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RESEARCH REPORT

Microarray validation of molecular and cellular signaling in *Homarus americanus* and *Penaeus monodon*

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Abstract

Previous studies have demonstrated that invertebrate neural tissues contain mammalian-like neurotransmitters, which activate specific cellular functions. Therefore, it was of interest to attempt to identify these molecules via Agilent gene expression microarrays. The array was used to analyze the transcriptional profiles of lobster and shrimp RNA samples. We show dopamine, serotonin, and acetylcholine genes and their corresponding receptors are significantly expressed in lobster and shrimp neural tissues with a signal to noise value greater than 2. These signal molecules are directly related to previously discovered molecules in invertebrates, suggesting that they first appeared earlier in evolution and are necessary for an animal's survival.

Key Words: lobster; shrimp; neurotransmitter; microarray; acetylcholine; biogenic amines

Introduction

Invertebrate neural tissues contain neurotransmitters, *i.e.,* biogenic amines, found in mammals (see (Stefano, 1982, 1990, 1992). In regard to catecholamines, the neural tissue of *Mytilus edulis* contains dopamine (DA) and norepinephrine (NE) as well as the indoleamine serotonin (Stefano and Aiello, 1975 Stefano *et al.*, 1976, 1977; Hidaka *et al.*, 1977; Twarog *et al.*, 1977; Malanga and Young, 1978; Satchell and Twarog, 1978; Stefano, 1982, 1990; Zhu *et al.*, 2005). These studies imply that elements of the neurotransmitter functions, *i.e.*, enzymes and receptors, are present in the neural tissues of this organism as well (Malanga and Aiello, 1971; Malanga, 1974 Stefano *et al.*, 1976, 1978; Catapane *et al.*, 1977, 1978, 1979, 1980 Twarog *et al*., 1977; Collins *et al.*, 1980; Hidaka *et al.*, 1977; Malanga and Young, 1978; Satchell and Twarog, 1978; Malanga and Poll, 1979; Stefano and Catapane, 1980).

Given this documentation we sought to use microarray technology to validate previous studies documenting the presence of these neurotransmitters in lobster and shrimp neural tissues. Previously we demonstrated cholinergic signaling elements, (acetylcholine and respective receptors in lobster (Zhu *et al.*, 2006) as well as chemical messengers associated with catecholamine metabolism (Casares *et al.*, 2005). We also performed a similar analysis in *Mytilus edulis* (Gerber *et al*., 2007; Mantione *et al*., 2009).

Thus, given their presence and action in invertebrate physiological systems it was of great interest to determine if human microarray chips would also show that they are present along with other processes associated with their signaling. The results of the present study support the observation that these signaling molecules appear to have evolved earlier than previously realized, given the many support processes now demonstrated, validating years of research on this topic.

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Materials and Methods

Homarus americanus and *Penaeus monodon* were purchased live commercially. Animals were then transported to the laboratory in chilled seawater (4 - 10 °C). In the laboratory, they were maintained as previously described in detail (Stefano *et al.*, 1994). Neural tissues, lobster and shrimp ventral nerve cords were dissected and kept on ice until needed.

Agilent microarray gene expression array

Agilent Human Genome Survey Arrays were used to analyze the transcriptional profiles of RNA

samples. The Agilent Human Genome Survey Array contains 31,700 60-mer oligonucleotide probes representing a set of 27, 868 individual human genes and more than 1,000 control probes. Sequences used for microarray probe design are from curated transcripts from the Celera Genomics Human Genome Database (www.celeradiscoverysystem.com), RefSeq transcripts that have been structurally curated from the LocusLink public database (http://ncbi.nlm.nih.bov/LocusLink/refseq.html), high-quality cDNA sequences from the Mammalian Gene Collection (MGC) (http://mgc.nci.nih.gov) and transcripts that were experimentally validated at Applied Biosystems. Total RNA from 1 lobster and 3 shrimp ventral nerve cords were isolated separately with the RNeasy Mini Kit (Qiagen, Valencia, CA, USA). The tissue was lysed in 600 µl buffer RLT and homogenized by passing the lysate 5 times through a 20-gauge needle fitted to a 3 ml syringe. The samples were then processed following the manufacturer's detailed instructions. In the final step, the RNA was eluted with 50 µl of RNase-free water by centrifugation for 1 min at 10,000 rpm. Quality of the RNA was analyzed using Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) using the total RNA nanochip according to manufacturer's protocol**.** RNA was reverse transcribed and the cDNA was transcribed and labeled with Cyanine-3-CTP following manufacturer's protocol. To each chip, 2 µg of labeled cRNA targets were hybridized at 55 ˚C for 18 h. Agilent microarray scanner software was used to extract assay signal and assay signal to noise ratio values from the microarray images. To determine expressed genes, the gene list was filtered by removing genes with a signal to noise value below two. The gene list was further filtered into moderately highly expressed and very highly expressed genes. Data sets were managed using Spotfire for Functional Genomics **(**TIBCO Software Inc., Palo Alto, CA, USA).

