as the time spent in the center region, the number of entries into the center region, the total distance traveled, and the average speed of the rapid ejaculator rats were lower than those of the normal rats, further confirming the anxiety state of the rapid ejaculator rats.

PE is the most common sexual dysfunction in men, with the pathogenesis involving both psychological and physiological factors, psychological factors include early sexual experiences, anxiety, depression, and sexual techniques, while physiological factors comprise 5-hydroxytryptamine (5-HT) receptor dysfunction, penile hypersensitivity, hyperexcitable ejaculatory reflex, genetic predisposition, prostatitis, endocrine diseases, and others [12–18]. The relationship between the basic state of the sympathetic nervous system and ejaculation is increasingly acknowledged. Animal experiments have demonstrated that inhibition of N-methyl-D-aspartic acid (NMDA) receptors in the paraventricular nucleus of the hypothalamus or activation of gamma-aminobutyric acid (GABA) receptors in the same area can inhibit sympathetic excitability and delay ejaculatory behavior in rapid ejaculator rats [19, 20]. Moreover, a multicenter clinical study on penile Neurophysiological Tests has objectively confirmed the hyperexcitability state of the sympathetic nervous system in patients with PPE [21].

Although considered two separate conditions, anxiety and depression are often comorbid. Approximately 85% of individuals with depression also experience significant anxiety, while 90% of individuals with anxiety disorders also suffer from depression [22, 23]. This suggests shared pathogenic mechanisms between anxiety and depression. Most depression-related symptoms are thought to be related to changes in the sympathetic nervous system, including anxiety, agitation, and insomnia [6]. Therefore, an abnormal state of the sympathetic nervous system may constitute the link between PPE and anxiety-depressive disorders, potentially explaining why rapid ejaculator rats are more prone to anxiety. Selective 5-hydroxytryptamine (5-HT) reuptake inhibitors and selective 5-HT norepinephrine reuptake inhibitors are first-line

medications for the treatment of anxiety disorders, while 5-HT reuptake inhibitors are also recommended for the treatment of PPE [24, 25]. The differential expression of 5-HT and norepinephrine associated with sympathetic nervous system excitability in PPE and anxiety-depressive disorders compared with the normal population further suggests a similar etiology between these conditions [20, 26]. Therefore, the increased susceptibility of rapid ejaculator rats to anxiety may be related to their sympathetic nervous system hyperexcitability.

Neurophysiological Test is an important method to diagnose PPE [21]. Neurophysiological Tests for PE consist of penile sympathetic skin response (PSSR), glans penis somatosensory evoked potentials (GPSEP), and dorsal nerve somatosensory evoked potentials (DNSEP) [21, 27–29]. According to the results of the Neurophysiological Tests, PE can be divided into 4 new types [21, 27–29]. Penile sympathetic hyperexcitability type is an important subtype among them, which reflects the hyperexcitability of sympathetic nerves in patients with PE [21]. Previous studies have also shown a higher prevalence of anxiety and depression in patients with PPE [30], and we speculate that this may be related to the sympathetic hyperexcitability in PPE patients.

BDNF is widely expressed in the central nervous system and plays a crucial role in synaptic modulation, learning, memory, and neuroprotection [31, 32]. Decreased BDNF in the hippocampus is associated with lower hippocampal mass, clinical anxiety and depressive disorders in humans, and anxiety-like and depressive-like behaviors in rodents [33–37]. BDNF and its receptor tyrosine kinase receptor B (TrkB) are also implicated in the pathophysiology of depression and the therapeutic mechanism of antidepressant drugs [38]. Animal experiments have demonstrated that central and serum BDNF levels are significantly lower in rapid ejaculator rats compared with normal rats, and this finding has been confirmed in patients with PPE [39, 40]. Our immunohistochemistry and Western blotting experiments on the hippocampus of the brain further confirmed that BDNF levels in the hippocampus of the rapid ejaculator rats were lower than those in the normal rats, which aligns with previous studies. This suggests that rapid ejaculator rats may share a similar pathogenesis with

anxiety and depressive disorders, indicating a potential link between rapid ejaculation and anxiety from a pathophysiological perspective.

For humans with advanced emotional cognition, a shortened intravaginal ejaculation latency time (IELT) often lead to negative personal consequences, such as frustration, annoyance, anxiety, and depression [1]. However, in rats, shortened IELT does not induce anxiety and depression since they lack higher emotional cognitive functions. Based on our results, we speculate that the anxiety state in rapid ejaculator rats may be related to their abnormal sympathetic nervous system state, suggesting a potential pathophysiological reason why patients with PPE may be more prone to anxiety and depression than the general population with normal ejaculation.

The study has certain limitations. Firstly, the animal experiments had a limited sample size due to the labor-intensive and time-consuming nature of screening rapid ejaculator rats as well as a low model yield of approximately 10%. Secondly, there is a lack of clinical research to verify the conclusions of this animal study. Future animal experiments with larger sample sizes and objective validation in clinical trials are warranted to further advance the understanding of this topic.

