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Alteration of neurogenesis-related gene expression in the hippocampus of male mice with pathology of aggressive behavior

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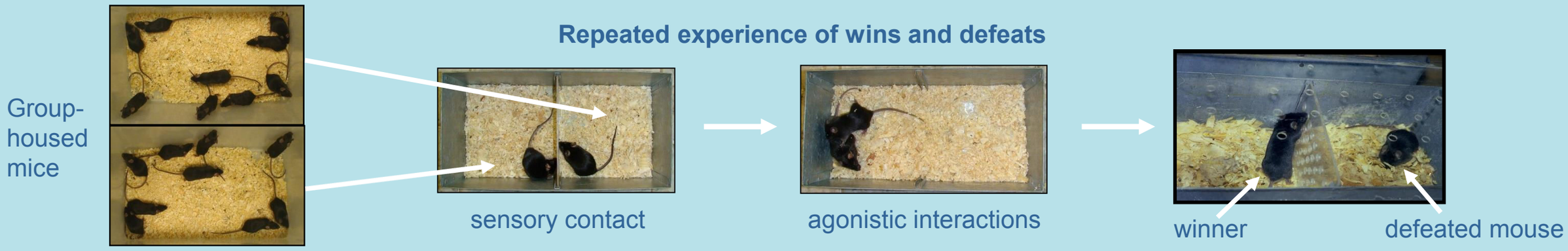
Introduction

Repeated experience of aggression in daily agonistic interactions leads to the development of behavioral pathology [1] similar to psychosis which is accompanied by enhanced aggressiveness and anxiety, hyperactivity, disturbances in social recognition etc. and by changes in neurotransmitters activity in different brain regions in male mice. It has been shown that cell proliferation (BrdU+ cells) and number of new immature neurons (DCX+ cells) were increased in the dentate gyrus of the hippocampus in chronically aggressive males [2]. The aim of the study was to analyze expression of genes involved in regulation of neurogenesis using RNA-Seq database in the hippocampus of male mice with enhanced aggression due to positive fighting experience.

Methods

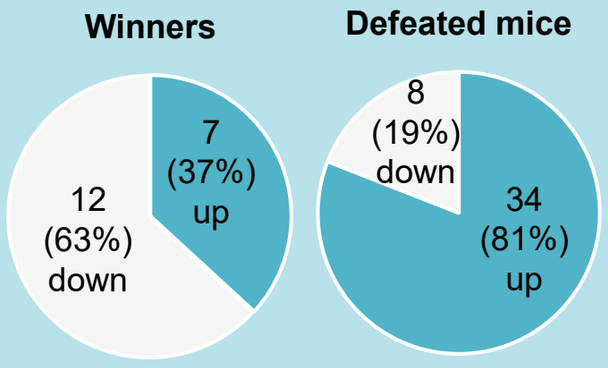


The sensory contact model [3] is used to generate mice who were defeated after 21 days of agonistic interactions (defeated mice) and aggressive males with repeated experiences of aggression accompanied by wins (winners). After three days of sensory contact the partition is removed for 10 min to allow agonistic interactions. Undoubted superiority of one of the partners is evident within 2 or 3 daily social encounters with the same opponent. Then, every day after the test, each defeated member of one pair is paired with a winning member of another pair behind the partition. The winners remain in their original cages.

The hippocampus was sequenced at JSC Genoanalytica (http://genoanalytica.ru, Moscow, Russia). The mRNA was extracted using the Dynabeads mRNA Purification Kit (Ambion, USA). cDNA libraries were constructed using NEBNext mRNA Library PrepReagent Set for Illumina (NEB, USA) following the manufacturer's protocol. The Cufflinks program was used to estimate the gene expression levels in fragments per kilobase of transcript per million mapped reads. The Mammalian Adult Neurogenesis Gene Ontology, (MANGO, http://mango.adult-neurogenesis.de) was used to identify the neurogenesis-related genes.

