The FGLamide-Allatostatins Influence Foraging Behavior in *Drosophila melanogaster*

Christine Wang, Ian Chin-Sang, William G. Bendena*

Department of Biology, Biosciences Complex, Queen’s University, Kingston, Ontario, Canada

Abstract

Allatostatins (ASTs) are multifunctional neuropeptides that generally act in an inhibitory fashion. ASTs were identified as inhibitors of juvenile hormone biosynthesis. Juvenile hormone regulates insect metamorphosis, reproduction, food intake, growth, and development. *Drosophila melanogaster* RNAi lines of PheGlyLeu-amide-ASTs (FGLa/ASTs) and their cognate receptor, Dar-1, were used to characterize roles these neuropeptides and their respective receptor may play in behavior and physiology. Dar-1 and FGLa/AST RNAi lines showed a significant reduction in larval foraging in the presence of food. The larval foraging defect is not observed in the absence of food. These RNAi lines have decreased for transcript levels which encodes cGMP-dependent protein kinase. A reduction in the *for* transcript is known to be associated with a naturally occurring allelic variation that creates a sitter phenotype in contrast to the rover phenotype which is caused by a *for* allele associated with increased for activity. The sitting phenotype of FGLa/AST and Dar-1 RNAi lines is similar to the phenotype of a deletion mutant of an AST/galanin-like receptor (NPR-9) in *Caenorhabditis elegans*. Associated with the foraging defect in *C. elegans npr-9* mutants is accumulation of intestinal lipid. Lipid accumulation was not a phenotype associated with the FGLa/AST and Dar-1 RNAi lines.

Citation: Wang C, Chin-Sang I, Bendena WG (2012) The FGLamide-Allatostatins Influence Foraging Behavior in *Drosophila melanogaster*. PLoS ONE 7(4): e36059.

Copyright: © 2012 Wang et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work is supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada Discovery Grant RGPIN 36481-08 to WGB. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: william.bendena@queensu.ca

Introduction

In insects, three differing allatostatin (AST) peptide structures have been isolated that inhibit juvenile hormone (JH) biosynthesis. These include the FGLamide (FGLa)/ASTs, the W(X)6Wamide/ASTs and the PISC/F/ASTs. Each unique peptide structure appears to inhibit JH biosynthesis in select insect species [1–3]. All three types of ASTs have been identified in *D. melanogaster*, but none have been identified as a regulator of JH biosynthesis [4–7].

The FGLa/ASTs are related to vertebrate somatostatin, galanin and opioid peptides and inhibit JH biosynthesis only in cockroaches, crickets, and termites [8–10]. In cockroaches, FGLa/ASTs also function to regulate gut contraction [11–13]. In *D. melanogaster* and other Diptera the FGLa/ASTs do not inhibit JH biosynthesis [7,14] and their function has yet to be determined. *D. melanogaster* FGLα/ASTs functionally interact with two galanin-like receptors Dar-1 and Dar-2 [4,5,16]. Dar-1 is primarily expressed in the larval CNS whereas Dar-2 appears to be expressed in the crop, midgut and hindgut [17].

A *Caenorhabditis elegans* AST-like peptide receptor, NPR-9, was identified in a BLAST search as the closest related GPCR to Dar-1 [18]. Analysis of a deletion mutant of *npr-9* revealed enhanced local search behavior and increased pivoting only in the presence of food. Mutant *npr-9* animals also displayed an increased level of intestinal fat. The foraging phenotype of *npr-9* is similar to a *D. melanogaster* mutation known as ‘sitter’ [19]. The sitter phenotype is due to a naturally occurring polymorphism in the foraging (*for*) gene. Two different alleles at the *for* locus characterize two different food-search behavioral phenotypes; *forS* = sitter, *forR* = rover. Rovers travel significantly greater distances when feeding as compared to the sitters, but no locomotor differences were seen between the two strains on non-nutrient media, suggesting that pathlength differences on food are not the result of a general locomotory defect [20]. The alleles differ by natural polymorphisms at the *dq2* [for] locus [21]. The *for* gene encodes a cGMP dependent protein kinase (PKG) and differences in PKG activity and *for* transcript levels are attributable to the differences in foraging behavior, where rovers have a significantly greater level of PKG activity and *for* transcript level when compared to sitters.

In this manuscript, we have identified an alteration in foraging behavior due to reduction in FGLa/ASTs or their receptor Dar-1. This is the first identified functional role for FGLa/ASTs or receptor Dar-1 in *D. melanogaster*.

Materials and Methods

Animals

*D. melanogaster* stocks were reared at 22°C, 12 hr light/dark cycle and 70%±5% relative humidity on standard medium containing 0.94% agar, 0.01% molasses, 8.2% cornmeal, 3.4% killed yeast, 0.18% benzoic acid, 0.66% propionic acid. Gal 4-UAS RNAi transgenic lines were obtained from Vienna *Drosophila* RNAi Center (VDRC, Vienna, Austria) [22]. These lines were created by transformation of isogenic strain *w*^1118^, which was used as a control in our experiments. Homozygous viable RNAi lines Dar-1 48496 and 101395 contained an insertion on chromosomes 1 and 2, respectively. Isogenic homozygous viable FGLa/AST RNAi lines...
103215 and 14397 contained insertions on chromosomes 2 and 3, respectively. Dar-2 RNAi lines 1326 and 1327 were not used in this study as they showed slow developmental growth and feeding defects. The ubiquitous driver line daughterless (DaGal4) and tissue specific driver 6986 were obtained from the Bloomington Stock center. The driver line 6986 expresses primarily in larval ring gland but also expresses in histoblasts, gut, Malpighian tubules, male accessory glands, testis sheath, and cyst cells [25].

**Larval Foraging Behavioral Assay**

Third instar larval foraging assays from each cross (RNAi lines and w^{1118} control crossed to DaGal4 or 6986 drivers) were examined using a modified procedure described [24], which is briefly outlined here. Third instar larvae (approximately 72 hours post-hatching) reared at 25°C were collected and washed with distilled water. Black rectangular Plexiglas plates (25 cm width×37 cm length×0.5 cm height) with 6 circular wells (0.5 mm deep with a 4.25 cm radius) were provided courtesy of the Sokolowski Lab (University of Toronto at Mississauga). Larvae were placed into the center of each of the 6 circular wells, which were filled with a thin layer of homogenized yeast paste (distilled water and Fleischmann’s Bakers’ Yeast; approximately 3:1 ratio by weight). Wells were then covered with standard Petri dish lids. After 5 minutes the foraging path lengths made within the yeast were traced, scanned, and quantified using the ImageJ program (http://rsb.info.nih.gov/ij/).

Third instar larvae foraging behavior was also analyzed off food. Standard Petri dishes were used and filled with a thin layer of 2% agar containing neutral red dye. Larvae were placed into the center of one of these agar filled Petri dishes and the foraging path lengths traced after 5 minutes and analyzed the same way as the on food foraging assay.

**Triglyceride Extraction and Quantification**

Triglyceride extraction and quantification of triglyceride and protein, respectively. The triglyceride levels were measured and normalized to Protein levels using the BCA Protein Assay (ThermoScientific). Triglyceride levels were normalized to Protein levels.

**RNA Extraction and Reverse Transcription**

RNA was extracted from 30 mg of D. melanogaster third instar larvae from each RNAi, DaGal4 or 6898 driver control, and w^{1118} lines using an RNaseasy kit (Qiagen). RNA was eluted with 30 µl of RNase-free water as opposed to the 50 µl suggested in the manual to concentrate the RNA extract further for reverse transcription. Remaining genomic DNA contamination was removed through the use of a DNA-free kit (Ambion), according to kit protocol.

All reverse transcription reactions were made with 8.0 µl volume of total isolated RNA at appropriate initial concentrations, as well as 83 µM dNTPs, 42 ng/µl oligo-d(T), 3 µl of 5× RT Buffer, 40 U RNSaeeOUT (Invitrogen), 10.5 mM DTT, and 150 U of SuperScript II Reverse transcriptase (Invitrogen) into a final reaction volume of 15 µl. The following procedures and conditions were used for all reaction: first step was incubating RNA with a primer mix of oligo-d(T) and dNTPs at 65°C for 5 minutes, 3 minutes on ice and then a master mix of 5× RT Buffer, RNSaeeOUT, DTT was added, followed by an incubation step for 2 minutes at 42°C. SuperScript II reverse transcriptase was added to the mixture and a final incubation session for 50 minutes at 42°C was carried out. The reaction was then terminated by heat inactivation at 70°C for 15 minutes and chilled on ice for 3 minutes. Immediately after, 60 µl of autoclaved water was added to each reaction tube, resulting in a 5 fold-dilution of cDNA samples.

**Quantitative PCR and Standard Curves using PCR Products**

For all qPCR experiments, primers were set at a 700 nM concentration and added with 5 µl of cDNA and 1× volume of 2× qPCR MasterMix Plus for SYBR Green I Low ROX (Eurogentec) to bring the final volume to 25 µl for each reaction. A mastermix of SYBR green, primers, and water was prepared to minimize variation. All qPCR reactions were performed in an Applied Biosystems 7500 Real Time PCR System (Foster City, USA) under the following conditions: pre-PCR denaturation and polymerase activation step for 15 minutes at 95°C, followed by 45 cycles of 15 second denaturation at 95°C, 1 minute hybridization step at variable temperatures (see hybridization temperatures of select primers below), and a 36 second elongation step at 72°C. Dissociation curve analyses were done to confirm the amplification of a single PCR product, under the following real time conditions: 15 second denaturation step at 95°C, followed by 1 minute at 60°C, and 15 seconds at 95°C. The forward primer 5’- ATTGTGGGAGCCGAAGGT-3’ and the reverse primer 5’-ATGATGTTCTGAAACCTGG-3’ were used at a hybridization temperature of 62°C. The forward and reverse sequences for the Dar-1 primers were 5’-GGAGCCACCTTACGTT- CATT-3’ and 5’-CTTCCACACCAGACACCTTT-3’, respectively and the hybridization temperature used was 62°C. The forward FGLa/AST primer 5’- CTACGGACCAGGACACGGAGA-3’ and the reverse primer 5’- CCCAGGGCAGAAATT- GAAAGG-3’ were used at a hybridization temperature of 62°C. The forward and reverse sequences for ribosomal protein gene Rp49 were 5’-GACGCTTCAAGGGACAGTATCTG-3’ and 5’-AAACGCGGTCTTGATGAGAG-3’, respectively and were used at a hybridization temperature of 56.8°C. Transcript levels for each gene were normalized to gene Rp49 and presented as relative expression levels compared to a control except for transcript levels which are presented as expression levels normalized to Rp49.

**Statistical Analysis**

For larval foraging behavioral assays, Image J was used to quantify foraging path lengths that were traced and scanned. T-tests assuming unequal variance were performed in Graphpad Prism to determine the statistical significance of the foraging path lengths, for transcript levels, and triglyceride levels between RNAi strains and their controls.

**Results**

**Confirmation of RNAi Knockdown in Dar-1 and FGLa/AST RNAi**

To assess the level of mRNA reduction/knockdown in Dar-1 and FGLa/AST RNAi lines (VDRC transformant IDs: 48496 , 101395; and 103215, 14397 respectively), each was crossed to driver lines (DaGal4 or 6896) or to w^{1118}. The relative transcript levels of each gene in the third instar larval stage was quantitated by real-time qPCR. Each Dar-1 and FGLa/AST RNAi line crossed to w^{1118} (Figure 1A, white bars) was compared DaGal4 crossed to w^{1118} (Figure 1A, black bar). In the absence of being crossed to DaGal4, the Dar-1 and FGLa/AST lines had significantly reduced RNA expression levels which ranged from approximately 12–18% (Figure 1A) suggesting that these RNAi lines without a driver exhibit some leaky gene knock down activity.
When crossed to the DaGal4 each RNAi line exhibited a further significant suppression of mRNA levels. Relative to their respective RNAi lines crossed to \( w^{1118} \), DaGal 4 expression in 48496 and 101395 Dar-1 RNAi lines resulted in mRNA suppression by 70 and 56\%, respectively. DaGal4 expression with FGLa/AST RNAi lines 14397 and 103215 resulted in mRNA levels being suppressed 76 and 70\% respectively. Dar 1 and FGLa/AST RNAi were also crossed to a tissue selective driver line 6896 and each displayed a reduction in their respective target gene mRNA levels of approximately 20\% relative to the same RNAi lines crossed to \( w^{1118} \) (Figure 1B).

Larval Foraging Assays for DaGal4 driven Dar-1 and FGLa/AST RNAi

In the larval foraging assay, a self-cross of non-transformed \( w^{1118} \) was compared to the driver DaGal4 X \( w^{1118} \) to confirm that no statistical difference in foraging resulted from the introduction of the DaGal4 driver (Figure 2). A cross of each RNAi line with the DaGal4 driver (eg. 48496 Dar-1 RNAi) was then compared to a cross of each RNAi line with \( w^{1118} \) in which RNAi should not be expressed (Figure 2A). On food, Dar-1 and FGLa/AST RNAi lines crossed to the DaGal4 driver showed significantly decreased foraging distances compared to the same RNAi lines crossed to \( w^{1118} \) (Figure 2A). Similarly, on food foraging of Dar-1 and FGLa/AST RNAi lines crossed to the DaGal4 driver was significantly reduced relative to DaGal4 X \( w^{1118} \) and the \( w^{1118} \) self-cross (Figure 2A).

When tested in the absence of food, no significant difference in foraging behavior was found between self-crossed \( w^{1118} \) and the DaGal4 driver line crossed to \( w^{1118} \). In the absence of food, no significant difference was found when RNAi lines for either Dar-1 or FGLa/AST crossed to the DaGal4 driver line were compared to their respective RNAi lines crossed to \( w^{1118} \) (Figure 2B).

Larval Foraging Assays for 6896 driven Dar-1 and FGLa/AST RNAi

The foraging assay was repeated using third instar larvae from Dar-1 and FGLa/AST RNAi lines crossed to the tissue selective driver 6896. No significant reduction in foraging path length in the presence (Figure 3A) or absence (Figure 3B) of food was noted when 6896 X Dar 1 or FGLa/AST RNAi lines were compared to their respective RNAi lines crossed to \( w^{1118} \).

Transcript Levels of Dar-1 and FGLa/AST Third Instar Larvae RNAi

Sitter and rover phenotypes differ in PKG activity and for transcript level, where sitters have lower PKG activity and for transcript levels compared to their rover counterparts [21]. This suggested that foraging defects in Dar-1 and FGLa/AST RNAi lines may result from reduced PKG due to alterations in the for transcript level. To examine this, we measured for transcript levels in each RNAi line.
The for transcript levels of Dar-1 and FGLa/AST RNAi lines crossed to DaGal4 driver line were significantly reduced compared to the same RNAi lines crossed to w¹¹¹⁸ (Figure 4). RNA extracted from larvae of Dar-1 and FGLa/AST RNAi lines crossed to w¹¹¹⁸ have reduced mRNA levels, with a significant reduction in the 14397 FGLa/AST RNAi line relative to DaGal4 X w¹¹¹⁸ self-cross and self-crossed w¹¹¹⁸ controls (Figure 4) which is consistent the RNAi lines without the drivers showing some knock down of the the Dar-1 and FGLa/AST genes. The tissue selective driver 6986 when crossed to w¹¹¹⁸, 101395 Dar-1 RNAi, or 103215 FGLa/AST

Figure 2. The foraging distance of third instar larvae (path length) A. in the presence of food and B. absence of food for 5 mins was measured for controls DaGal4 x w¹¹¹⁸ (black bar), w¹¹¹⁸ self-cross (grey bar) and Dar-1 and FGLa/AST RNAi lines crossed to DaGal4 (patterned bar) or w¹¹¹⁸ (white bar); N = 30–34. Asterisks indicate a significant difference * = p<0.05; ** = p<0.001 and *** = p<0.0001. Only the significance in comparison with DaGal4 X w¹¹¹⁸ was equivalent.
doi:10.1371/journal.pone.0036059.g002

Figure 3. The foraging distance of third instar larvae (path length) A. in the presence of food and B. absence of food for 5 mins was measured for controls 6896 x w¹¹¹⁸ (black bar), w¹¹¹⁸ self-cross (grey bar) and Dar-1 and FGLa/AST RNAi lines crossed to 6896 (patterned bar) or w¹¹¹⁸ (white bar); N = 30–34.
doi:10.1371/journal.pone.0036059.g003
RNAi lines did not have any significant alteration in for mRNA levels relative to \( w^{118} \) self-cross (Figure 4). This is consistent with 6986 crosses failing to influence foraging behavior (Figure 3).

## Triglyceride Levels of Dar-1 and FGLa/AST Third Instar Larvae RNAi

*C. elegans* npr-9 mutants showed both local search behavior defects and an increase in intestinal lipid accumulation compared to wild type worms [18]. Dar-1 and FGLa/AST RNAi lines showed foraging defects similar to that of npr-9 mutants, which would suggest that foraging defects may be tied into decreased metabolic rate or increased food uptake efficiency. In order to assess this we measured the levels of total triglyceride in third instar larval RNAi lines that showed foraging defects.

In contrast to our hypothesis, there was no significant difference in triglyceride levels between the Dar-1 and FGLa/AST RNAi lines crossed to DaGal4 compared to both RNAi lines crossed to \( w^{118} \) (Figure 5).

### Discussion

FGLa/AST peptides were first identified as JH biosynthesis inhibitors from brain extracts of *Diploptera punctata* [8, 26]. In *D. punctata*, FGLa/ASTs have been shown to elicit myoinhibitory effects in the hindgut and activate midgut \( \alpha \)-amylase secretion [12, 27]. Injection of FGLa/ASTs into *Blattella germanica* females inhibited food consumption, linking FGLa/ASTs with the regulation of digestive or feeding processes [28]. In *D. melanogaster*, FGLa/AST-specific antibodies reveal expression in interneurons, motorneurons and endocrine cells in the midgut [7]. FGLa/ASTs do not inhibit JH biosynthesis or innervate the ring gland/corpora allata in *Diptera* [3]. Based on immunohistochemical localization and mRNA expression, FGLa/ASTs are referred to as brain-gut peptides [29] but their function remains unclear. Work in *C. elegans* provided a testable hypothesis that *D. melanogaster* FGLa/ASTs and their CNS-localized receptor Dar-1 may be associated with foraging behavior [18]. Foraging behaviors in *D. melanogaster*, has been associated with naturally occurring variations in the *for* gene that encodes PKG [19, 30]. On food, the rover phenotype (*for*) exhibits greater mobility than the sitter phenotype (*for*), however, both genotypes move at similar speeds in the absence of food [31, 32]. PKG has an evolutionary conserved function in the regulation of foraging behavior in fruit flies, the honeybee *Apis mellifera*, red harvester ant *Pogonomyrmex barbatus*, and the nematode *C. elegans* [33–35]. Nurse honey bees were found to have lower PKG activity levels and lower *Amfor* RNA levels (ortholog of *for*) than forager honey bees, as well, nurse honey bees can change to foragers when fed an activator of PKG [33]. Interestingly, the difference in PKG activity is reversed when comparing dwellers to roamers in *C. elegans*, where a loss-of-function mutation in PKG (egl-4) caused an increase in roaming behavior in the presence of food. This suggests that PKG has a conserved function among these organisms even though the effect it has may differ between them.

Our results show that the ubiquitous expression of FGLa/AST RNAi or Dar-1 RNAi is related to a decrease in foraging behavior of *D. melanogaster* third instar larvae in the presence of food. Foraging behavior, under these conditions, is not altered in the absence of food. This alteration in foraging behavior appears to be related to a decrease in for transcript levels of Dar-1 and FGLa/AST RNAi lines crossed to DaGal4 compared to both RNAi lines crossed to \( w^{118} \). This suggests that *D. melanogaster* Dar-1 and its FGLa/AST ligand directly or indirectly activates or stabilizes for...
gene expression, as reduction of Dar-1 or its FGLa/AST ligand significantly reduces for mRNA levels causing a reduced foraging (for) phenotype. RNAi lines crossed to w1118 (i.e. no driver) appeared to have reduced for transcripts relative to controls DgG4 X w1118 and the w1118 control, however, this reduction was only significant in the case of 14397 FGLa/AST X w1118 and may be explained, in part, by all RNAi lines X w1118 having ‘leaky’ expression which led to a significant reduction in their respective gene expression. The decrease in foraging behavior on food was not found when the reduction in FGLa/AST and Dar-1 mRNA levels were reduced in a tissue selective manner. This is consistent with the observation that the tissue specific RNAi did not reduce Dar-1 or FGLa/AST gene expression to the levels caused by the ubiquitously expressed DaGal4 driver. Expression of Dar-1 and FGLa/AST RNAi under the direction of the tissue selective driver 6986 did not alter for mRNA levels relative to controls. It is also likely that foraging behavior is only affected when FGLa/ASTs interact with Dar-1 and alter for expression in select cellular localizations.

C. elegans npr-9 mutants showed a significant increase in intestinal lipid accumulation compared to N2 wild type worms, which would suggest that the increased local search behavior seen in these mutant worms may also increase food uptake efficiency [18]. Since Dar-1 and FGLa/AST RNAi lines showed foraging defects similar to that of npr-9 mutants, we hypothesized that similar increase in triglycerides would also be seen. However, our results show no significant difference in triglyceride levels between the Dar-1 and FGLa/AST RNAi lines crossed to either the DgG4 driver or w1118 control. The lack of lipid accumulation when Dar-1 or FGLa/AST levels are reduced is similar to lipid accumulation in D. melanogaster forS larvae. In the presence of food, forS larvae ingest less food, exhibit higher glucose absorption and preferential glucose allocation to lipids rather than sugars. forR larvae thus have higher lipid levels than forS larvae [36, 37]. This contrasts with A. mellifera, where foraging bees have reduced lipid levels in comparison to nurse bees [30].

FGLa/ASTs and receptor Dar-1 do not participate in the regulation of juvenile hormone biosynthesis in D. melanogaster [39]. The alteration in foraging behavior and direct or indirect influence on the for transcript is the first function assigned to the D. melanogaster FGLa/ASTs and its receptor Dar-1. Future work will be directed at defining where FGLa/ASTs interact with Dar-1 and how this interaction influences for gene expression.

**Author Contributions**

Conceived and designed the experiments: CW WGB. Performed the experiments: CW. Analyzed the data: CW ICS WGB. Contributed reagents/materials/analysis tools: WGB. Wrote the paper: CW WGB.

**References**

1. Bendena WG, Garaside CS, Yu CG, Tohe SS (1997) Allatostatins: Diversity in structure and function of an insect neuropeptide family. Annu N Y Acad Sci 814: 53–66.

2. Bendena WG, Donly BC, Tohe SS (1999) Allatostatins: A growing family of neuropeptides with structural and functional diversity. Annu N Y Acad Sci 897: 311–329.

3. Stay B, Tohe SS (2007) The role of allatostatins in juvenile hormone synthesis in insects and crustaceans. Annu Rev Entomol 52: 277–299.

4. Bregel N, Weise C, Krienskamp HJ, Richer D (1999) Reverse physiology in Drosophila. Identification of a novel allatostatin-like neuropeptide and its cognate receptor structurally related to the mammalian somatostatin/galanin/oid peptide family. EMBO J 18(21): 5892–5900.

5. Williamson M, Lenz C, Winther AME, Nassel DR, Grimmelikhuijzen CJP (2001) Molecular cloning, genomic organization, and expression of a B-type (cricket-type) allatostatin preprohormone from Drosophila melanogaster. Biochem Biophys Res Commun 281(2): 344–350.

6. Williamson M, Lenz C, Winther AME, Nassel DR, Grimmelikhuijzen CJP (2001) Molecular cloning, genomic organization, and expression of a C-type (Manduca sexta-type) allatostatin preprohormone from Drosophila melanogaster. Biochem Biophys Res Commun 282(1): 124–130.

7. Yoon JG, Stay B (1995) Immunocytochemical localization of Dilpophila punctata allatostatin-like peptide in Drosophila. J Comp Neurol 363(3): 475–488.

8. Woodhead AP, Stay B, Sedel SL, Khan MA, Tohe SS (1989) Primary structure of four allatostatins: Neuropeptide inhibitors of juvenile hormone synthesis. Proc Natl Acad Sci U S A 86(15): 5997–6001.

9. Lorenz MW, Kollmair M, Hoffmann KH (1995) Identification of two allatostatins from the cricket, Gryllus bimaculatus: Evidence for the existence of cricket allatostatins. Regul Pept 60(1): 107–116.

10. Reichwald K, Unnithan GC, Davis NT, Agricola H, Feyereisen R (1994) Identification and expression of allatostatins in the gut of Reticulitermes flavipes (Isoptera, Rhinotermitidae) larvae thus have higher lipid levels than forS larvae. Proc Natl Acad Sci U S A 101(4): 1339–1342. 10.1073/pnas.0709492105.

11. Pereira HS, Macdonald DE, Hnilicka AJ, Sokolowski MB (1995) Chaser (csr), a new gene affecting larval foraging behavior in Drosophila melanogaster. Proc Natl Acad Sci U S A 92(11): 5044–5049.

12. Sokolowski MB, Hansell KP (1992) The foraging locus - behavioral tests for normal muscle movement in ror and sitter Drosophila melanogaster larvae. Heredity 58(3): 203–209.

13. Osborne KA, Robichon A, Burgess E, Butland S, Shaw RA, et al. (1997) Natural behavior polymorphism due to a cGMP-dependent protein kinase of Drosophila. Science 277(5327): 834–836.

14. Dietz G, Chen D, Schnorrer F, Su KC, Barinova Y, et al. (2007) A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. Nature 448(7150): 151–156.

15. Manseau L, Baradaran A, Brower D, Budhu A, Elefant F, et al. (1997) GAL4 enhancer traps expressed in the embryo, larval brain, imaginal discs, and ovary of Drosophila. Dev Dyn 209(3): 310–322.

16. Lenz C, Williamson M, Grimmelikhuijzen CJP (2000) Molecular cloning and genomic organization of a second probable allatostatin receptor from Drosophila melanogaster. Biochem Biophys Res Commun 273(2): 571–577.

17. Chantapatradi VR, Wang J, Dow JAT (2007) Using FlyAtlas to identify better drosophila melanogaster models of human disease. Nat Genet 39(6): 713–720.

18. Bendena WG, Boudreau JR, Papanicolaou T, Malby M, Tohe SS, et al. (2008) A Carausobatinae allatostatin/galanin-like receptor NPR-9 inhibits local search behavior in response to feeding cues. Proc Natl Acad Sci U S A 105(4): 1339–1342. 10.1073/pnas.0709492105.

19. Pereira HS, Sokolowski MB (1993) Mutations in the larval foraging gene affect adult locomotory behavior after feeding in Drosophila melanogaster. Proc Natl Acad Sci U S A 90(11): 5044–5049.

20. Sokolowski MB, Hansell KP (1992) The foraging locus - behavioral-tests for normal muscle movement in ror and sitter Drosophila melanogaster larvae. Genetica 85(3): 203–209.

21. