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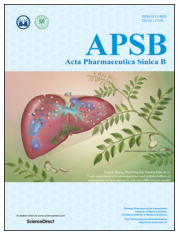

SHORT COMMUNICATION

The effect of 8-OH-DPAT and dapoxetine on gene expression in the brain of male rats during ejaculation

Xijun Qin[a], Xiaojun Ma[a,*], Dongping Tu[a], Zuliang Luo[a], Jie Huang[b], Changming Mo[c]

[a]*Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100193, China*
[b]*Guangxi University of Chinese Medicine, Nanning 530200, China*
[c]*Guangxi Branch of Institute of Medicinal Plant Development, Chinese Academy of Medical Science, Nanning 530023, China*

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KEY WORDS

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Abstract The 5-HT$_{1A}$ receptor agonist 8-hydroxy-2-[di-*n*-propylamino] tetralin (8-OH-DPAT) promotes ejaculation of male rats, whereas dapoxetine delays this process. However, the gene expression profile of the brain at ejaculation following administrationof these two compounds has not been fully elucidated. In the present study, a transcriptomic BodyMap was generated by conducting mRNA-Seq on brain samples of male Sprague–Dawley rats. The study included four groups: pre-copulatory control (CK) group, ejaculation (EJ) group, 0.5 mg/kg 8-OH-DPAT-ejaculation group (DPAT), and 60 mg/kg dapoxetine-ejaculation (DAP) group. The resulting analysis generated an average of approximately 47 million sequence reads. Significant differences in the gene expression profiles of the aforementioned groups were observed in the EJ (257 genes), DPAT (349 genes) and the DAP (207 genes) compared with the control rats. The results indicate that the expression of *Drd1* and *Slc6a3* was significantly different after treatment with 8-OH-DPAT, whereas the expression of *Drd4* was significantly different after treatment with dapoxetine. Other genes, such as *Wnt9b*, *Cdkn1a* and *Fosb*, exhibited significant differences in expression after the two treatments and are related to bladder cancer, renal cell carcinoma and sexual addiction. The present study reveals the basic pattern of gene expression that was activated at ejaculation in the presence of 8-OH-DPAT or dapoxetine, providing preliminary gene expression information during rat ejaculation.

*Corresponding author. Tel.: +86 13501187416; fax: +86 21 57643271.
E-mail address: mayixuan10@163.comb (Xiaojun Ma).

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1. Introduction

Ejaculation is the physiological process that leads to the expulsion of sperm from the urethra, which consists of two different stages, an emission and an expulsion phase[1]. Ejaculation is the strongest part of sexual behavior and usually corresponds with an orgasm[2]. The neurophysiological mechanism of ejaculation is extremely complicated and is regulated by a variety of neuronal and neurochemical systems that are not fully understood, notably in terms of genetic origin. A number of brain neurotransmitters and hormones, as well as their receptors affect both animal and human ejaculation, including dopamine (DA), serotonin (5-HT), norepinephrine (NE), prolactin (PRL), oxytocin (OXT) and endogenous opioid peptides[3-9]. DA and 5-HT have been of considerable interest among the different central neurotransmitters that are involved in mediating the neural control of the ejaculation process[10]. DA is considered as a "trigger" for sex, whereas 5-HT as a "brake"[11].

Dysfunction of ejaculation and/or the bisexual orgasm is influenced by 5-HT receptor agonists and selective 5-HT uptake inhibitors (SSRI)[12]. 8-Hydroxy-2-[di-*n*-propylamino] tetralin (8-OH-DPAT) is a 5-HT_{1A}–specific agonist that is administered peripherally or centrally and decreases the number of intromissions prior to ejaculation and decreases ejaculation latencies. In addition, 8-OH-DPAT increases the rate of ejaculation[13-17]. The mechanism of 8-OH-DPAT–mediated effects has yet to be established. A study has shown that 8-OH-DPAT promotes ejaculation by stimulating the presynaptic 5-HT_{1A} receptor and diminishing 5-HT release into the synaptic cleft[11]. A considerable decrease in the expression of the genes *5-Ht1a*, *5-Ht2a,* and *Tph-2* has been noted with chronic administration of 8-OH-DPAT. Furthermore, the administration of 8-OH-DPAT has been shown to decrease the functional activity of the 5-HT_{1A} receptor[18]. In contrast to these observations, two studies have demonstrated that the effect of 8-OH-DPAT on the ejaculation of rats is a central process and is possibly mediated by the D_2-like receptors as opposed to the 5-HT_{1A} receptors[15,19].

Selective serotonin re-uptake inhibitors can inhibit sexual orgasm and induce transient inhibition of sexual desire[20]. Dapoxetine is a novel, fast-acting SSRI that has considerable efficacy in the treatment of premature ejaculation (PE)[21]. The administration of dapoxetine at 1–3 h prior to the sexual process prolongs the intravaginal ejaculation latency time (IELT)[22,23]. The mechanism of action of dapoxetine is still unclear, although it is suggested that dapoxetine inhibits the serotonin transporter and subsequently enhances the action of serotonin at the pre- and postsynaptic receptors[24]. Furthermore, the study conducted by Clement et al.[23] on anaesthetized male rats demonstrated that the acute administration of dapoxetine inhibits ejaculation by modulating the activity of neurons involved in the brain network related to the ejaculation process.

In light of the observation that the gene expression profile of the brain at ejaculation after the administrationof 8-OH-DPAT and dapoxetine has not been fully elucidated, the present study utilized a high-throughput transcriptome sequencing process to investigate the association between the gene expression profile in the brain and the ejaculation behavior of male rats that were treated with 8-OH-DPAT and dapoxetine.