Results

Neurogenesis-related DEG in the winners and defeated mice



Correlation matrix of 8 down-regulated DEG involved in the neurogenesis regulation in the winners

Variables	*Heyl*	*Nog*	*Rara*	*Cit*	*Pou3f3*	*Foxg1*	*Neurod2*	*Vgf*
Heyl	**1**	**0,783**	**0,733**	**0,750**	0,600	**0,833**	0,450	0,583
Nog	**0,783**	**1**	**0,717**	**0,933**	**0,783**	**0,833**	0,683	**0,917**
Rara	**0,733**	**0,717**	**1**	0,583	0,600	**0,767**	**0,700**	0,600
Cit	**0,750**	**0,933**	0,583	**1**	**0,783**	**0,900**	0,583	**0,883**
Pou3f3	0,600	**0,783**	0,600	**0,783**	**1**	**0,800**	**0,900**	**0,867**
Foxg1	**0,833**	**0,833**	**0,767**	**0,900**	**0,800**	**1**	0,667	**0,750**
Neurod2	0,450	0,683	**0,700**	0,583	**0,900**	0,667	**1**	**0,750**
Vgf	0,583	**0,917**	0,600	**0,883**	**0,867**	**0,750**	**0,750**	**1**

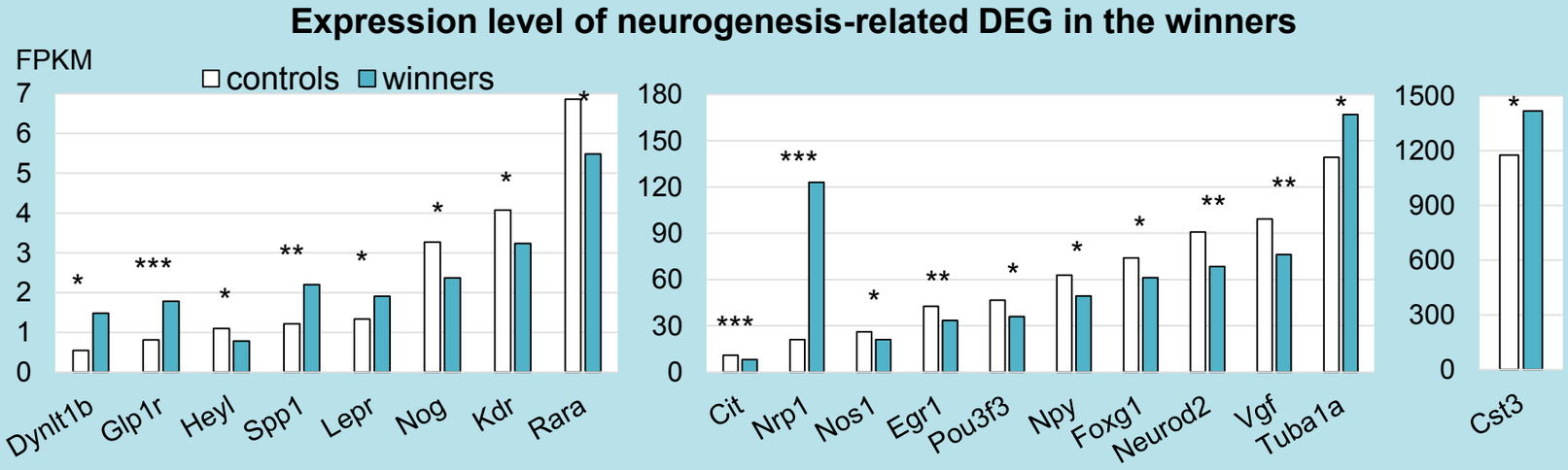
Values in bold are different from 0 with a significance level alpha = 0.05

Changes in the expression of neurogenesis-related DEG in the winners

Gene	Gene product	Changes
Dynlt1b	dynein light chain Tctex-type 1B (Dynlt1b)	△
Glp1r	glucagon-like peptide 1 receptor (Glp1r)	△△△△
Heyl	hairy/enhancer-of-split related with YRPW motif-like (Heyl)	▼
Spp1	secreted phosphoprotein 1 (Spp1)	△△
Lepr	leptin receptor (Lepr)	△
Nog	noggin (Nog)	▼
Kdr	kinase insert domain protein receptor (Kdr)	▼
Rara	retinoic acid receptor, alpha (Rara)	▼
Cit	citron (Cit)	▼▼▼
Nrp1	neuropilin 1 (Nrp1)	△△△△
Nos1	nitric oxide synthase 1, neuronal (Nos1)	▼
Egr1	early growth response 1 (Egr1)	▼▼
Pou3f3	POU domain, class 3, transcription factor 3 (Pou3f3)	▼
Npy	neuropeptide Y (Npy)	▼
Foxg1	forkhead box G1 (Foxg1)	▼
Neurod2	neurogenic differentiation 2 (Neurod2)	▼▼
Vgf	VGF nerve growth factor inducible (Vgf)	▼▼
Tuba1a	tubulin, alpha 1A (Tuba1a)	△
Cst3	cystatin C (Cst3)	△

▼ – decreased expression; △ – increased expression *vs* the controls; △ – $p < 0.05$; △ △ – $p < 0.01$; △ △ △ △ – $p < 0.0001$; ▼ – $p < 0.05$; ▼ ▼ – $p < 0.01$; ▼ ▼ ▼ – $p < 0.001$.

Expression level of neurogenesis-related DEG in the winners



DEG - differentially expressed genes; * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$ *vs* the controls.

There were analyzed more than 300 genes important for the neurogenesis presented in the MANGO database and other resources. DEG with the majority of decreased expression (12 of 19) were detected in the aggressive mice as compared to the controls. The downregulated genes were *Heyl, Nog, Kdr, Rara, Cit, Nos1, Egr1, Pou3f3, Npy, Foxg1, Neurod2,* and *Vgf* encoded the proteins which have different functions and some of them are transcriptional regulation factors acting as repressors. Upregulated genes were *Dynlt1b, Glp1r, Spp1, Lepr, Nrp1, Tuba1a* and *Cst3* mostly encoding different receptors. Correlation analysis revealed reliable functional interactions between expression of most downregulated genes, but not upregulated ones. In particular, the expression of *Foxg1 and Heyl* genes that may act as transcriptional repressors is highly correlated with each other and with other genes.

Conclusions

These data show differentially expressed genes in aggressive male mice with activated neurogenesis in the hippocampus. We can assume that disturbances of transcription, in particular, the *Foxg1* and *Heyl* genes which act as the transcriptional repressors, may contribute to the increased neurogenesis, stimulating cell proliferation which as consequence, is accompanied by growth of new neurons as was shown earlier [2]. Close association between expression of most downregulated genes may indicate common disturbances in transcription processes in the hippocampus. This study may be useful for understanding the molecular mechanisms of the control of neurogenesis processes in the brain.

[1] Kudryavtseva, N.N., 2006. Psychopathology of repeated aggression: a neurobiological aspect, in: Perspectives on the Psychology of Aggression, ed. Morgan J.P. NY: Nova Science Publichers, Inc. Ch. 2., pp. 35-64.

[2] Smagin, D.A., Park J-H, Michurina, T.V., Peunova, N., Glass, Z., Sayed, K., Bondar, N.P., Kovalenko, I.L., Kudryavtseva, N.N. and Enikolopov, G., 2015. Altered hippocampal neurogenesis and amygdalar neuronal activity in adult mice with repeated experience of aggression. Front Neurosci 9:443.

[3] Kudryavtseva, N.N., Smagin, D.A., Kovalenko, I.L., Vishnivetskaya, G.B., 2014. Repeated positive fighting experience in male inbred mice. Nature Protoc 9(11), 2705-17.

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