Results

The previously discovered invertebrate neurotransmitter molecules include dopamine, as well as other biogenic amines, acetylcholine and serotonin. Tables 1-4 list the associated genes that were significantly expressed as analyzed by the Gene Survey microarray (Agilent) with a signal to noise value greater than 2 in untreated lobster nervous tissue.

Table 1 *Homarus americanus* dopamine and serotonin pathway genes present

Dopamine receptors present	
DRD1	dopamine receptor D1
DRD2	dopamine receptor D2
DRD3	dopamine receptor D3
DRD4	dopamine receptor D4
DRD5	dopamine receptor D5
Serotonin receptors present	
HTR1A	5-hydroxytryptamine (serotonin) receptor 1A, G protein-coupled
HTR1B	5-hydroxytryptamine (serotonin) receptor 1B, G protein-coupled
HTR1D	5-hydroxytryptamine (serotonin) receptor 1D, G protein-coupled
HTR1E	5-hydroxytryptamine (serotonin) receptor 1E, G protein-coupled
HTR1F	5-hydroxytryptamine (serotonin) receptor 1F, G protein-coupled
HTR2A	5-hydroxytryptamine (serotonin) receptor 2A, G protein-coupled
HTR2B	5-hydroxytryptamine (serotonin) receptor 2B, G protein-coupled
HTR2C	5-hydroxytryptamine (serotonin) receptor 2C, G protein-coupled
HTR3A	5-hydroxytryptamine (serotonin) receptor 3A, ionotropic
HTR3B	5-hydroxytryptamine (serotonin) receptor 3B, ionotropic
HTR4	5-hydroxytryptamine (serotonin) receptor 4, G protein-coupled
HTR5A	5-hydroxytryptamine (serotonin) receptor 5A, G protein-coupled
HTR6	5-hydroxytryptamine (serotonin) receptor 6, G protein-coupled
HTR7	5-hydroxytryptamine (serotonin) receptor 7, adenylate cyclase-coupled
Dopamine and serotonin metabolism genes present	
COMT	catechol-O-methyltransferase
DBH	dopamine beta-hydroxylase (dopamine beta-monooxygenase)
DDC	dopa decarboxylase (aromatic L-amino acid decarboxylase)
MAOA	monoamine oxidase A
MAOB	monoamine oxidase B
TDO2	tryptophan 2,3-dioxygenase
TH	tyrosine hydroxylase
TPH1	tryptophan hydroxylase 1
TPH2	tryptophan hydroxylase 2
Dopamine and serotonin transporters present	
SLC6A3	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3
SLC6A4	solute carrier family 6 (neurotransmitter transporter, serotonin), member 4

Other dopamine and serotonin related genes present	
ALDH5A1	aldehyde dehydrogenase 5 family, member A1
BDNF	brain-derived neurotrophic factor
CALY	calcyon neuron-specific vesicular protein
CYP2D6	cytochrome P450, family 2, subfamily D, polypeptide 6
EPHB1	EPH receptor B1
GDNF	glial cell derived neurotrophic factor
GFAP	glial fibrillary acidic protein
MOXD1	monooxygenase, DBH-like 1
NR4A1	nuclear receptor subfamily 4, group A, member 1
NR4A3	nuclear receptor subfamily 4, group A, member 3
PDYN	prodynorphin
PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
SLC18A1	solute carrier family 18 (vesicular monoamine), member 1
SLC18A2	solute carrier family 18 (vesicular monoamine), member 2
SYN2	synapsin II