2. Materials and methods

2.1. Materials

The reagents (suppliers) are as follows: estradiol benzoate (2 mg/mL, Xianju Pharma, Zhejiang, China), progesterone (20 mg/mL, from Xianju Pharma, Zhejiang, China), 8-OH-DPAT (Sigma, USA), dapoxetine ([(*S*)-*N,N*-dimethyl-3-(naphthalen-1-yloxy)-1-phenylpropan-1-amine, Sigma, USA), Trizol reagent (Invitrogen, USA), DNase I (Promega, USA), Oligo (dT) magnetic beads (Invitrogen, USA), ECL (Electro-Chemi-Luminescence) reagents (Millipore, USA).

2.2. Animal handling

2.2.1. Animals

A total of 80 male and female adult Sprague–Dawley rats were used for all experiments. A total of 40 male rats were sexually experienced (age: 10 week; weight: 300 ± 20 g), whereas the remaining 40 female rats were ovariectomized (age: 10 week; weight: 220 ± 20 g). The animals were obtained from the Vital River Laboratory Animal Technology Co., Ltd. and kept in a cage with access to food and water *ad libitum* (12-h light/dark cycle) at 22 ± 2 °C and 45%–50% humidity. All interventions and animal care procedures were carried out in accordance with the Guidelines and Policies for Animal Surgery of Chinese Academy of Medical Science (Beijing, China) and approved by the institutional Animal Use and Care Committee.

2.2.2. Preparation of sexually receptive females

Estrus in female rats was induced by hormones using the following method[25]. Briefly, 100 μg/mL of estradiol benzoate and 5 mg/mL of progesterone were prepared by adding sesame oil to the crystalline forms of each compound. The mixtures were then heated to 60 °C for 1 h and shaken thoroughly for use. Estradiol benzoate was injected s.c. at approximately 52 h prior to copulation and progesterone was administered at approximately 4 h prior to the process. Both hormones were injected in a volume of 0.2 mL/rat.

2.2.3. 8-OH-DPAT and dapoxetine treatments

The two compounds 8-OH-DPAT and dapoxetine were used for all experiments. Solutions of 8-OH-DPAT and dapoxetine were prepared in 0.9% saline prior to the copulatory test. 8-OH-DPAT was injected i.p. to male rats at doses of 0.5 mg/kg, 1.0 mg/kg and 2.0 mg/kg 20 min prior to copulation, and dapoxetine was injected i.p. at doses of 30, 45 and 60 mg/kg 1 h prior to the copulation.

2.2.4. Behaviour observations

The room where all animal experiments were carried out was illuminated with red lighting (approximately 30 lx). The experiments were carried out during the period of 19:00–22:00. The male rats were placed in the experimental cage and allowed to adapt to the environment for 15 min before the experiment. The male rats were paired with estrus females (1:1) in the cage. The following sexual behavior indexes of the male rats were recorded using a high-definition camera (DVR H.264), including mount latency (ML), time of the first male sexual event in the absence of intromission, the intromission latency (IL), time of the first intromission at the beginning of

male copulation, the intromission frequency (IF), number of intromission before the first ejaculation, the ejaculation latency (EL), the time from the first intromission to the ejaculation, the post-ejaculatory interval (PEI), the time from ejaculation to the next intromission. Each observation period lasted for 40 min. The experiment was terminated early if intromission had not occurred within the first 10 min after pairing. The vaginal sperm and the vaginal suppository were observed following the first ejaculation in order to verify the behavioral observations.

2.3. Methods

2.3.1. Experimental design and collection of brain tissue

Male rats were randomly divided into four groups: (1) Control group (CK), where male rats were placed in the experimental cage alone, in the absence of the initiation of the sexual behavior; (2) Ejaculation group (EJ), where male rats were placed in the experimental cage with a female, in the absence of treatment; (3) 0.5 mg/kg 8-OH-DPAT-ejaculation group (DPAT), where male rats were administered 8-OH-DPAT and placed in the experimental cage with a female; (4) 60 mg/kg dapoxetine-ejaculation group (DAP), where male rats were placed after administration of 8-OH-DPAT and dapoxetine in the experimental cage with a female. Following completion of the first ejaculation, the male rats were immediately sacrificed *via* spinal dislocation and the brain tissues were rapidly obtained and immediately frozen in liquid nitrogen. All materials were stored at $-80\ ^{\circ}C$ until further processing.

2.3.2. RNA isolation and preparation for RNA-Seq

Prior to RNA extraction, brain tissues were homogenized into a fine powder using a mortar and a pestle under continuous liquid N_2 chilling. The brain tissue powder was stored at $-80\ ^{\circ}C$. Total RNA was extracted using Trizol reagent with 60 mg of brain tissue. All RNA samples were sent to the Beijing Genomics Institute (BGI, Beijing, China) for sequencing. The total RNA was incubated with DNase I and magnetic beads with Oligo (dT) were used to isolate mRNA. The mRNA was then mixed with fragmentation buffer (Invitrogen, USA) in order to obtain short fragments. The cDNA was synthesized using the mRNA fragments as the templates. The short fragments were purified and resolved with EB buffer (Tris–HCl, 10 mmol/L, pH

