Journal of Invertebrate Pathology 128 (2015) 37-43

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

Transcriptome analysis of the brain of the silkworm *Bombyx mori* infected with *Bombyx mori* nucleopolyhedrovirus: A new insight into the molecular mechanism of enhanced locomotor activity induced by viral infection

Guobao Wang^a, Jianjia Zhang^a, Yunwang Shen^a, Qin Zheng^a, Min Feng^a, Xingwei Xiang^b, Xiaofeng Wu^{a,*}

^a College of Animal Science, Zhejiang University, Hangzhou 310029, China
^b Zhejiang Marine Development Research Institute, Zhoushan 316000, China

ARTICLE INFO

Article history: Received 6 February 2015 Revised 2 April 2015 Accepted 8 April 2015 Available online 22 April 2015

Keywords: Bombyx mori Brain Transcriptome Viral infection Enhanced locomotor activity

ABSTRACT

Baculoviruses have been known to induce hyperactive behavior in their lepidopteran hosts for over a century. As a typical lepidopteran insect, the silkworm *Bombyx mori* displays enhanced locomotor activity (ELA) following infection with B. mori nucleopolyhedrovirus (BmNPV). Some investigations have focused on the molecular mechanisms underlying this abnormal hyperactive wandering behavior due to the virus; however, there are currently no reports about B. mori. Based on previous studies that have revealed that behavior is controlled by the central nervous system, the transcriptome profiles of the brains of BmNPV-infected and non-infected silkworm larvae were analyzed with the RNA-Seg technique to reveal the changes in the BmNPV-infected brain on the transcriptional level and to provide new clues regarding the molecular mechanisms that underlies BmNPV-induced ELA. Compared with the controls, a total of 742 differentially expressed genes (DEGs), including 218 up-regulated and 524 down-regulated candidates, were identified, of which 499, 117 and 144 DEGs could be classified into GO categories, KEGG pathways and COG annotations by GO. KEGG and COG analyses, respectively. We focused our attention on the DEGs that are involved in circadian rhythms, synaptic transmission and the serotonin receptor signaling pathway of B. mori. Our analyses suggested that these genes were related to the locomotor activity of B. mori via their essential roles in the regulations of a variety of behaviors and the down-regulation of their expressions following BmNPV infection. These results provide new insight into the molecular mechanisms of BmNPV-induced ELA.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

An intriguing phenomenon in which parasites manipulate the behavior of their hosts to increase parasite transmission rates has been observed in a variety of organisms (Beckage, 1997; Moore, 2002; Lefèvre et al., 2009). Parasites are capable of imposing dramatic behavioral influences on their hosts that include alterations in foraging, location and locomotor activity (Moore, 2002). Some parasitoid wasps coerce spiders into spinning 'sleeping bags' that are suspended from branches and provide a safe pupating site for the wasp (Eberhard, 2000). Aedes aegypti mosquitoes carrying Dengue virus (*Flaviviridae*) display a 50% increase in locomotor activity compared with uninfected mosquitoes (Lima-Camara

et al., 2011) and exhibit longer probing and feeding times (Platt et al., 1997). Many of these behavioral changes help to increase parasite transmission rates and are thought to be conversed strategies of the parasite for manipulating their hosts' behaviors (Moore, 1995). Although numerous examples of such manipulations are known, the molecular mechanisms underlying these phenomena are still largely enigmatic, particularly regarding the changes that occur in the host.

Research on the parasitic manipulation of behavior has focused on the effects of parasites on host neural function. This emphasis is reasonable given that behavior is controlled by the central nervous system (Moore, 2002; Klein, 2003). Because the brain is a behaviorregulating center, parasites control their hosts' behavior by manipulating brain activity to a certain extent (Adamo, 2002; Thomas et al., 2005). For example, the phenomenon in which the gammarid host *Gammarus lacustris* is infected by the acanthocephalan







^{*} Corresponding author. Tel.: +86 571 88982198; fax: +86 571 88982130. *E-mail address:* wuxiaofeng@zju.edu.cn (X. Wu).

Polymorphus paradoxus and subsequently displays abnormal escape behavior has been proven to result from the adverse effect exert by the parasite on the host's serotonergic system in the brain (Maynard et al., 1996). Several lines of research have applied proteomic technology to explore the molecular mechanisms underlying the phenomena of parasite manipulation of the host brain at the protein level (Biron et al., 2005, 2006; Ponton et al., 2006; Lefèvre et al., 2007a,b). However, a detailed report about the molecular mechanisms in the host brain at the gene expression level is lacking.

Baculoviruses have been known to induce hyperactive behavior in their lepidopteran hosts for over a century (Goulson, 1997; Hoover et al., 2011; Houte et al., 2012). The diseased larvae often display enhanced locomotor activity (ELA), which leads them to migrate to the upper plant foliage and subsequently die. The decaying corpse will contaminate a larger surface area of the host plant resulting in increased dispersal and transmission of the virus. Similar to baculovirus hosts, the silkworm (*Bombyx mori*), which is a typical lepidopteran insect that has been domesticated for over 5000 years, displays enhanced locomotor activity after infection by the *B. mori* nucleopolyhedrovirus (BmNPV). Although Kamita et al. (2005) and Katsuma et al. (2012) found that the protein tyrosine phosphatase (*ptp*) gene of BmNPV plays an essential role in this ELA phenomenon, there are no further investigations of the molecular mechanisms of this ELA phenomenon in *B. mori*.