Table 2 *Homarus americanus* signal transduction pathway genes present

cAMP/PKA pathway activity	
ADCY1	adenylate cyclase 1
ADCY2	adenylate cyclase 2
ADCY3	adenylate cyclase 3
ADCY5	adenylate cyclase 5
CASP3	caspase 3, apoptosis-related cysteine peptidase
CDK5	cyclin-dependent kinase 5
CREB1	cAMP responsive element binding protein 1
DUSP1	dual specificity phosphatase 1
FOS	FBJ osteosarcoma oncogene
MAPK1	mitogen-activated protein kinase 1
PPP1R1B	protein phosphatase 1, regulatory (inhibitor) subunit 1B
PRKACA	protein kinase, cAMP-dependent, catalytic, alpha
P13K/AKT pathway activity	
AKT1	thymoma viral proto-oncogene 1
AKT2	thymoma viral proto-oncogene 2
AKT3	thymoma viral proto-oncogene 3
GSK3A	glycogen synthase kinase 3 alpha
PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha
PIK3CG	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit gamma
PLA2 pathway activity	
ALOX12	arachidonate 12-lipoxygenase
PDE10A	phosphodiesterase 10A
PDE4A	phosphodiesterase 4A
PDE4B	phosphodiesterase 4B
PDE4C	phosphodiesterase 4C
PDE4D	phosphodiesterase 4D
PLA2G5	phospholipase A2, group V
PLC pathway activity	
ITPR1	inositol 1,4,5-trisphosphate receptor 1
PLCB1	phospholipase C, beta 1
PLCB2	phospholipase C, beta 2
PLCB3	phospholipase C, beta 3
G-protein coupled receptor regulation activity	
ADRB1	adrenoceptor beta 1
ADRB2	adrenoceptor beta 2
ADRBK1	adrenergic, beta, receptor kinase 1
ADRBK2	adrenergic, beta, receptor kinase 2
APP	amyloid beta (A4) precursor protein
ARRB1	arrestin, beta 1
ARRB2	arrestin, beta 2
GRK4	G protein-coupled receptor kinase 4
GRK5	G protein-coupled receptor kinase 5
GRK6	G protein-coupled receptor kinase 6
SNCA	synuclein, alpha (non A4 component of amyloid precursor)
SNCAIP	synuclein, alpha interacting protein

Table 3 *Homarus americanus* cell signaling genes present

ACVR2B	activin A receptor, type IIB
BMP1	bone morphogenetic protein 1
CCL1	chemokine (C-C motif) ligand 1
CCL13	chemokine (C-C motif) ligand 13
CCL15	chemokine (C-C motif) ligand 15
CCL19	chemokine (C-C motif) ligand 19
CCL23	chemokine (C-C motif) ligand 23
CCL24	chemokine (C-C motif) ligand 24
CCNC	cyclin C
CCND3	cyclin D3
CCNL1	cyclin L1
CCR6	chemokine (C-C motif) receptor 6
CCR9	chemokine (C-C motif) receptor 9
CCRL2	chemokine (C-C motif) receptor-like 2
CDC14A	CDC14 cell division cycle 14 homolog A
CDC23	cell division cycle 23 homolog
CDC25A	cell division cycle 25 homolog A
CDK6	cyclin-dependent kinase 6
CSF2RB	colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)
CXCL12	chemokine (C-X-C motif) ligand 12
CXCR3	chemokine (C-X-C motif) receptor 3
DAPK1	death-associated protein kinase 1
DOCK1	dedicator of cytokinesis 1
EPOR	erythropoietin receptor
GDF2	growth differentiation factor 2
GDF8	growth differentiation factor 8
IFNK	interferon, kappa
IL11RA	interleukin 11 receptor, alpha
IL15	interleukin 15
IL16	interleukin 16
IL18BP	interleukin 18 binding protein
IL23R	interleukin 23 receptor
IL31RA	interleukin 31 receptor A
IL7	interleukin 7
LATS1	LATS, large tumor suppressor, homolog 1
LEPR	leptin receptor
LIF	leukemia inhibitory factor
MOBK1B	MOB kinase activator 1A
MYH11	myosin, heavy polypeptide 11, smooth muscle
NRP1	neuropilin 1
OSM	oncostatin M
PARD3	par-3 partitioning defective 3 homolog
PARD6A	par-6 partitioning defective 6 homolog alpha
NAMPT	nicotinamide phosphoribosyltransferase
PF4	platelet factor 4
PIN1	peptidylprolyl cis/trans isomerase, NIMA-interacting 1
PRC1	protein regulator of cytokinesis 1
STAT1	signal transducer and activator of transcription 1
TLR2	toll-like receptor 2
TNFRSF11A	tumor necrosis factor receptor superfamily, member 11A
TNFRSF25	tumor necrosis factor receptor superfamily, member 25