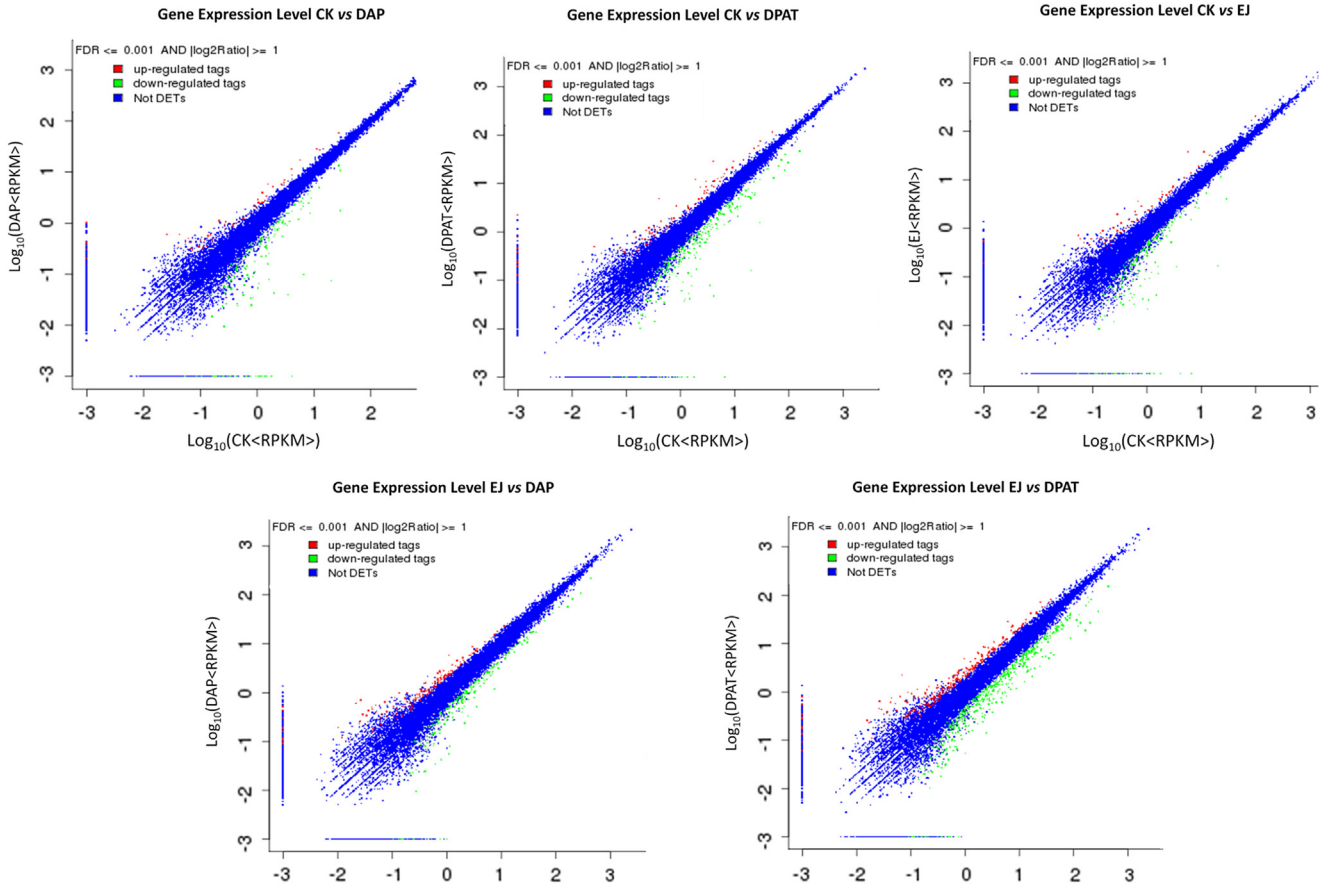

Figure 1 Differences in rat transcriptomic profiles. The results are depicted as scatter plots and indicate the gene expression values that were expressed as $\log_2$ (RPKM), for CK (*x* axis) and the other groups of rats and the concentration of compounds administered in a log scale (*y* axis). The plots indicate the gene expression profiles between the CK and the other groups of rats in the three different treated ejaculation stages. Red: up-regulated; Green: down-regulated. The mean value of gene expression exhibits a significant difference (FDR $\leq$ 0.001, fold change $\geq$ 2), whereas the blue color corresponds to a non-significant difference. CK, control; EJ, ejaculation; DPAT, 0.5 mg/kg 8-OH-DPT-ejaculation; DAP, 60 mg/kg dapoxetine-ejaculation.

8.5, Qiagen, Germany) for end-preparation and single nucleotide A (adenine) addition. The short fragments were then connected with adapters. Following agarose gel electrophoresis, suitable fragments were selected as the templates for PCR amplification. An Agilent 2100 Bioanaylzer (Agilent, USA) and an ABI StepOnePlus Real-Time PCR Systems (ABI, USA) were used for quantification and quality control of the sample library. Sequencing was conducted using IlluminaHiSeqTM 2000 (Illumina, USA).

2.3.3. Analysis of differentially expressed genes (DEGs)

The level of gene expression was calculated using RPKM (reads per kilo base of transcriptome per million mapped reads) according to the following formula:

$$\mathrm{RPKM} = \frac{10^6 C}{NL/10^3} \quad (1)$$

where RPKM represents the expression of the gene, C represents the number of reads that are uniquely aligned to the gene, N represents the total number of reads that are uniquely aligned to all genes, and L represents the number of bases in the gene.

In the present study, "FDR ≤ 0.001 was used and the absolute value of $\log_2$ ratio was set at ≥ 1" as the threshold required to measure the significance of differences in the gene expression.