B. mori is not only an important economic insect but is also a rising model organism. *B. mori* possesses a variety of advantages, such as ease of rearing and experimental manipulation as compared with other Lepidoptera insects. Moreover, the whole-genome sequencing of *B. mori* has been completed (Xia et al., 2004). Recently, RNA-sequencing (RNA-Seq), which is a powerful tool for transcriptome analyses and is based on deep sequencing technology, has been used to explore the molecular mechanisms of multiple physiological events in the silkworm (Li et al., 2012; Nie et al., 2014).

In the present study, RNA-Seq was applied to the polyadenylate-enriched mRNAs from the silkworm brains to better understand the complexity of the molecular mechanisms underlying BmNPV-induced ELA in *B. mori*. We obtained both up- and downregulated genes by comparing the brain transcriptome profiles between BmNPV-infected and non-infected silkworm groups. We also screened the ELA-related genes from the DEGs via bioinformatic analyses. We believe that our data will provide useful information that will expand our current knowledge about the molecular mechanisms underlying BmNPV-induced ELA in *B. mori*.

2. Materials and methods

2.1. Viruses and silkworm strain

BmNPV was stored in our laboratory. The Dazao silkworm strain was used in the study. The larvae were reared on fresh mulberry leaves in an environment with a 12-h light/12-h dark at 25 ± 1 °C and 70–85% relative humidity. Newly molted larvae of the fifth instar were used for the experiments.

2.2. Oral infection of the silkworms with B. mori nucleopolyhedrovirus (BmNPV) for transcriptome analyses

Approximately 15 newly molted silkworm larvae of the 5th-instar from each of a total of 10 groups were placed in 10 petri dishes and deprived of food prior to infection. For the oral infections, the silkworms were fed mulberry leaves with added BmNPV polyhedra. The infection dose was calculated to be 2×10^4 polyhedra per larva. By 3 h, most of the viral meal was ingested. The larvae were then transferred to a large petri dish with normal mulberry leaves. For the non-infected controls, the same volume of 0.9% physiological saline was mixed in the feed for the silkworms, and the rearing conditions were identical to those of the BmNPV-infected groups. All experiments were performed in three independent biological replications.

2.3. RNA extraction

The larval brains were dissected from the BmNPV-infected and non-infected groups (Fig. 1) at 96 h post-infection when the BmNPV-induced silkworms began to display abnormal hyperactive wandering behaviors and were then immediately snap9-frozen in liquid nitrogen. Total RNA was extracted from each sample using Trigol reagent (DingGuo Biotechnology, Beingjing, China) following the manufacturer's instructions. The RNA was quantified by measuring the absorbance at 260 nm using a NanoVue UV–Vis spectrophotometer (Bio-Science). The purities of all RNA samples were assessed at absorbance ratios of $OD_{260/280}$ and $OD_{260/230}$. The RNA integrity number (RIN) values (>8.0) of the samples were assessed using an Agilent 2100 Bioanalyzer (Santa Clara, CA, USA).

2.4. cDNA library construction and Illumina RNA-Seq

Libraries construction and RNA-Seq were performed by the Biomarker Biotechnology Corporation (Beijing, China). Briefly, the mRNAs were enriched with a NEBNext Poly (A) mRNA Magnetic Isolation Module (NEB, E7490). The enriched mRNAs were used as templates, and cDNA libraries were constructed using the NEBNext mRNA Library Prep Master Mix Set for the Illumina (NEB, E6110) and NEBNext Multiplex Oligos for the Illumina (NEB, E7500) following the manufacturer's instructions. The inserts sizes were detected with 1.8% agarose gel electrophoresis. cDNA library quantification was performed with quantitative RT-PCR using the Library Quantification Kit-Illumina GA Universal (Kapa, KK4824) according to the protocol. Clusters of the cDNA libraries were generated on an Illumina cBot. Finally, the cDNA libraries were sequenced on an Illumina HiSeq[™] 2000.

2.5. RNA-Seq data analysis

The clean reads were filtered from the raw reads by removing the reads with only adaptors and unknown nucleotides >5% and those that were of low quality. The clean reads were then mapped to the silkworm genome sequence obtained from the SilkDB



Fig. 1. BmNPV-infected and non-infected silkworms. (A) A BmNPV-infected silkworm. (B) A non-infected silkworm. Bar = 1 cm.

database (http://silkworm.swu.edu.cn/silkdb/) using the TopHat software (Trapnell et al., 2009). The gene expression levels were calculated using the fragments per kilobase of exon per million fragments mapped (FPKM) method (Mortazavi et al., 2008). A FPKM >0.1 threshold was applied to ensure that the genes were expressed in two groups. The criteria of a fold change (FC) ≥ 2 and a false discovery rate (FDR) < 0.01 were used to isolate the differentially expressed genes. The ratios of gene expression between the two groups (i.e. the BmNPV and control groups) with values greater than 2.0 were taken to indicate up-regulation, and those below 0.5 were taken to indicate down-regulation. The sequencing data generated in this study were deposited in the NCBI Sequence Read Archive (SRA, http://www.ncbi.nlm.nih.gov/sra) with accession number SRR1928169.

2.6. Bioinformatics analysis

The sequences of the differentially expressed transcripts were subjected to BLAST queries against the Ami gene ontology (GO) database (http://amigo.geneontology.org/cgi-bin/amigo/blast.cgi). The corresponding GO terms were extracted from the most homologous genes using a Perl program. The GO annotation results were plotted with the Web Gene Ontology Annotation Plot (WEGO) (http://wego.genomics.org.cn/cgi-bin/wego/index.pl) tool bv uploading compiled WEGO-native format files containing the obtained GO terms. The FASTA gene sequences of the differentially expressed transcripts were searched against the online Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http:// www.genome.jp/tools/blast/). The corresponding KEGG pathways were extracted. The pathways were classified according to the definitions of the KEGG (http://www.genome.ad.jp/kegg/pathway. html). Additionally, the gene sequences of the differentially regulated transcripts were subjected to the Clusters of Orthologous Groups (COG) database (http://www.ncbi.nlm.nih.gov/COG/), and the corresponding COG annotation results were extracted.

2.7. Validation of RNA-Seq by quantitative RT-PCR

To validate the transcriptome data, 10 genes (7 down-regulated and 3 up-regulated) were selected for qPCR. The primer sequences and related information are shown in Table S1.

The total RNAs isolated from both the BmNPV-infected and non-infected groups were reverse transcribed using a GoScriptTM Reverse Transcription System (A5001, Promega) following the manufacturer's instructions. According to the SYBR Premix Ex TaqTM Kit (TaKaRa) protocol, the reactions were run on an ABI7300 real-time PCR system using a 20-µL reaction system with reaction procedures of 40 cycles of 94 °C for 3 s and 60 °C for 30 s followed by a dissociation for the quality control of the amplified products. All samples were examined in triplicate. The expression level of each gene was calculated with the $2^{-\triangle Ct}$ method (Livak and Schmittgen, 2001) and normalized to the silkworm house-keeping gene *actinA3*. Following normalization, the ratios were expressed as fold changes compared with the samples from the control group.

3. Results

3.1. General transcriptional patterns

Whole genome mRNA sequencing was used to monitor the global changes in gene expression in the silkworm brain. After filtering, 64.05% and 87.85% clean reads from the two groups were matched to the silkworm genome using Tophat software; the proportions of the unique mapped reads accounted for the mapped reads were 95.92% and 96.48%, respectively (Table 1). Alignment against the silkworm genomic sequence revealed 742 differentially expressed genes (DEGs) that included 218 up-regulated and 524 down-regulated genes in the BmNPV-infected group (Table S2).

3.2. Gene ontology (GO) analysis of the differentially expressed genes

The DEGs were assigned to various GO categories to determine their functional classifications (Fig. 2). A total of 499 genes (123 up-regulated and 376 down-regulated, Table S3) with GO annotations were classified into the following 3 functional categories: cellular component, molecular function and biological process. Overall, 17, 12 and 22 catalogs of cellular component, molecular function and biological process were clustered, respectively. The majority of the annotated DEGs were assigned to the cell, membrane and organelle catalogs in the cellular component category. The widest distribution of the DEGs was associated with binding and catalytic activity in the molecular function category. In the category of biological process, the majority of the DEGs were involved in metabolic process, cellular process, multicellular organismal process, developmental process, response to stimulus and biological regulation.

3.3. KEGG pathway analysis of the differentially expressed genes

A total of 117 DEGs were assigned to 87 different pathways in this study (Table S4). As shown in Fig. 3, the pathways were classified into the following six categories: metabolism, genetic information processing, environmental information processing, cellular processes, organismal systems and human diseases. In the mapped pathways of the up-regulated genes, the abundant genes mapped onto carbohydrate, nucleotide and amino acid metabolisms. Similarly, the down-regulated genes were also primarily related to carbohydrate metabolism. However, it is noteworthy that there were numerous down-regulated genes that were associated with signal transduction and interactions between signaling molecules, suggesting that abnormal signal transmission occurred in the brains of the silkworms following infection with BmNPV.

3.4. COG analysis of the differentially expressed genes

COG is a database in which orthologous gene products are classified. COG analysis of the DEGs provided informations about their possible functions (Table S5 and Fig. 4). A total of 144 DEGs with 19 functional definitions were obtained. Down-regulated genes accounted for a large proportion of this set, and observations that amino acid transport and metabolism, carbohydrate transport and metabolism and signal transduction mechanisms were prevalent in the down-regulated genes corresponded well with the results

Table 1	
Statistical analyses of the transcriptome sequence data.	

