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Transcriptional profiling of ventral tegmental area of male mice with alternative patterns of social behaviors

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Abstract — The activity of ventral tegmental area (VTA) neurons plays a crucial role in the reward circuit, emotional and addictive behaviors in animals and humans. ***To elucidate the molecular mechanisms underlying the functional changes in VTA neurons under stress arising from social interactions, RNA-Seq analysis was used to compare the transcriptome profiles in the VTA of three groups of male mice: chronically winning mice with positive social experience in daily agonistic interactions, chronically defeated mice with negative social experience in daily agonistic interactions, and control mice having no experience of agonistic interactions.*** **The data obtained showed that both winning and defeated mice experience stress, however, in defeated animals, the repeated agonistic interactions have a stronger effect and cause more significant changes in the levels of gene transcription.** ***Several genes have been identified that may be involved in the determination of alternative behavioral phenotypes in groups of male mice with alternative social experience.***

Keywords — ventral tegmental area (VTA), repeated agonistic interactions, RNA-Seq, winners and defeated mice

Introduction

The mesocortical and mesolimbic dopaminergic pathways originating from the ventral tegmental area (VTA) neurons play a crucial role in reward circuit, emotional and addictive behaviors. Induction of VTA dopaminergic activity is associated with the development of a number of psychiatric disorders. Repeated positive or negative social experience in daily agonistic interactions can be considered as an effective experimental approach to elucidate the molecular mechanisms underlying the activity of VTA neurons [1]. The repeated winning or defeated experience of mice leads to the formation of distinctive patterns of behavior which might be characterized as the winning mice having experience of social victories and developing enhanced aggressiveness, and defeated mice having a chronic experience of defeats and developing a mixed anxiety/depression-like state [2-4]. The current study was aimed at searching for the key VTA genes that may be responsible for the formation of alternative behaviors in winning and defeated mice exposed to the repeated agonistic interactions.

Methods

Animals

C57BL/6J male mice aged 10-12 weeks were used. Mice were kept under standard conditions with food and water given *ad libitum*. The study was approved by Scientific Council N 9 of the Institute of Cytology and Genetics SB RAS of March, 24, 2010, N 613 (Novosibirsk). Alternative forms of social behavior were generated as a result of daily agonistic interactions of male mice as described previously [3]. Three groups of animals were used: (1) Controls – mice without the experience of agonistic interactions; (2) chronically winning mice; (3) chronically defeated mice. VTA samples from 3 animals of each group were collected-and stored in RNAlater (Life Technologies, USA) at -70°C until sequencing.

RNA-Seq analysis

The frozen VTA samples were sent to JSC Genoanalytica (Moscow, Russia, http://genoanalytica.ru) where the RNA-Seq analysis was performed. All samples were analyzed as biological replicates. The sequencing data were mapped to the mouse reference genome sequence GRCm38.p3. Cufflinks/Cuffdiff programs were run to identify the differentially expressed genes (DEGs) in the experimental and control groups. Genes were considered differentially expressed at a false discovery rate (FDR) <5%. Only annotated gene sequences were used in the analysis.

Databases used

The Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/) Pathway Database was used to identify the significantly ($p<0.05$) enriched metabolic pathways. To define the association of DEGs with behavior/neurological phenotype the Neurological Disease Portal (Phenotypes, Mouse) in Rat Genome Database (https://rgd.mcw.edu/rgdweb/portal/home.jsp?p=7) was employed. The functional annotation of DEGs was performed by the Database for Annotation, Visualization and Integrated Discovery gene annotation tool (http://david.abcc.ncifcrf.gov/). Atlas of combinatorial transcriptional regulation in mouse and man [5] was used to reveal the DEGs encoding the transcription factor genes.

Statistical methods

The obtained RNA-Seq data (in FPKM values) were analyzed using partial-least squares discriminant analysis (PLS-DA) [6] following by the Pearson correlation between gene expression levels and the coordinates of animals along the first functionally meaningful synthetic PLS-DA Axis 1. A set of expressed genes, which were characterized by the highest correlation coefficients, was considered as genes contributing the most to inter-group differences.

Results

Analysis of gene expression in VTA from winning mice compared to the control revealed 44 differentially expressed genes (DEGs), 14 of which are known as associated with the behavior and neurological phenotype. Nine DEGs encode transcription factors. The results obtained in this comparison suggest an increase in the synthesis and transport of serotonin in VTA of aggressive mice compared to the control.

Analysis of gene expression in VTA of defeated mice compared to the control revealed 188 differentially expressed genes, 64 of which are known as associated with the behavior/neurological phenotype, including six DEGs (*Camk2a, Gad2, Slc6a2, Slc6a3, Slc6a4, Tph2*) known as associated with abnormal depression-related behavior. 28 DEGs encode transcription factors. Based on changes in the level of transcription of a number of genes in VTA of defeated mice as compared to control ones, activation of calcium signaling processes, increased synthesis of GABA (gamma-aminobutyric acid), increased synthesis and transport of serotonin and dopamine, and decreased transport of norepinephrine and glycine can be assumed.

A comparison of the DEG lists revealed 23 common genes that significantly changed the level of transcription in both winning and defeated mice compared to the control. 22 of these genes either increased or decreased the level of transcription unidirectionally in both experimental groups, and only one gene (*Nrgn*, neurogranin) changed the level of transcription in winning and defeated animals in different directions with respect to the control. Accordingly, we can conclude that, regardless of whether the animals are winning or defeated, the level of transcription of a large number of genes in their VTA changes unidirectionally, which is probably the result of the reaction of the animal organism to social stress conditions. It can be assumed that genes whose expression level varies in different directions can make the contribution to the formation of alternative behavioral phenotypes in mice with alternative social experiences in daily agonistic interactions. One of these genes is probably the *Nrgn* gene. In order to identify additional genes that changed the transcription level in winners and defeated mice in different directions, a comparative analysis of gene expression in winners compared to defeated animals was performed.

When comparing the level of gene expression in VTA of winning mice versus the defeated, 207 DEGs were determined. Among them, 6 genes bidirectionally changing the level of transcription in experimental groups of mice relative to the control were found. According to technology based on PLS-DA analysis, four of them (*Six3, Ercc2, Otx2* and *Nrgn*) can make the maximum contribution to intergroup differences when comparing winning and defeated mice. Three of these genes *Ercc2, Otx2*, and *Nrgn* are associated with behavior/neurological phenotype. The most highly expressed of these genes are the *Ercc2* and *Nrgn*. Gene Ontology analysis showed that the *Ercc2* gene is associated with biological processes such as neurogenesis (gliogenesis), regulation of transcription from RNA polymerase II promoter (regulation of gene expression), programmed cell death, stem cell differentiation, response to stimulus, response to hypoxia, immune system development, aging, and the *Nrgn* gene is associated with biological processes such as nervous system development, cell-cell signaling, trans-synaptic signaling, behavior, cognition, regulation of signaling, learning or memory, learning, response to stimulus, signal transduction, regulation of synapse structure or activity, modulation of synaptic transmission, regulation of synaptic plasticity, associative learning, intracellular signal transduction. We suggest that the expression patterns of these genes may be involved in the determination of behavioral differences in mice with alternative social experiences in daily agonistic interactions.

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