2.3.4. Gene ontology (GO) and pathway enrichment analysis of DEGs

GO includes molecular functions, cellular components, and biological processes. The basic unit of GO is the GO-term. Every GO-term belongs to a type of ontology. The method utilized in the present study involves the following formula:

$$p = 1 - \sum_{i=0}^{m-1} \frac{\binom{M}{i}\binom{M-N}{N-i}}{\binom{N}{n}} \quad (2)$$

where N represents the number of all genes with the GO annotation, n represents the number of DEGs in N, M represents the number of all genes that are annotated to certain GO terms and m represents the number of DEGs in M. The calculated p-value was subjected to the Bonferroni correction using a corrected p value ≤ 0.05 as the threshold. The GO terms fulfilling this condition are defined as significantly enriched in DEGs.

Pathway-based analysis aids in the investigation of the biological functions of the specific genes. The KEGG (Kyoto Encyclopedia of Genes and Genomes, the major public pathway-related database) was used to carry out the pathway enrichment analysis of the DEGs. This analysis identifies metabolic pathways or signal transduction pathways that are significantly enriched in the DEGs compared to the whole genome. The pathway-based analysis requires the same formula utilized in the GO analysis. In this case, however, N represents the number of all the genes with the KEGG annotation, n represents the number of the DEGs in N, M represents the number of all the genes annotated to specific pathways, and m represents the number of the DEGs in M.

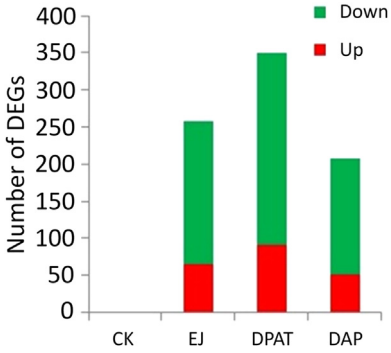

Figure 2 Percentages of differentially expressed genes (DEGs). DEG values were transformed according to the following thresholds: $|\log_2\mathrm{Ratio}| \geq 1$ and FDR $\leq$ 0.001. A 2-fold change or higher was considered as an up-regulation. CK, control; EJ, ejaculation; DPAT, 0.5 mg/kg 8-OH-DPT-ejaculation; DAP, 60 mg/kg dapoxetine-ejaculation.

2.3.5. RT-qPCR analysis of DEGs

RT-qPCR was carried out using RNA isolated from rat brain tissues. A total of four candidate DEG$_S$ (*Drd1*, *Drd4, Slc6a3*, and *Slc6a4*) were selected. The PCR primers that were used are listed as follows: *Drd1* (F: 5′CCATAGAGACGGTGAGCATT3′, R: 5′GCTATTCCAACCAGCCTCTTC3′), *Drd4* (F: 5′ACCTGGCT GGGCTATGTCAA3′, R: 5′TTGCGGAAGACACTTCGAAAC T3′), *Slc6a3* (F: 5′GGACTTCTACCGACTCTGTGA3′, R: 5′ GTGGTGATGATTGCGTCTCT3′), *Slc6a4* (F: 5′GCGGAGATG AGGAATGAAGA3′, R: 5′CAATCCCAGCGATTAACA3′), *β*-actin (R: 5′AGGGAAATCGTGCGTGACAT3′, R: 5′GAACCGC TCATTGCCGATAG3′). A total of three replicates were used. The analysis of variance was conducted using SPSS 17.0 (IBM Analytics, Chicago, Illinois, USA).

2.3.6. Western blotting

Following total protein extraction, the BCA (bicinchoninic acid) method was used for the quantitative analysis of the protein content. The Western blot analysis was conducted as follows: The samples were subjected to SDS-PAGE (80 V × 30 min+ 100 V × 60 min) and the gels were subsequently placed in Western blotting buffer (0.025 mol/L Tris, 0.25 mol/L glycine and 0.1% SDS, *w/v*). The transfer of the proteins onto the PVDF membranes was conducted at 100 V for 40 min in ice-cold buffer (48 mmol/L Tris, 39 mmol/L glycine, 0.37 g/L SDS and 20% methanol, *v/v*). The membranes were subsequently transferred to TBST buffer containing 5% powdered skim milk and were incubated for 1 to 2 h at 25 °C. The membranes were washed three times in TBST (Tris-buffered saline and Tween 20) for 5 min. The samples were incubated overnight with the primary antibodies at 4 °C with gentle shaking. The following morning the samples were washed three times with TBST for 5 min. The samples were incubated with the secondary antibodies for 1 h at 25 °C and washed with TBST three times for 10 min. Detection was carried out using ECL reagents and the analysis was conducted using Odyssey software (Li-COR Company, USA) for image analysis.

2.3.7. Statistical analysis

The data are presented as mean ± standard deviation ($\bar{x} \pm s$) for all variables measured in the experimental groups. The statistical analysis was carried out by SPSS17.0 (IBM Analytics, Chicago, Illinois, USA). A P value <0.05 was considered as statistically significant.