It is hypothesized that the increased rate of anxiety in patients with PPE may not solely be a consequence of shortened IELT. Instead, patients with PPE may have a pathophysiological predisposition to experience anxiety and depression. These findings underscore the potential importance of considering anxiolytic therapy in the treatment of PPE. It is probably that clinicians should evaluate the anxiety and depression of PPE patients at the first visit. Therefore, active anxiety and depression treatment may be considered for patients with anxiety and depression, while active prevention may be carried out on patients without anxiety and depression, such as psychological counseling and active PPE treatment.

## DATA AVAILABILITY

All data generated or analyzed during this study can be obtained by contacting me via email (13913957628@163.com).

## REFERENCES

- Moreira ED Jr, Brock G, Glasser DB, Nicolosi A, Laumann EO, Paik A, et al. Help-seeking behaviour for sexual problems: the global study of sexual attitudes and behaviors. *Int J Clin Pract.* 2005;59:6–16.
- Serefoglu EC, McMahon CG, Waldinger MD, Althof SE, Shindel A, Adairan G, et al. An evidence-based unified definition of lifelong and acquired premature ejaculation: report of the second International Society for Sexual Medicine Ad Hoc Committee for the Definition of Premature Ejaculation. *Sex Med.* 2014;2:41–59.
- Alwaal A, Breyer BN, Lue TF. Normal male sexual function: emphasis on orgasm and ejaculation. *Fertil Steril.* 2015;104:1051–60.
- Rowland DL, Crawford SB. Idiosyncratic heart rate response in men during sexual arousal. *J Sex Med.* 2011;8:1383–9.
- Zorba OU, Cicek Y, Uzun H, Çetinkaya M, Önem K, Rifaioğlu MM. Autonomic nervous system dysfunction in lifelong premature ejaculation: analysis of heart rate variability. *Urology.* 2012;80:1283–6.
- Guinjoan SM, Bonanni Rey RA, Cardinali DP. Correlation between skin potential response and psychopathology in patients with affective disorders. *Neuropsychobiology.* 1995;31:24–3.
- Owens A, Low D, Iodice V, Mathias C, Critchley H. Emotion and the autonomic nervous system—a two-way street: insights from affective, autonomic and dissociative disorders. In: Reference module in neuroscience and biobehavioral psychology. Elsevier; Amsterdam; 2017. p. 1–15.
- Pattij T, de Jong TR, Uitterdijk A, Waldinger MD, Veening JG, Cools AR, et al. Individual differences in male rat ejaculatory behaviour: searching for models to study ejaculation disorders. *Eur J Neurosci.* 2005;22:724–34.
- Morris RGM. Spatial localization does not require the presence of local cues. *Learn Motiv.* 1981;12:239–60.
- Hall C. Emotional behavior in the rat. I. Defecation and urination as measures of individual differences in emotionality. *J Comp Psychol.* 1934;18:385–403.
- Bartikofsky D, Hertz MJ, Bauer DS, Altschuler R, King WM, Stewart CE. Balance beam crossing times are slower after noise exposure in rats. *Front Integr Neurosci.* 2023;17:1196477.
- Waldinger MD. The neurobiological approach to premature ejaculation. *J Urol.* 2002;168:2359–67.
- Guo L, Liu Y, Wang X, Yuan M, Yu Y, Zhang X, et al. Significance of penile hypersensitivity in premature ejaculation. *Sci Rep.* 2017;7:10441.
- Chen X, Wang FX, Hu C, Yang NQ, Dai JC. Penile sensory thresholds in subtypes of premature ejaculation: implications of comorbid erectile dysfunction. *Asian J Androl.* 2018;20:330–5.
- Huang Y, Zhang X, Gao J, Tang D, Gao P, Peng D, et al. Association of STIN2 VNTR polymorphism of serotonin transporter gene with lifelong premature ejaculation: a case-control study in Han Chinese subjects. *Med Sci Monitor.* 2016;22:3588–94.
- Shamloul R, el-Nashaar A. Chronic prostatitis in premature ejaculation: a cohort study in 153 men. *J Sex Med.* 2006;3:150–4.
- Zhang Y, Li X, Zhou K, Zhou M, Xia K, Xu Y, et al. Influence of experimental autoimmune prostatitis on sexual function and the anti-inflammatory efficacy of celecoxib in a rat model. *Front Immunol.* 2020;11:574212.
- Cinar O, Durmus N, Aslan G, Demir O, Evcim AS, Gidener S, et al. Effects of the dopamine D3 receptor agonist 7-hydroxy-2-(di-N-propylamino) tetralin in hyperthyroidism-induced premature ejaculation rat model. *Andrologia.* 2018. <https://doi.org/10.1111/and.12956>.
- Xia JD, Chen J, Yang BB, Sun HJ, Zhu GQ, Dai YT, et al. Differences in sympathetic nervous system activity and NMDA receptor levels within the hypothalamic paraventricular nucleus in rats with differential ejaculatory behavior. *Asian J Androl.* 2018;20:355–9.
- Zhang QJ, Yang BB, Yang J, Wang YM, Dai YT, Song NH, et al. Inhibitory role of gamma-aminobutyric receptors in paraventricular nucleus on ejaculatory responses in rats. *J Sex Med.* 2020;17:614–22.
- Yang B, Hong Z, Luse DC, Han Y, Sun G, Feng Y, et al. The diagnostic role of neurophysiological tests for premature ejaculation: a prospective multicenter study. *J Urol.* 2022;207:172–82.
- Voltas N, Hernández-Martínez C, Arija V, Canals J. The natural course of anxiety symptoms in early adolescence: factors related to persistence. *Anxiety Stress Coping.* 2017;30:671–86.
- Ghandour RM, Sherman LJ, Vladutiu CJ, Ali MM, Lynch SE, Bitsko RH, et al. Prevalence and treatment of depression, anxiety, and conduct problems in US children. *J Pediatr.* 2019 Mar;206:256–e3.
- Katzman MA, Bleau P, Blier P, Chokka P, Kjernisted K, Van Ameringen M, et al. Canadian clinical practice guidelines for the management of anxiety, posttraumatic stress and obsessive-compulsive disorders. *BMC Psychiatry.* 2014;14:51.
- Althof SE, McMahon CG, Waldinger MD, Serefoglu EC, Shindel AW, Adairan PG, et al. An update of the International Society of Sexual Medicine's Guidelines for the diagnosis and treatment of premature ejaculation (PE). *Sex Med.* 2014;2:60–90.
- Blier P. Neurobiology of depression and mechanism of action of depression treatments. *J Clin Psychiatry.* 2016;77:e319.
- Xia JD, Zhou LH, Han YF, Chen Y, Wang R, Dai YT. A reassessment of penile sensory pathways and effects of prilocaine-lidocaine cream in primary premature ejaculation. *Int J Impot Res.* 2014;26:186–90.
- Xia JD, Han YF, Zhou LH, Xu ZP, Chen Y, Dai YT. Sympathetic skin response in patients with primary premature ejaculation. *Int J Impot Res.* 2014;26:31–34.
- Xia J, Chen T, Chen J, Han Y, Xu Z, Zhou L, et al. The sympathetic skin response located in the penis as a predictor of the response to sertraline treatment in patients with primary premature ejaculation. *J Sex Med.* 2014;11:2801–8.
- Yang Y, Lu Y, Song Y, Chen H, Liu X. Correlations and stratification analysis between premature ejaculation and psychological disorders. *Andrologia.* 2019;51:e13315.
- Lu B, Nagappan G, Guan X, Nathan PJ, Wren P. BDNF-based synaptic repair as a disease-modifying strategy for neurodegenerative diseases. *Nat Rev Neurosci.* 2013;14:401–16.
- Yin JB, Wu HH, Dong YL, Zhang T, Wang J, Zhang Y, et al. Neurochemical properties of BDNF-containing neurons projecting to rostral ventromedial medulla in the ventrolateral periaqueductal gray. *Front Neural Circuits.* 2014;8:137.
- Hempstead BL. Brain-derived neurotrophic factor: three ligands, many actions. *Trans Am Clin Climatol Assoc.* 2015;126:9–19.
- Deltheil T, Guizard BP, Cerdan J, David DJ, Tanaka KF, Repérant C, et al. Behavioral and serotonergic consequences of decreasing or increasing hippocampus brain-derived neurotrophic factor protein levels in mice. *Neuropharmacology.* 2008;55:1006–14.
- Qiao H, An SC, Xu C, Ma XM. Role of proBDNF and BDNF in dendritic spine plasticity and depressive-like behaviors induced by an animal model of depression. *Brain Res.* 2017;1663:29–37.

36. Ieraci A, Mallei A, Popoli M. Social isolation stress induces anxious-depressive-like behavior and alterations of neuroplasticity-related genes in adult male mice. *Neural Plast.* 2016;2016:6212983.
37. Casagrande BP, Ribeiro AM, Pisani LP, Estadella D. Hippocampal BDNF mediated anxiety-like behaviours induced by obesogenic diet withdrawal. *Behav Brain Res.* 2023;436:114077.
38. Zhang JC, Yao W, Hashimoto K. Brain-derived neurotrophic factor (BDNF)-TrkB signaling in inflammation-related depression and potential therapeutic targets. *Curr Neuropharmacol.* 2016;14:721–31.
39. Huang Y, Peng D, Geng H, Dai Y, Jiang H, Zhang X. Expression of brain-derived neurotrophic factor in rapid ejaculator rats: a further study. *Andrologia.* 2021;53:e14134.
40. Atik YT, Gokce A, Halis F, Cimen HI. Can low serum brain-derived neurotrophic factor levels be associated with lifelong premature ejaculation?; A pilot study. *Andrologia.* 2020;52:e13746.

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## AUTHOR CONTRIBUTIONS

Chunlu Xu drafted the manuscript. Tao Song carried out the Immunohistochemistry and Western Blot and performed the statistical analysis. Ning Wu carried out the

Copulation test and participated in the revision work. Yutian Dai conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

## COMPETING INTERESTS

The authors declare no competing interests.

## ADDITIONAL INFORMATION

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