Table 4 *Homarus americanus* other neurotransmitter related genes present

ACHE	Acetylcholinesterase
BCHE	Butyrylcholinesterase
CHRM1	cholinergic receptor, muscarinic 1
CHRM2	cholinergic receptor, muscarinic 2
CHRM3	cholinergic receptor, muscarinic 3
CHRM4	cholinergic receptor, muscarinic 4
CHRM5	cholinergic receptor, muscarinic 5
CHRNA1	cholinergic receptor, nicotinic, alpha 1 (muscle)
CHRNA10	cholinergic receptor, nicotinic, alpha 10 (muscle)
CHRNA2	cholinergic receptor, nicotinic, alpha 2 (muscle)
CHRNA3	cholinergic receptor, nicotinic, alpha 3 (muscle)
CHRNA4	cholinergic receptor, nicotinic, alpha 4 (muscle)
CHRNA5	cholinergic receptor, nicotinic, alpha 5 (muscle)
CHRNA6	cholinergic receptor, nicotinic, alpha 6 (muscle)
CHRNA9	cholinergic receptor, nicotinic, alpha 9 (muscle)
CHRNB1	cholinergic receptor, nicotinic, beta 1 (muscle)
CHRNB2	cholinergic receptor, nicotinic, beta 2 (muscle)
CHRNB3	cholinergic receptor, nicotinic, beta 3 (muscle)
CHRNB4	cholinergic receptor, nicotinic, beta 4 (muscle)
CHRND	cholinergic receptor, nicotinic, delta (muscle)
CHRNE	cholinergic receptor, nicotinic, epsilon (muscle)
CHRNG	cholinergic receptor, nicotinic, gamma (muscle)
COLQ	collagen-like tail subunit (single strand of homotrimer) of asymmetric acetylcholinesterase
SLC18A3	solute carrier family 18 (vesicular monoamine), member 3
SLC5A7	solute carrier family 5 (choline transporter), member 7
SLC6A2	solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2

Tables 5 - 8 list the associated genes that were significantly expressed as analyzed by the Gene Survey microarray (Agilent) with a signal to noise value greater than 2 in untreated shrimp nervous tissue. Gene sequences detected with signal to noise values greater than 2 were considered to be present. Given the logarithmic analysis supplied by the SpotFire for functional genomics program (SpotFire, Somerville, Maine, USA), any positive signal to noise value indicates gene presence is in greater amounts than background noise. Furthermore, the gene copy number of the human transcriptome on the microarray chip is not identical to the gene copy number of the respective animal.

According to this criterion, most of the genes detected in the lobster were between the signal to noise ratio of 2 to 3. The ratios for the CHRNA5, HTR3A, MAOA and TH genes were between 3 and 4. The most highly expressed genes, all with a signal to noise greater than 10, were the CCL24, CHRNE, COMT, CXCR3, DRD4, MOXD1, PDE4B, and PLCB2.

For the shrimp genes detected, most of the genes detected were between the signal to noise ratio of 2 to 3. The ratios for the CCND3, CHRM1, DRD5, HTR1B, HTR1E, HTR3A, PDE4C, PLCB1, PRC1, PRKACA, and SNCA genes were between 3 and 4. Finally, the most highly expressed genes, all with a signal to noise greater than 10, were the ACVR2B, AKT2, CCL19, CCL24, CHRM2, CHRM5, CHRNB2, CHRNB3, CHRNE, CYP2D6, DRD4, GFAP, HTR3A, IL16, IL18BP, PLCB2, TH, and TPH2.

Discussion

As noted earlier, invertebrate ganglia contain biogenic amines, serotonin and acetylcholine as validated by gene expression microarray (Mantione *et al.*, 2009). Based on these findings, which validate the current results, one can surmise that these chemical messengers emerged early during the course of evolution and were maintained (Ottaviani *et al.*, 1991, 1988, 1995, 2007; Ottaviani and Franceschi, 1996; Stefano *et al.*, 2009).

In another invertebrate, biogenic amines in *Mytilus* tissues have been demonstrated not only in the tissues but to exhibit pharmacological specificity in regard to tissue excitation and inhibition. Dopamine, serotonin and acetylcholine have been implicated in the regulation of cilia activity, smooth muscle regulation and foot control (Twarog and Cole, 1972; Hidaka and Twarog, 1977; Hidaka *et al.*, 1977; Twarog *et al.*, 1977; Catapane *et al.*, 1978, 1979; Malanga and Young, 1978; Satchell and Twarog, 1978; Malanga and Poll, 1979; Aiello *et al.*, 1981). These studies demonstrate that the respective receptor mediated systems, exhibiting high specificity to various related agonists and antagonists occur in specific tissues.

Table 5 *Penaeus monodon* dopamine and serotonin pathway genes present

Dopamine receptors present	
DRD1	dopamine receptor D1
DRD2	dopamine receptor D2
DRD3	dopamine receptor D3
DRD4	dopamine receptor D4
DRD5	dopamine receptor D5
Serotonin receptors present	
HTR1A	5-hydroxytryptamine (serotonin) receptor 1A, G protein-coupled
HTR1B	5-hydroxytryptamine (serotonin) receptor 1B, G protein-coupled
HTR1D	5-hydroxytryptamine (serotonin) receptor 1D, G protein-coupled
HTR1E	5-hydroxytryptamine (serotonin) receptor 1E, G protein-coupled
HTR1F	5-hydroxytryptamine (serotonin) receptor 1F, G protein-coupled
HTR2A	5-hydroxytryptamine (serotonin) receptor 2A, G protein-coupled
HTR2B	5-hydroxytryptamine (serotonin) receptor 2B, G protein-coupled
HTR2C	5-hydroxytryptamine (serotonin) receptor 2C, G protein-coupled
HTR3A	5-hydroxytryptamine (serotonin) receptor 3A, ionotropic
HTR3B	5-hydroxytryptamine (serotonin) receptor 3B, ionotropic
HTR4	5-hydroxytryptamine (serotonin) receptor 4, G protein-coupled
HTR5A	5-hydroxytryptamine (serotonin) receptor 5A, G protein-coupled
HTR6	5-hydroxytryptamine (serotonin) receptor 6, G protein-coupled
HTR7	5-hydroxytryptamine (serotonin) receptor 7, adenylate cyclase-coupled
Dopamine and serotonin metabolism genes present	
COMT	catechol-O-methyltransferase
DBH	dopamine beta-hydroxylase (dopamine beta-monooxygenase)
DDC	dopa decarboxylase (aromatic L-amino acid decarboxylase)
TH	tyrosine hydroxylase
MAOA	monoamine oxidase A
MAOB	monoamine oxidase B
TDO2	tryptophan 2,3-dioxygenase
TPH1	tryptophan hydroxylase 1
TPH2	tryptophan hydroxylase 2
Dopamine and serotonin transporters present	
SLC6A3	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3
SLC6A4	solute carrier family 6 (neurotransmitter transporter, serotonin), member 4
Other dopamine and serotonin related genes present	
ALDH5A1	aldehyde dehydrogenase 5 family, member A1
BDNF	brain-derived neurotrophic factor
CALY	calcyon neuron-specific vesicular protein
CYP2D6	cytochrome P450, family 2, subfamily D, polypeptide 6
EPHB1	EPH receptor B1
GDNF	glial cell derived neurotrophic factor
GFAP	glial fibrillary acidic protein
MOXD1	monooxygenase, DBH-like 1
NR4A1	uclear receptor subfamily 4, group A, member 1
NR4A3	nuclear receptor subfamily 4, group A, member 3
PDYN	Prodynorphin
PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
SLC18A1	solute carrier family 18 (vesicular monoamine), member 1
SLC18A2	solute carrier family 18 (vesicular monoamine), member 2
SYN2	synapsin II

In previous and current research, measures are taken to confirm gene expression including TaqMan Probes and molecular methods as well as Western blotting (Hauton *et al.*, 2005). The ability of microarray to corroborate with and/or confirm an expanse of previous research is demonstrated in this study of neurotransmitter molecules found in these invertebrates. Given the comprehensive nature of a single microarray chip and the accuracy and precision of the data expressed by these chips, this research indicates that the use of microarray could be independently sufficient for determining gene expression given the validating preexisting data (Nachmansohn, 1964; Nagabhushanam, 1966;