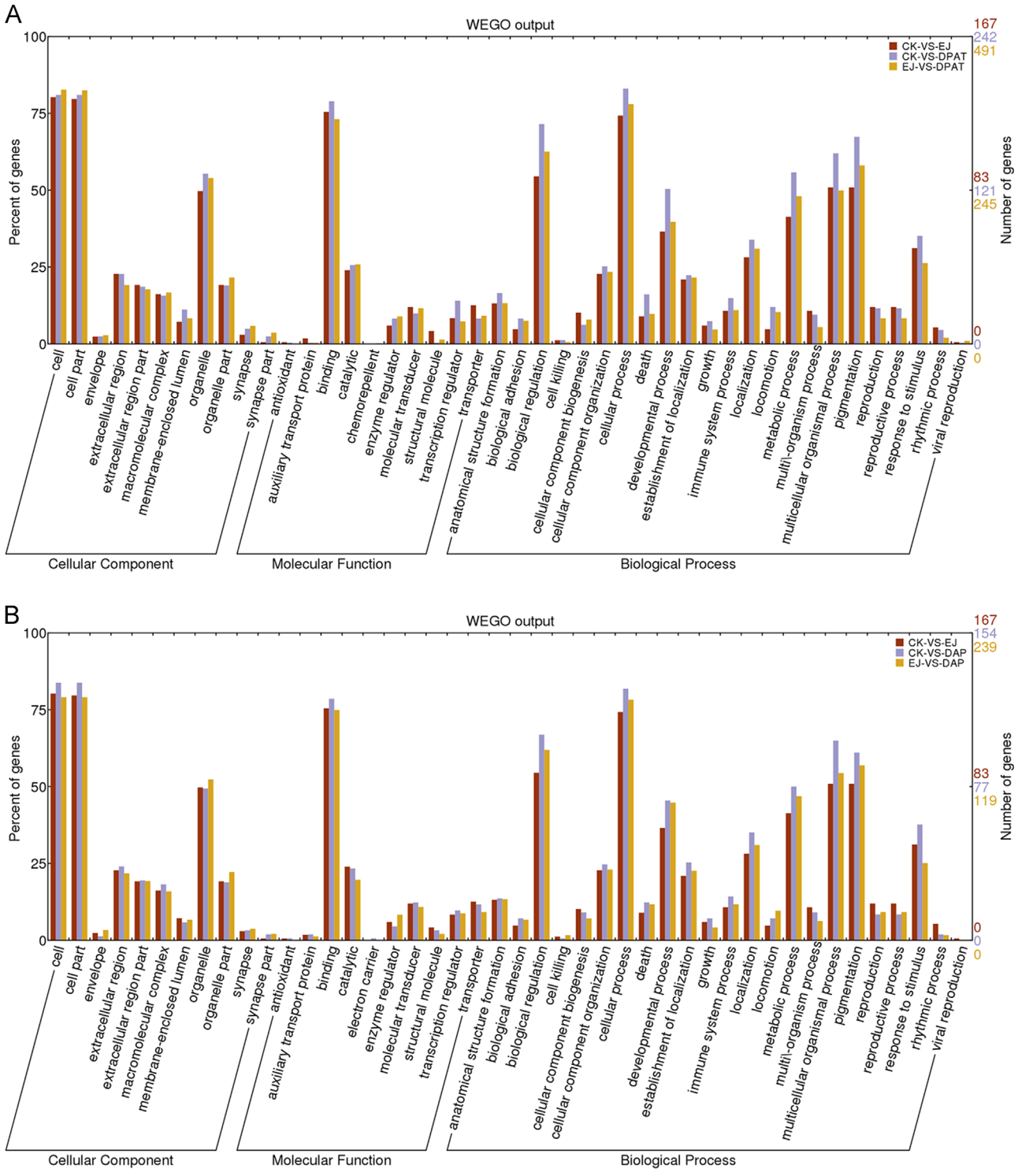

Figure 3 Histogram of gene ontology classification. The results are summarized in three main categories: cellular component, molecular function and biological process. The right *y*-axis indicates the number of genes in a category. The left *y*-axis indicates the percentage of a specific category of genes in the main category. CK, control; EJ, ejaculation; DPAT, 0.5 mg/kg 8-OH-DPT-ejaculation; DAP, 60 mg/kg dapoxetine-ejaculation.

3. Results

3.1. The effects of different doses of 8-OH-DPAT and dapoxetine on ejaculation

Initial experiments were carried out in order to determine the appropriate dose for administration of the compounds. The results demonstrated that 0.5 and 1.0 mg/kg of 8-OH-DPAT stimulated ejaculation as demonstrated by a statistically significant reduction in the latency of mounts, intromissions and ejaculation. Dapoxetine significantly reduced the ejaculation performance at a dose of 60 mg/kg by delaying the latency of mounts and decreasing the latency of ejaculation and post-ejaculatory interval. The results were consistent with previously reported data[23]. As a result,

0.5 mg/kg of 8-OH-DPAT and 60 mg/kg of dapoxetine were selected for the experimental conditions.

3.2. *Overview of the landscape of the rat transcriptome*

A total of four RNA-Seq libraries were constructed from four RNA samples derived from 40 male rats in order to study the male rat brain transcriptome following 8-OH-DPAT and dapoxetine administration. The RNA libraries were also sequenced in order to provide insight in the rat brain transcriptome. An average of 47 million sequence reads per sample was generated by removing the raw reads containing adapters, the high N ($>10\%$) and/or the large number of low-quality reads (*i.e.*, when the percentage of low quality bases was over 50% in a read and the sequencing quality was less than 10).

In this transcriptome sequencing, an average of 21,255 genes was expressed in each sample. Although a large variance in gene expression was observed, the gene expression profiles ($|\log_2\text{Ratio}| \geq 1$ and FDR ≤ 0.001, fold change above twice) between the groups analyzed (EJ, DPAT, DAP and CK) exhibited a differential (Fig. 1). Differential expression was noted using statistical analysis for a total of 257 genes (66 up-regulated and 191 down-regulated) in the EJ group, 349 genes (91 up-regulated and 258 down-regulated) in the DPAT group and 207 genes (52 up-regulated and 155 down-regulated) in the DAP group, respectively (Fig. 2). Twenty DEGs, which present the most significantly different expression ($|\log_2\text{Ratio}| \geq 1$ and FDR ≤ 0.001, fold change ≥ 2), with their up or down expression of the three groups each (EJ, DPAT and DAP) is shown in Supplementary Table 1S.

3.3. *Gene ontology (GO) classification*

GO assignments were used to classify the functions of the predicted DEGs. All DEG sequences can be categorized into 44 functional groups based on the sequence homology (Fig. 3A and B). The terms "cell", "cell part", "binding" and "cellular process" are predominantly used in each of the three main categories (cellular component, molecular function and biological process) of the GO classification in the DPAT and the DAP groups (Fig. 3A and B). A high percentage of genes that corresponded to the "organelle", "biological regulation", "multicellular organism process", "metabolic process" and "pigmentation" categories were noted, whereas a lesser percentage of genes corresponded to the "synapse part", "chemorepellent", and "viral reproduction" categories.

3.4. *KEGG Enrichment analysis*

The KEGG[26,27] was used for pathway enrichment analysis of DEGs (FDR ≤ 0.05). This analysis identified significantly enriched metabolic pathways or signal transduction pathways in DEGs compared to the whole genome background. The pathway enrichment analysis of DEGs was conducted in four transcriptomes that were annotated in Ref-Seq. A total of 21 signaling pathways were enriched, including neurotransmitter synapses, endocrine hormone signals, muscle contraction, addiction, and disease pathways. Changes in the number of DEGs were measured in all these pathways (Fig. 4). The results of the analysis indicated that both number of DEGs in dopaminergic and serotonergic

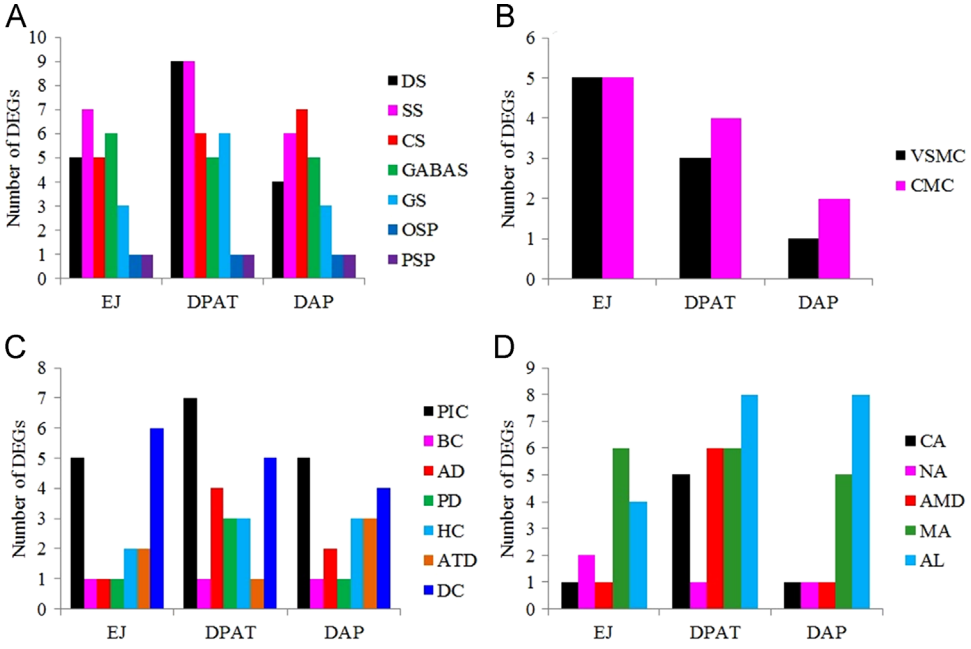

Figure 4 The changes in the number of the DEGs associated with various signaling pathways during ejaculation induced by different treatments. (A) A total of seven pathways related to the transmitter synapse and the endocrine hormone signaling pathways were notably enriched. (B) Two pathways related to muscle contraction were notably enriched. (C) Seven pathways related to the pathways in cancer, bladder cancer, Alzheimer's disease, Parkinson's disease, Hepaitis C, autoimmune thyroid and dilated cardiomyopathy were notably enriched. (D) Five pathways related to addiction were notably enriched. DS, dopaminergic synapse; SS, serotonergic synapse; CS, cholinergic synapse; GABAS, GABAergic synapse; GS, glutamatergic synapse; OSP, oxytocin signaling pathway; PSP, prolactin signaling pathway; VSUC, vascular smooth muscle contraction; CMC, cardiac muscle contraction; PIC, pathways in cancer; BC, Bladder cancer; AD, Alzheimer's disease; PD, Parkinson's disease; HC, Hepaitis C; ATD, autoimmune thyroid disease; DC, dilated cardiomyopathy; CA, Cocaine addiction; NA, Nicotine addiction; AMD, amphetamine addiction; MA, morphine addiction; AL, alcoholism. CK, control; EJ, ejaculation; DPAT, 0.5 mg/kg 8-OH-DPT; DAP, 60 mg/kg dapoxetine.

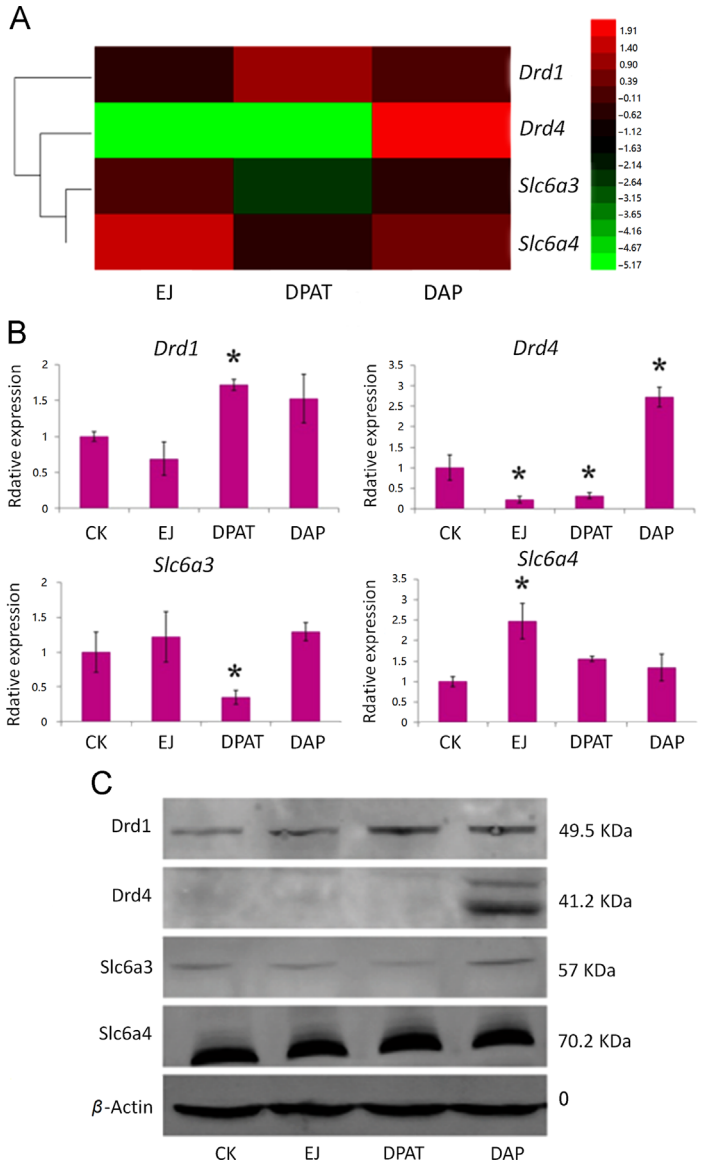

Figure 5 Changes of *Drd1*, *Drd4*, *Slc6a3* and *Slc6a4* expression. (A) Clustering analysis of four genes measured in RNA-Seq. In RNA-Seq, expression was calculated with the log scale of RPKM values. The significant difference between CK and other groups was justified by $|\log_2 \text{Ratio}| \geq 1, p < 0.05$. (B) Relative expression of four genes measured in RT-qPCR. Gene expression was calculated using the $2^{-\Delta\Delta C}$ algorithm with CK as the control ($^{*}P < 0.05$). (C) Four genes corresponding to protein measured in Western blot. The resulting four protein bands were detected clearly. CK, control; EJ, ejaculation; DPAT, 0.5 mg/kg 8-OH-DPT; DAP, 60 mg/kg dapoxetine.

synapses following 8-OH-DPAT administration increased to 9, compared with the EJ group that exhibited a lower number of DEGs (4 and 7). The expression of DEGs of the remaining signaling pathways exhibited no significant difference. All DEGs of the signaling pathways in the dapoxetine group produced no significant difference (Fig. 4A). Consequently, the focus of the investigation was on the dopaminergic and serotonergic synapses. In addition, a considerable number of DEGs were related to diseases (including cancer, Alzheimer's disease, Parkinson's disease, Hepaitis C, autoimmune thyroid and dilated cardiomyopathy) and addiction pathways (Fig. 4C). For example, the number of DEGs that are included in the pathways associated with cancer, Alzheimer's disease and Parkinson's disease increased to 7, 3 and 3 respectively in the DPAT group compared with that in the EJ group (5, 1 and 1). No significant difference was noted in the DAP group. With regard to addiction, the DEGs related to cocaine and amphetamine addiction and alcoholism increased to 5, 6 and 8 respectively in the DPAT group, compared with that in EJ group (1, 1 and 4; Fig. 4D).

3.5. *Analysis of changes in DEGs*

An analysis of the gene expression patterns was carried out using the $\log_2$ Ratio (FPKM) (up-regulation and down-regulation, CK baseline = 1, $|\log_2 \text{Ratio}| \geq 1$, FDR ≤ 0.001) in order to evaluate the differential gene expression in the four transcriptomes. The differences associated with neurotransmitter receptors, transporters, and genes involved in hormonal metabolism were evaluated in the present study. The analysis revealed a differential expression

for the following four genes: *Drd1*, *Drd4*, *Slc6a3*, and *Slc6a4* (Fig. 5). The expression of *Drd4* was significantly down-regulated (fold change: −5.17) during induction of ejaculation by 8-OH-DPAT treatment and up-regulated (fold change: 2.42) during inhibition of ejaculation by dapoxetine compared with the control group. The genes *Drd1* and *Slc6a3* were differentially expressed following induction of ejaculation by the administration of 8-OH-DPAT. Specifically, *Drd1* and *Slc6a3* exhibited a 1.13-fold up-regulation and a −2.28-fold down-regulation. The expression of *Slc6a4* was evident at ejaculation (EJ group) (Fig. 5A). RT-qPCR and Western blot analysis were used to validate these changes in the expression of the genes identified. The results of the expression analysis revealed similar trends in the expression of these four genes compared with the sequencing results (Fig. 5B). For example, the relative expression of *Drd1* mRNA and the corresponding protein increased following stimulation of ejaculation by 8-OH-DPAT. The results of RT-qPCR and Western blot analysis suggest that gene transcription and translation to functional protein occur in the three different treatment groups at ejaculation.

4. Discussion

The effects of 8-OH-DPAT and dapoxetine on the sexual behavior of male rats were investigated in this study. The data reported in the present study suggest that the treatment of male rats with 0.5 mg/kg of 8-OH-DPAT accelerated ejaculation, whereas treatment with 60 mg/kg of dapoxetine delayed ejaculation. The results were in accordance with studies conducted by Ahlenius (0.25 mg/kg s.c.)[13] and Clement (300 mg/kg *p.o.*)[23], although there is dose variation.

The effects of 8-OH-DPAT and dapoxetine on the gene expression profile in the brain during the ejaculation process have been addressed by a limited number of *in vivo* studies. A transcriptomicBodyMap was generated using mRNA-Seq in the brain of male rats. This approach identified the number and the variety of DEGs that were differentially expressed. In addition, the dopamine receptor genes *Drd1*, *Drd4* and the transporter gene *Slc6a3* were found to exhibit significantly different expression, whereas the 5-HT receptor and/or transporter genes, with the exception of *Slc6a4*, did not reveal a significant difference in expression.

The compound 8-OH-DPAT is believed to promote the ejaculation process. In contrast to the present observations, several studies have suggested that 8-OH-DPAT displays a moderate affinity for the D_2-like receptors (D_2, D_3, and D_4)[28,29], whereas stimulation of ejaculation is dependent on the D_2 receptor, as opposed to the 5-HT_{1A} receptor[15,19]. Dupre and coworkers[30] suggested that 8-OH-DPAT could directly stimulate substantia nigra striatum D_1 receptor expression, in part by regulation of the D_2 or DA-mediated release of 5-HT_{1A}. The present study revealed that the D_1 receptor gene *Drd1* was up-regulated, whereas the DA transporter gene *Slc6a3* was down-regulated in male rats following 8-OH-DPAT treatment. Furthermore, genetic polymorphisms of the DA receptor and the transporter genes *Drd4* and *Slc6a3* might affect sexual function, including sexual desire, arousal and premature ejaculation[31,32]. The results presented in the current study revealed that 8-OH-DPAT may stimulate two genes responsible for ejaculation.

Dapoxetine is a novel SSRI that has been considered as a potential 5-HT transport inhibitor[33]. To date, the evidence reported is insufficient to support a direct association of dapoxetine with the dopamine system that is required for the ejaculation process. In the present study, *Drd4* was significantly up-regulated (fold change: 2.42) following dapoxetine treatment whereas no such trend was noted regarding other 5-HT receptor or transporter genes.

It is noteworthy that a number of DEGs were found to be associated with addiction pathways, such as cocaine, amphetamine, morphine, nicotine and alcohol. Ejaculation, which constitutes a potent part of sexual behavior is a reward behavior. At present, the addiction to sex is a well-characterized process and it has been adequately described according to the reward-reinforcement model[34,35], because they share the same biomolecular mechanisms that induce addiction to drugs[34,36], and especially the mechanisms[35,36] apply to sexual addiction. Δ*FosB* (*FosB*'s truncated splice variants) is the most significant transcription factor that is involved in addiction[37] and notably in sexual addiction[35]. In the present study, *Fosb* was up-regulated during the ejaculation process (fold change 1.56). The administration of 8-OH-DPAT during ejaculation increased the expression of *Fosb* (fold change 2.68). This result revealed that 8-OH-DPAT may alter or enhance sexual addiction.

It is important to note that a significant body of data has demonstrated that sex reduces the risk for a number of diseases, including cancer[38,39]. For example in our study, the expression of *Wnt3a* is significantly elevated (fold change 7.53) at ejaculation (EJ group), and *Wnt9b* and *Cdkn1a* are up-regulated at ejaculation that is stimulated by 8-OH-DPAT and inhibited by dapoxetine treatment. The data reported by previous studies[40] have confirmed that *Wnt3a* suppresses cancer cell proliferation, and both *Wnt9b* and *Cdkn1a* suppress cancer progression[41-43].

In conclusion, the present study indicates that a substantial number of genes are up-regulated or down-regulated in the central nervous system at three different states of ejaculation. It is suggested that the acute administration of the compounds 8-OH-DPAT and dapoxetine altered the expression of certain genes, such as *Drd1*, *Drd4* and *Slc6a3* which belong to the class of dopamine receptor and transporter genes. In addition, the expression of addiction- and disease-related genes such as *Fosb*, *Wnt3a, Wnt9b* and *Cdkn1a* was altered in the *in vivo* model used. The present study provides a reliable system to identify the differences in gene expression involved in the ejaculation process stimulated by 8-OH-DPAT and dapoxetine. Despite these promising findings, future studies are required in order to fully elucidate the profile of genes involved in sexual behavior. The obtained results may be applied for clinical diagnosis and treatment of sexual disorders in human patients.

Appendix A. Supporting material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.apsb.2016.11.004.

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