Clean reads	Total reads	40,765,270	42,239,674
	Total bases	4,076,243,285	4,223,650,782
	GC%	42.79	44.31
	Q30%	87.50	91.17
Mapping to genome	Mapped reads Unique mapped reads Multiple mapped reads Pair mapped reads Single mapped	26,110,238 (64.05%) 25,305,377 (96.92%) 804,861 (3.08%) 23,239,822 (89.01%) 2,192,092 (9.40%)	37,106,733 (87.85%) 36,106,158 (97.30%) 1,000,575 (2.70%) 34,079,941 (91.84%) 2,215,837 (6.07%)



Fig. 2. GO categories of the differentially expressed genes (DEGs). The annotated DEGs were classified into the cellular component, molecular function and biological process categories by WEGO according to the GO terms. The right panel shows the gene numbers mapped to the GO terms. The left panel shows the proportions of up-regulated and down-regulated genes according to the GO terms. The right panel shows the numbers of up- and down-regulated genes.



Fig. 3. Pathway categories of the 117 DEGs according to the KEGG pathway taxonomy. The pathways were clustered into metabolism, genetic information processing, environmental information processing, cellular processes, organismal systems and human diseases. The left panel shows the numbers of genes that mapped to each of the pathways.

of the KEGG analysis. Among the up-regulated genes, nucleotide transport and metabolism, translation, ribosomal structure and biogenesis were highly represented, suggesting abnormal metabolism in the brains of the *B. mori* that were infected with BmNPV at the transcriptional and protein levels. Our COG analysis results were consistent with the results of our GO and pathway analyses.

3.5. Validation of the data reliability by qRT-PCR

To verify the RNA-seq results, we selected 10 DEGs by designing specific primers for quantitative RT-PCR. Our results indicated that the changes observed in the expression levels of these genes (Fig. 5) mimicked the changes measured with RNA-seq. Hence, the qPCR results confirmed the reliability of our RNA-seq data.

4. Discussion

B. mori nucleopolyhedrovirus is an exclusive pathogen of the silkworm that frequently causes serious damage to sericulture production. The BmNPV-induced ELA of *B. mori* often occurs during the late stage of infection and is characterized by irregular movements of the infected larvae. It is believed that the virus induces this behavior so that after death, the decaying cadaver will release virions and contaminate a larger surrounding area, resulting in



Fig. 4. Clusters of orthologous group classifications of the differentially expressed genes. The up-regulated and down-regulated genes according to the BmNPV-group.



Fig. 5. Quantitative RT-PCR analyses were used to validate the differentially expressed genes according to RNA-seq. Ten genes were selected for analysis and exhibited changes that were similar to those measured by RNA-seq. The Ct values of each reaction were normalized to the endogenous control *actinA3*. CRY1, cryptochrome 1; ACE1, acetylcholinesterase type 1; CaMKII, Ca²⁺/calmodulin-dependent protein kinase II; TAR, tyramine receptor; Ser4, serotonin receptor 4; P450, cytochrome P450; TnT, troponin T; CDA, cytidine deaminase; CDK2, cyclin-dependent kinase 2.

increased dispersal and transmission of the virus (Hoover et al., 2011).

As the main component of the central nervous system, the brain plays essential roles in receiving and transmitting external signals and coordinating different types of behavior (Straussa and Heisenberg, 1993). Intrigued by the relationship between infection and brain activity and hence hyperactive locomotion, we used RNA-Seq to investigate the changes in the BmNPV-infected brains of *B. mori* on the transcriptional level. A high dose $(2 \times 10^4 \text{ polyhe})$ dra per larva) was used to infect the larvae and ensure that they acquired the NPV disease during the fifth instar. The time point at which the silkworms displayed enhanced locomotor activity (96 h post-inoculation) was selected to compare the genes in the BmNPV-infected brains that were differentially expressed relative to the controls. At 96 h post-inoculation, a total of 742 DEGs were identified. These DEGs during infection provide potential insights into the complex regulatory phenomena of the response to viral infection at the transcriptional level. In this study, we focused on the DEGs that were related to the locomotion of B. mori, and we classified them into three categories that included circadian rhythms, synaptic transmission and the serotonin receptor signaling pathway (Table 2).

4.1. Differentially expressed genes related to circadian rhythms

Disturbances in circadian rhythms are known to affect a variety of behaviors. Easton et al. (2003) showed that mutations in the circadian gene *clock* lead to increased exploratory activity and escape-seeking behavior. We found two down-regulated genes

Table 2

Classification	Gene ID	NCBI blast annotation	Log ₂ FC	Regulated
Circadian rhythm	BGIBMGA000031	Ebony [Bombyx mori]	-1.52	Down
	BGIBMGA007140	Cryptochrome 1 [Bombyx mori]	-1.01	Down
Synaptic transmission	BGIBMGA000943	Synaptobrevin-like [Bombyx mori]	-1.78	Down
	BGIBMGA013466	PREDICTED: vesicular acetylcholine transporter-like [Bombyx mori]	-6.32	Down
	BGIBMGA010205	PREDICTED: vesicle-associated membrane protein 2-like [Bombyx mori]	-1.70	Down
	BGIBMGA006590	PREDICTED: syntaxin-1A-like [Bombyx mori]	-1.02	Down
	BGIBMGA003321	Acetylcholinesterase type 1 [Bombyx mori]	-1.27	Down
	BGIBMGA010643	PREDICTED: vesicular glutamate transporter 2.2-like [Bombyx mori]	-1.74	Down
	BGIBMGA005724	PREDICTED: growth/differentiation factor 8-like [Bombyx mori]	-1.31	Down
	BGIBMGA000408	Ca ²⁺ /calmodulin-dependent protein kinase II [Bombyx mori]	-2.77	Down
	BGIBMGA006298	PREDICTED: peripheral plasma membrane protein CASK-like [Bombyx mori]	-1.35	Down
Serotonin receptor signaling pathway	BGIBMGA011067	Tyramine receptor [Bombyx mori]	-2.06	Down
	BGIBMGA006929	Serotonin receptor 4 [Bombyx mori]	-1.09	Down
	BGIBMGA002316	Octopamine receptor [Bombyx mori]	-2.13	Down

