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Research report

Profiles of brain central nervous system gene expression associated with ejaculation behavior in male rats

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HIGHLIGHTS

- Genes related to neurotransmitter receptors, transporters and hormones were significant difference in expression at ejaculation.
- Related to sexual addiction gene *Fosb* was notably up-expressed at ejaculation.
- Related to cancer genes present differently expressing at ejaculation.

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ABSTRACT

The male rat has been used extensively as a model for evaluating the neurophysiology of sexual behavior. However, gene expression in the brain throughout the process of sexual intercourse has yet to be elucidated. In the present study, we created a transcriptomic BodyMap by performing mRNA-Seq on brain samples from pre-copulatory control (CK), fourth intromission (CR4), ejaculation (EJ) and post-ejaculatory interval 1-min (PEI1) Sprague Dawley rats ($n = 40$, all male, each 10). The resulting analysis generated an average of approximately 47 million sequence reads, indicating changes in roughly 21,255 genes for each sample. Among of them, significant differences in gene expression relative to control rats were observed in the CR4 (139 genes), EJ (257 genes), and PEI1 (130 genes) groups. KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis identified 22 pathway-related genes. We further identified eight important genes related to neural pathways using RT-qPCR and Western blot, ruling out the possibility of false positives. The results of the present study not only revealed the basic pattern of gene expression during male rate sexual activity but also provide preliminary data and methodology for further research regarding animal sexual activity.

1. Introduction

Ejaculation is an intensely pleasurable physiological phenomenon for higher-functioning animals and the most reinforcing part of sexual intercourse, usually occurring in conjunction with orgasm [1]. Ejaculation is important for both reproduction and general health and is closely associated with subjective pleasurable feelings [2] and systemic reactions that may reduce death rate [3] as well as the occurrence of many diseases, including heart disease, depression, and prostate cancer [4–7]. Moreover, research has revealed that ejaculation can improve sleep [8], relieve pain [9], and reduce social anxiety [9,10]. Studies of male rat sexual intercourse are particularly suitable for the analysis of the behavioral and neurobiological mechanisms underlying the motivation to engage in a given behavior in the absence of deprivation or physiological need [11].

The brain is responsible for issuing the ejaculation "command", which is associated with extremely complex underlying neurophysiological mechanisms and regulated by various neuronal and neurochemical systems. A number of brain neurotransmitters and hormones as well as their receptors affect both animal

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and human ejaculation, including dopamine (DA), serotonin (5-HT), norepinephrine (NE), prolactin (PRL), oxytocin (OXT), endogenous opioid peptides, and others [12–19]. They coordination of activity in this vast array of regulatory transmitters significantly influences male ejaculation behavior [20].

Sexologists have utilized electrophysiological and anatomical physiology techniques in order to study the neural mechanisms underlying ejaculation and orgasm, including such methods as positron emission tomography (PET) and functional magnetic resonance imaging (fMRT), both of which allow for the exploration of subcutaneous neural activity. Previous research has revealed that males and females exhibit neurological differences with regard to sexual arousal. Indeed, human studies have revealed significant differences between men and women in hypothalamus, amygdala, and hippocampus activation during the viewing of pornographic films and images [21,22]. Imaging studies of female orgasms have further reported that most brain systems are in an active state during orgasm [17]. Although no such evidence has been observed in rats, immunohistochemical studies have revealed similar regions of excitation during sexual intercourse, including the amygdala as well as the paraventricular nucleus (PVN) and medical preoptic area (MPOA) of the hypothalamus [17]. However, such imaging studies have only measured hemodynamic effects caused by neural activity rather than directly assessing neural activity in the brain.

Given that the process of ejaculation is quite rapid while genetic responses occur far more slowly than neurotransmission, previous researchers had expressed little inclination with regard to studying gene expression in the brain during ejaculation. However, a number of recent studies have indicated that polymorphisms in neurotransmitter receptor and transporter genes may influence sexual function including arousal, libido, and premature ejaculation (PE), such as dopamine D4 receptor gene (*DRD4*), dopamine transporter gene (*SLC6A3*), serotonin receptor genes (*HTR-1A*, *HTR-1B*, *HTR-2C*), and oxytocin receptor genes [23–26]. In light of this research, the present study aimed to investigate the association between gene expression in the brain and ejaculation behavior in male rats using high-throughput transcriptome sequencing.

2. Materials and methods

2.1. Animals

Sexually experienced 60 male (10 week-age, weight: 300 ± 20 g) and 60 ovariectomized female (10 week-age, weight: 220 ± 20 g) adult Sprague Dawley rats were used for all experiments, all of which were obtained from Vital River Laboratory Animal Technology Co., Ltd. Animals were kept four per cage, with ad libitum access to food and water (12-h light/dark cycle), at 22 ± 2 °C and 45–50%. All interventions and animal care procedures were performed in accordance with the Guidelines and Policies for Animal Surgery provided by our institute (Chinese Academy of Medical Science, Beijing, China) and were approved by the institutional Animal Use and Care Committee.

2.2. Preparation of sexually receptive females

We used the following method for hormonal induction of estrus in female rats: 100 μg/ml of estradiol benzoate and 5 mg/ml of progesterone (from XIANJU PHARMA, Zhejiang, China) was respectively prepared by adding sesame oil to crystalline forms of each. The mixture was then heated to 60 °C for 1 h and thoroughly shaken. Estradiol benzoate was injected about 52 h before the females were to be used and progesterone about 4 h before copulation [11].

2.3. Behavioral observations

The testing house was decorated with red lighting (approximately 30 lux). All testing occurred between 19:00 and 22:00. Males were placed in the testing house and allowed to adapt to the environment 15 min prior to the experiment. Later, they were folded with estrus females (1:1) in a rectangular box (40 × 60 × 40 cm high). The following components of male rat sexual behavior were recorded using a high-definition camera (DVR H.264): *mount latency* (*ML*), the first time of mounting females–no intromission; *intromission latency* (*IL*), the first intromission into females; *intromission frequency* (*IF*); *ejaculation latency* (*EL*), the time from the first intromission until ejaculation; and *post-ejaculatory interval* (*PEI*), the time from ejaculation to the following intromission. Each observation period lasted 40 min. However, the experiment was ended prematurely if intromission had not occurred within the first 10 min after the presentation of the female to the male. In order to verify our behavioral observations of ejaculation behavior, we subsequently analyzed vaginal sperm and vaginal suppository following the first ejaculation.

2.4. Experimental design and collection of brain tissue

Rats were divided into four groups at random: (1) a *Control group* (*CK*), in which the male had been placed in the testing box alone without initiation of sexual behavior; (2) a *fourth intromission group* (*CR4*), in which the rats had been placed in the testing box at the time of the fourth intromission; (3) an *ejaculation group* (*EJ*), in which the rats had been placed in the testing box at the time of the male's first ejaculation; and (4) a *post-ejaculatory interval group* (*PEI1*), in which the rats had been placed in the testing box 1-min after the first ejaculation. Following completion of the experiments, male rats were immediately sacrificed via spinal dislocation. Whole brain tissues were rapidly obtained under ice and immediately frozen in liquid nitrogen. All materials were stored at −80 °C until further processing.

2.5. RNA isolation and preparation for RNA-Seq

Each whole brain was individually ground into a fine powder using a mortar and pestle under continuous liquid N_2 chilling prior to RNA extraction. Ground brain tissue was stored at −80 °C. Total RNA was extracted from ~60 mg of ground tissue using the Trizol reagent. All RNA samples were sent to the Beijing Genomics Institute (BGI) for sequencing. The total RNA was treated using DNase I, and magnetic beads with Oligo (dT) were used to isolate mRNA. The mRNA was then mixed with the fragmentation buffer in order to obtain short fragments, following which cDNA was synthesized using the mRNA fragments as templates. Short fragments were purified and resolved with EB buffer for end reparation and single nucleotide A (adenine) addition. The short fragments were then connected with adapters. After agarose gel electrophoresis, suitable fragments were selected for use as templates in PCR amplification. During the quality control steps, Agilent 2100 Bioanaylzer and ABI StepOnePlus Real-Time PCR System were used in quantification and qualification of the sample library. Sequencing was performed using Illumina HiSeqTM 2000 (Illumina Company, USA).

2.6. Analysis of differentially expressed genes (DEGs)

We calculated levels of gene expression using RPKM (Reads per kilo base of transcriptome per million mapped reads) according to the following formula:

$$\mathrm{RPKM} = \frac{10^6 C}{NL/10^3}$$