Table 6 *Penaeus monodon* signal transduction pathways genes present

cAMP/PKA pathway activity	
ADCY1	adenylate cyclase 1
ADCY2	adenylate cyclase 2
ADCY3	adenylate cyclase 3
ADCY5	adenylate cyclase 5
CASP3	caspase 3, apoptosis-related cysteine peptidase
CDK5	cyclin-dependent kinase 5
CREB1	cAMP responsive element binding protein 1
DUSP1	dual specificity phosphatase 1
FOS	FBJ osteosarcoma oncogene
MAPK1	mitogen-activated protein kinase 1
PPP1R1B	protein phosphatase 1, regulatory (inhibitor) subunit 1B
PRKACA	protein kinase, cAMP-dependent, catalytic, alpha
P13K/AKT pathway activity	
AKT1	thymoma viral proto-oncogene 1
AKT2	thymoma viral proto-oncogene 2
AKT3	thymoma viral proto-oncogene 3
GSK3A	glycogen synthase kinase 3 alpha
GSK3B	glycogen synthase kinase 3 beta
PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha
PIK3CG	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit gamma
PLA2 pathway activity	
ALOX12	arachidonate 12-lipoxygenase
PDE10A	phosphodiesterase 10A
PDE4A	phosphodiesterase 4A
PDE4B	phosphodiesterase 4B
PDE4C	phosphodiesterase 4C
PDE4D	phosphodiesterase 4D
PLA2G5	phospholipase A2, group V
PLC pathway activity	
ITPR1	inositol 1,4,5-trisphosphate receptor 1
PLCB1	phospholipase C, beta 1
PLCB2	phospholipase C, beta 2
PLCB3	phospholipase C, beta 3
G-protein coupled receptor regulation activity	
ADRBK1	adrenergic, beta, receptor kinase 1
ADRBK2	adrenergic, beta, receptor kinase 2
APP	amyloid beta (A4) precursor protein
ARRB1	arrestin, beta 1
ARRB2	arrestin, beta 2
GRK4	G protein-coupled receptor kinase 4
GRK5	G protein-coupled receptor kinase 5
GRK6	G protein-coupled receptor kinase 6
SNCA	synuclein, alpha (non A4 component of amyloid precursor)
SNCAIP	synuclein, alpha interacting protein

Hildebrand *et al.*, 1974; Marder, 1974; Sullivan *et al.*, 1977; Davis and Ocorr and Berlind, 1983; Cournil *et al.*, 1984, 1994; Siwicki *et al.*, 1987; Juorio and Sloley, 1988; Chiba and Tazaki, 1992; Ma *et al.*, 1992; Ma and Weiger, 1993; Cournil *et al*., 1995; Rodriguez *et al.*, 1995; Destoumieux *et al*., 1997, 1999; Mancillas *et al.*, 1998; Scholz *et al.*, 1998; Heinrich *et al.*, 2000; Peeke *et al.*, 2000; Antonsen and Paul, 2001; Harzsch, 2003; Pulver *et al.*, 2003; Casares *et al.,* 2005; Cheng *et al.*, 2005; Tiu *et al.*, 2005; Casares *et al.*, 2006; Zhu *et al.*, 2006; Chang *et al.*, 2007; Brown-Peterson *et al.*, 2008; Leelatanawit *et al.*, 2008; Li and Brouwer, 2009; Tinikul *et al.*, 2011). This preexisting data serves as a validation of the current microarray results.

In summary, it appears neural communication, which occurs in invertebrate neural tissues, including those innervating peripheral tissues originated earlier in evolution and was maintained

Table 7 *Penaeus monodon* cell signaling genes present

ACVR2B	activin A receptor, type IIB
BMP1	bone morphogenetic protein 1
CCL1	chemokine (C-C motif) ligand 1
CCL13	chemokine (C-C motif) ligand 13
CCL15	chemokine (C-C motif) ligand 15
CCL19	chemokine (C-C motif) ligand 19
CCL23	chemokine (C-C motif) ligand 23
CCL24	chemokine (C-C motif) ligand 24
CCNC	cyclin C
CCND3	cyclin D3
CCNL1	cyclin L1
CCR6	chemokine (C-C motif) receptor 6
CCR9	chemokine (C-C motif) receptor 9
CCRL2	chemokine (C-C motif) receptor-like 2
CDC14A	CDC14 cell division cycle 14 homolog A
CDC23	cell division cycle 23 homolog
CDC25A	cell division cycle 25 homolog A
CDK6	cyclin-dependent kinase 6
CSF2RB	colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)
CXCL12	chemokine (C-X-C motif) ligand 12
CXCR3	chemokine (C-X-C motif) receptor 3
DAPK1	death-associated protein kinase 1
DOCK1	dedicator of cytokinesis 1
EPOR	erythropoietin receptor
GDF2	growth differentiation factor 2
GDF8	growth differentiation factor 8
IFNK	interferon, kappa
IL11RA	interleukin 11 receptor, alpha
IL15	interleukin 15
IL16	interleukin 16
IL18BP	interleukin 18 binding protein
IL23R	interleukin 23 receptor
IL31RA	interleukin 31 receptor A
IL7	interleukin 7
LATS1	LATS, large tumor suppressor, homolog 1
LEPR	leptin receptor
LIF	leukemia inhibitory factor
MOBK1B	MOB kinase activator 1A
MYH11	myosin, heavy polypeptide 11, smooth muscle
NRP1	neuropilin 1
OSM	oncostatin M
PARD3	par-3 partitioning defective 3 homolog
PARD6A	par-6 partitioning defective 6 homolog alpha
NAMPT	nicotinamide phosphoribosyltransferase
PF4	platelet factor 4
PIN1	peptidylprolyl cis/trans isomerase, NIMA-interacting 1
PRC1	protein regulator of cytokinesis 1
STAT1	signal transducer and activator of transcription 1
TLR2	toll-like receptor 2
TNFRSF11A	tumor necrosis factor receptor superfamily, member 11A
TNFRSF25	tumor necrosis factor receptor superfamily, member 25

Table 8 *Penaeus monodon* other neurotransmitter related genes present

ACHE	Acetylcholinesterase
BCHE	Butyrylcholinesterase
CHRM1	cholinergic receptor, muscarinic 1
CHRM2	cholinergic receptor, muscarinic 2
CHRM3	cholinergic receptor, muscarinic 3
CHRM4	cholinergic receptor, muscarinic 4
CHRM5	cholinergic receptor, muscarinic 5
CHRNA1	cholinergic receptor, nicotinic, alpha 1 (muscle)
CHRNA10	cholinergic receptor, nicotinic, alpha 10 (muscle)
CHRNA2	cholinergic receptor, nicotinic, alpha 2 (muscle)
CHRNA3	cholinergic receptor, nicotinic, alpha 3 (muscle)
CHRNA4	cholinergic receptor, nicotinic, alpha 4 (muscle)
CHRNA5	cholinergic receptor, nicotinic, alpha 5 (muscle)
CHRNA6	cholinergic receptor, nicotinic, alpha 6 (muscle)
CHRNA9	cholinergic receptor, nicotinic, alpha 9 (muscle)
CHRNB1	cholinergic receptor, nicotinic, beta 1 (muscle)
CHRNB2	cholinergic receptor, nicotinic, beta 2 (muscle)
CHRNB3	cholinergic receptor, nicotinic, beta 3 (muscle)
CHRNB4	cholinergic receptor, nicotinic, beta 4 (muscle)
CHRND	cholinergic receptor, nicotinic, delta (muscle)
CHRNE	cholinergic receptor, nicotinic, epsilon (muscle)
CHRNG	cholinergic receptor, nicotinic, gamma (muscle)
COLQ	collagen-like tail subunit (single strand of homotrimer) of asymmetric acetylcholinesterase
CTRL	chymotrypsin-like
SLC18A3	solute carrier family 18 (vesicular monoamine), member 3
SLC5A7	solute carrier family 5 (choline transporter), member 7
SLC6A2	solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2

(Stefano, 1988). Certainly, the opposite has also been shown, *i.e.*, stimulation vs. inhibition, that neuropeptides can alter and direct invertebrate immune actions (see Stefano *et al.*, 1996). The present study adds new insight as to the origins of many processes that have been used to enhance survival by many animals and advances the hypothesis that these mechanisms evolved earlier in evolution.

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