Note: FC indicates the fold change (BmNPV-/Control-) of gene expression in FPKM.

that are involved in the circadian rhythms of insects, i.e. BGIBMGA000031 and BGIBMGA007140 (which encode ebony protein and cryptochrome-1, respectively) (Table 2). The ebony protein can be detected in the hypodermis, visual system and other brain regions (Richardt et al., 2002) and plays essential roles in cuticle tanning and sclerotization and nervous system functions such as vision and behavior (Richardt et al., 2003). Previous biochemical investigations have led to the conclusion that ebony has a β -alanyl-dopamine synthetase function and that mutations in ebony, among other effects, selectively perturb phototaxis and locomotor activity rhythm and cause arrhythmicity in Drosophila melanogaster (Newby and Jackson, 1991; Suh and Jackson, 2007). In insects, cryptochromes act as components of the circadian clock that control daily physiological and behavioral rhythms and as photoreceptors that mediate the entrainment of the circadian clock to light (Cashmore, 2003). In our study, it is possible that the down-regulations of ebonv and cryptochrome-1 caused by infection with BmNPV resulted in the disturbance of the locomotor activity rhythm and gave rise to the abnormal behavior of *B. mori*.

4.2. Differentially expressed genes in synaptic transmission

The nervous system exerts its functions through synaptic transmission. Neurons receive information from sensory organs, send information to motor organs and share information with other neurons. Neurotransmitters contained in synaptic vesicles are transmitted to the various parts of the organism via synaptic transmission and regulate the different types of behavior exhibited by an organism (Calabresi et al., 2000). In our study, nine DEGs involved in synaptic transmission were down-regulated in the BmNPV-infected brains (Table 2).

Vesicle-associated membrane protein (also known as synaptobrevin) is a type II membrane protein of small synaptic vesicles and is essential for neuroexocytosis because its proteolysis by tetanus and botulinum neurotoxins types B, D, F and G blocks neurotransmitter release (Washbourne et al., 1995). An important step in cholinergic transmission involves the vesicular storage of acetylcholine, and this process is mediated by the vesicular acetylcholine transporter (VAChT). Prado et al. (2006) found that VAChT deficiency in mice alters synaptic vesicle filling and affects Ach release and that VAChT-knockdown severely impairs object and social recognition behavior in mice. Acetylcholinesterase (AChE) is known to play important roles in the maintenance of the normal transmission of neural impulses in synaptic clefts by catalyzing the hydrolysis of the neurotransmitter acetylcholine (Lang et al., 2010). Jensen et al. (1997) reported on the relationship between AChE inhibition and locomotor behavior in the carabid beetle Pterostichus cupreus and noted that AChE activity affects the behavioral sensitivity of P. cupreus. Additionally, Gupta and Sundararaman (1991) observed a relationship between AChE activity and burrowing capability in earthworms that had been exposed to a carbamate insecticide. These results suggest critical roles of VAChT and AChE in the regulation of a variety of behaviors in animals. Syntaxin-IA is a synaptic membrane protein that is localized to axons and synapses in the nervous system. The complete absence of syntaxin-1A causes subtle morphological defects in the peripheral and central nervous systems that affect nonneural secretory events and entirely abolishes neurotransmitter release in D. melanogaster (Schulze et al., 1995). Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) is one of the most abundant kinases in the brain and is also a major calcium-regulated signal transducer that regulates many neuronal systems, such as the synthesis and release of neurotransmitters (Schulman, 1993). Kadivar et al. (2014) found that CaMKII activity is involved in chronic morphine-induced behavioral plasticity in the rat. Thus, we conclude that the down-regulations of the DEGs (i.e. BGIBMGA000943, BGIBMGA013466, BGIBMGA010205,

BGIBMGA006590, *BGIBMGA003321*, *BGIBMGA010643*, *BGIBMGA005724*, *BGIBMGA000408* and *BGIBMGA006298*) might have affected synaptic transmission in the BmNPV-induced brains and resulted in abnormal signal transduction and hence abnormal behavior.

4.3. Differentially expressed genes in the serotonin receptor signaling pathway

In insects, the signaling of the biogenic amine serotonin (5-hydroxytryptamine, 5-HT) controls a variety of behaviors including those related to circadian rhythms, aggression, behavioral gregarization and phototaxis in locusts, honeybees and fruit flies (Dierick and Greenspan, 2007; Anstey et al., 2009; Thamm et al., 2010; Ott et al., 2012). The specific functions of serotonin are mediated by its binding to and subsequent activation of membrane receptors. Yuan et al. (2005) showed that serotonin acting via the D. melanogaster 5-HT_{1B} receptor affects circadian rhythms. The activation of the 5-HT₂ receptor with DOI has been shown to decrease overall aggression in D. melanogaster (Johnson et al., 2009). Silva et al. (2014) employed the Gal4-UAS technique to pan-neuronally express RNAis directed to the transcripts of each of the cloned 5-HT receptors in Drosophila larvae via the use of the elav-Gal4 driver and found that the animals that expressed RNAis for 5-HT_{1B}, 5-HT₂ and 5-HT₇ receptors in the mushroom bodies exhibited increased motor behavior. Here, three related genes, BGIBMGA011067, BGIBMGA006929 and BGIBMGA002316 (annotated as the tyramine receptor, serotonin receptor 4 and octopamine receptor, respectively) were down-regulated in the BmNPV-infected brains. Previous studies have reported that tyramine and octopamine modulate the locomotion of D. melanogaster larvae (Saraswati et al., 2004) and behavioral responses to addictive drugs, such as ethanol (Scholz, 2005). We speculate that the down-regulation of these DEGs might have altered the levels of biogenic amines (BA) in the brain. The increased locomotion observed in the BmNPV-infected silkworm might be involved in the modification of BA levels.

Additionally, a rather large number of DEGs were involved in some other biological processes and functional categories (Table S3), which indicates a series of major physiological and pathological changes in the silkworm brain following infection with BmNPV. In conclusion, our results provide new avenues to explore the molecular mechanisms underlying the ELA phenomenon caused by BmNPV in *B. mori*. Furthermore, we obtained genes with unknown functions, which should not be neglected because they might make important contributions to this pathogen-host interaction.

Acknowledgments

This work was supported by the National Basic Research Program of China (2012CB114600) and the Natural Science Foundations of Zhejiang Province (LZ14C170001, LQ14C170001) and China (31272506, 31472146).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jip.2015.04.001.

References

Adamo, S.A., 2002. Modulating the modulators: parasites, neuromodulators and host behavioral change. Brain Behav. Evol. 60, 370–377.

Anstey, M.L., Rogers, S.M., Ott, S.R., Burrows, M., Simpson, S.J., 2009. Serotonin mediates behavioral gregarization underlying swarm formation in desert locusts. Science 323, 627–630.

- Beckage, N.E., 1997. Parasites and Pathogens-Effects on Host Hormones and Behavior. Chapman and Hall, New York.
- Biron, D.G., Marché, L., Ponton, F., Loxdale, H.D., Galéotti, N., Renault, L., Joly, C., Thomas, F., 2005. Behavioural manipulation in a grasshopper harbouring hairworm: a proteomics approach. Proc. Biol. Sci. 272, 2117–2126.
- Biron, D.G., Ponton, F., Marché, L., Galeotti, N., Renault, L., Demey-Thomas, E., Poncet, J., Brown, S.P., Jouin, P., Thomas, F., 2006. 'Suicide' of crickets harbouring hairworms: a proteomics investigation. Insect Mol. Biol. 15, 731–742.
- Calabresi, P., Centonze, D., Gubellini, P., Marfia, G.A., Pisani, A., Sancesario, G., Bernardi, G., 2000. Synaptic transmission in the striatum: from plasticity to neurodegeneration. Prog. Neurobiol. 61, 231–265.
- Cashmore, A.R., 2003. Cryptochromes: enabling plants and animals to determine circadian time. Cell 114, 537–543.
- Dierick, H.A., Greenspan, R.J., 2007. Serotonin and neuropeptide F have opposite modulatory effects on fly aggression. Nat. Genet. 39, 678–682.
- Easton, A., Arbuzova, J., Turek, F.W., 2003. The circadian *clock* mutation increases exploratory activity and escape-seeking behavior. Genes, Brain Behav. 2, 11–19.
- Eberhard, W.G., 2000. Spider manipulation by a wasp larva. Nature 406, 255–256. Goulson, D., 1997. Wipfelkrankheit: modification of host behaviour during baculoviral infection. Oecologia 109, 219–228.
- Gupta, S.K., Sundararaman, V., 1991. Correlation between burrowing capability and AChE activity in the earthworm, *Pheretima posthuma*, on exposure to carbaryl. Bull. Environ. Contam. Toxicol. 46, 859–865.
- Hoover, K., Grove, M., Gardner, M., Hughes, D.P., McNeil, J., Slavicek, J., 2011. A gene for an extended phenotype. Science 333, 1401.
- Houte, S.V., Ros, V.I.D., Mastenbroek, T.G., Vendrig, N.J., Hoover, K., Spitzen, J., Oers, M.M.V., 2012. Protein tyrosine phosphatase-induced hyperactivity is a conserved strategy of a subset of baculoviruses to manipulate lepidopteran host behavior. PLoS ONE 7, e46933.
- Jensen, C.S., Garsdal, L., Baatrup, E., 1997. Acetylcholinesterase inhibition and altered locomotor behavior in the carabid beetle *Pterostichus cupreus*. A linkage between biomarkers at two levels of biological complexity. Environ. Toxicol. Chem. 16, 1727–1732.
- Johnson, O., Becnel, J., Nichols, C.D., 2009. Serotonin 5-HT₂ and 5-HT_{1A}-like receptors differentially modulate aggressive behaviors in *Drosophila melanogaster*. Neuroscience 158, 1292–1300.
- Kadivar, M., Farahmandfar, M., Ranjbar, F.E., Zarrindast, M.R., 2014. Increased calcium/calmodulin-dependent protein kinase II activity by morphinesensitization in rat hippocampus. Behav. Brain Res. 267, 74–82.
- Kamita, S.G., Nagasaka, K., Chua, J.W., Shimada, T., Mita, K., Kobayashi, M., Shimada, T., 2005. A baculovirus-encoded protein tyrosine phosphatase gene induces enhanced locomotory activity in a lepidopteran host. PNAS 102, 2584–2589.
- Katsuma, S., Koyano, Y., Kang, W.K., Kokusho, R., Kamita, S.G., Shimada, T., 2012. The baculovirus uses a captured host phosphatase to induce enhanced locomotory activity in host caterpillars. PLoS Pathog, 8, e1002644.
- Klein, S.L., 2003. Parasite manipulation of the proximate mechanisms that mediate social behaviour in vertebrates. Physiol. Behav. 79, 441–449.
- Lang, G.J., Shang, J.Y., Chen, Y.X., Cui, Y.C., Wang, Q., Tang, Z.H., Zhang, C.X., 2010. Expression of the housefly acetylcholinesterase in a bioreactor and its potential application in the detection of pesticide residues. World J. Microbiol. Biotechnol. 26, 1795–1801.
- Lefèvre, T., Thomas, F., Ravel, S., Patrel, D., Renault, L., Le Bourligu, L., Cuny, D., Biron, D.G., 2007a. *Trypanosoma brucei brucei* induces alteration in the head proteome of the tsetse fly vector *Glossina palpalis gambiensis*. Insect Mol. Biol. 16, 651–660.
- Lefèvre, T., Thomas, F., Schwartz, A., Levashina, E., Blandin, S., Brizard, J.P., Bourligu, L.L., Demettre, E., Renaud, F., Biron, D.G., 2007b. Malaria Plasmodium agent induces alteration in the head proteome of their Anopheles mosquito host. Proteomics 7, 1908–1915.
- Lefèvre, T., Adamo, S.A., Biron, D.G., Missé, D., Hughes, D., Thomas, F., 2009. Invasion of the body snatchers: the diversity and evolution of manipulative strategies in host-parasite interactions. Adv. Parasitol. 68, 45–83.
- Li, Y., Wang, G.Z., Tian, J., Liu, H.F., Yang, H.P., Yi, Y.Z., Wang, J.H., Shi, X.F., Yao, B., Zhang, Z.F., 2012. Transcriptome analysis of the silkworm (*Bombyx mori*) by high-throughput RNA sequencing. PLoS ONE 7, e43713.
 Lima-Camara, T.N., Bruno, R.V., Luz, P.M., Castro, M.G., Lourenço-de-Oliveira, R.,
- Lima-Camara, T.N., Bruno, R.V., Luz, P.M., Castro, M.G., Lourenço-de-Oliveira, R., Sorgine, M.H.F., Peixoto, A.A., 2011. Dengue infection increases the locomotor activity of *Aedes aegypti* females. PLoS ONE 6, e17690.
- Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{- Δ Ct} method. Methods 25, 402–408.
- Maynard, B., DeMartini, L., Wright, W., 1996. Gammarus lacustris harboring Polymorphus paradoxus show altered patterns of serotonin-like immunoreactivity. J. Parasitol. 82, 663–666.
- Moore, J., 1995. The behavior of parasitized animals. Bioscience 45, 89-96.

- Moore, J., 2002. Parasites and the Behavior of Animals. Oxford University Press, Oxford.
- Mortazavi, A., Williams, B.A., McCue, K., Schaeffer, L., Wold, B., 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat. Methods 5, 621–628.
- Newby, L.M., Jackson, F.R., 1991. Drosophila ebony mutants have altered circadian activity rhythms but normal eclosion rhythms. J. Neurogenet. 7, 85–101.
- Nie, H., Liu, C., Cheng, T., Li, Q., Wu, Y., Zhou, M., Zhang, Y., Xia, Q., 2014. Transcriptome analysis of integument differentially expressed genes in the pigment mutant (*quail*) during molting of silkworm, *Bombyx mori*. PLoS ONE 9, e94185.
- Ott, S.R., Verlinden, H., Rogers, S.M., Brighton, C.H., Quah, P.S., Vleugels, R.K., Verdonck, R., Broeck, J.V., 2012. Critical role for protein kinase A in the acquisition of gregarious behavior in the desert locust. PNAS 109, E381–E387.
- Platt, K.B., Linthicum, K.J., Myint, K.S.A., Innis, B.L., Lerdthusnee, K., Vaughn, D.W., 1997. Impact of dengue virus infection on feeding behavior of *Aedes aegypti*. Am. J. Trop. Med. Hyg. 57, 119–125.
- Ponton, F., Lefèvre, T., Lebarbenchon, C., Thomas, F., Loxdale, H.D., Marché, L., Renault, L., Perrot Minnot, M.J., Biron, D., 2006. Do distantly related parasites rely on the same proximate factors to alter the behavior of their hosts? Proc. Biol. Sci. 273, 2869–2877.
- Prado, V.F., Martins-Silva, C., Castro, B.M., Lima, R.F., Barros, D.M., Amaral, E., Ramsey, A.J., Sotnikova, T.D., Ramirez, M.R., Kim, H.G., Rossato, J.R., Koenen, J., Quan, H., Cota, V.R., Moraes, M.F.D., Gomez, M.V., Guatimosim, C., Wetsel, W.C., Kushmerick, C., Pereira, G.S., Gainetdinov, R.R., Izquierdo, I., Caron, M.G., Prado, M.A.M., 2006. Mice deficient for the vesicular acetylcholine transporter are myasthenic and have deficits in object and social recognition. Neuron 51, 601– 612.
- Richardt, A., Rybak, A., Strortkuhl, K.F., Meinertzhagen, L.A., Hovemann, B., 2002. Ebony protein in the *Drosophila* nervous system: optic neuropile expression in glial cells. J. Comp. Neurol. 452, 93–102.
- Richardt, A., Kemme, T., Wagner, S., Schwarzer, D., Marahiel, M.A., Hovemann, B.T., 2003. Ebony, a novel nonribosomal peptide synthetase for beta-alanine conjugation with biogenic amines in *Drosophila*. J. Biol. Chem. 278, 41160–41166.
- Saraswati, S., Fox, L.E., Soll, D.R., Wu, C.F., 2004. Tyramine and octopamine have opposite effects on the locomotion of *Drosophila* larvae. J. Neurobiol. 58, 425– 441.
- Scholz, H., 2005. Influence of the biogenic amine tyramine on ethanol-induced behaviors in *Drosophila*. J. Neurobiol. 63, 199–214.
- Schulman, H., 1993. The multifunctional Ca²⁺/calmodulin-dependent protein kinases. Curr. Opin. Cell Biol. 5, 247–253.
- Schulze, K.L., Broadie, K., Perin, M.S., Belien, H.J., 1995. Genetic and electrophysiological studies of *Drosophila* syntaxin-IA demonstrate its role in nonneuronal secretion and neurotransmission. Cell 80, 311–320.
- Silva, B., Goles, N.I., Varas, R., Campusano, J.M., 2014. Serotonin receptors expressed in *Drosophila* mushroom bodies differentially modulate larval locomotion. PLoS ONE 9, e89641.
- Straussa, R., Heisenberg, M., 1993. A higher control center of locomotor behavior in the Drosophila Brain. J. Neurosci. 13, 1852–1861.
- Suh, J., Jackson, F.R., 2007. Drosophila ebony activity is required in glia for the circadian regulation of locomotor activity. Neuron 55, 435–447.
- Thamm, M., Balfanz, S., Scheiner, R., Baumann, A., Blenau, W., 2010. Characterization of the 5-HT_{1A} receptor of the honeybee (*Apis mellifera*) and involvement of serotonin in phototactic behavior. Cell. Mol. Life Sci. 67, 2467–2479.
- Thomas, F., Adamo, S.A., Moore, J., 2005. Parasitic manipulation: where are we and where should we go? Behav. Process. 68, 185–199.
- Trapnell, C., Pachter, L., Salzberg, S.L., 2009. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 25, 1105–1111.
- Washbourne, P., Schiavo, G., Montecucco, C., 1995. Vesicle-associated membrane protein-2 (synaptobrevin-2) forms a complex with synaptophysin. Biochem. J. 305, 721–724.
- Xia, Q., Zhou, Z., Lu, C., Cheng, D., Dai, F., Li, B., Zhao, P., Zha, X., Cheng, T., Chai, C., Pan, G., Xu, J., Liu, C., Lin, Y., Qian, J., Hou, Y., Wu, Z., Li, G., Pan, M., Li, C., Shen, Y., Lan, X., Yuan, L., Li, T., Xu, H., Yang, G., Wan, Y., Zhu, Y., Yu, M., Shen, W., Wu, D., Xiang, Z., Yu, J., Wang, J., Li, R., Shi, J., Li, H., Li, G., Su, J., Wang, X., Li, G., Zhang, Z., Wu, Q., Li, J., Zhang, Q., Wei, N., Xu, J., Sun, H., Dong, L., Liu, D., Zhao, S., Zhao, X., Meng, Q., Lan, F., Huang, X., Li, Y., Fang, L., Li, C., Li, D., Sun, Y., Zhang, Z., Yuang, Y., Xi, Y., Qi, Q., He, D., Huang, H., Zhang, X., Wang, Z., Li, W., Cao, Y., Yu, Y., Yu, H., Li, J., Ye, J., Chen, H., Zhou, Y., Liu, B., Wang, J., Ye, J., Ji, H., Li, S., Ni, P., Zhang, J., Zhang, Y., Zheng, H., Mao, B., Wang, W., Ye, C., Li, S., Wang, J., Wong, G.K., Yang, H., 2004. A draft sequence for the genome of the domesticated silkworm (*Bombyx mori*). Science 306, 1937–1940.
- Yuan, Q., Lin, F., Zheng, X., Sehgal, A., 2005. Serotonin modulates circadian entrainment in *Drosophila*. Neuron 47, 115–127.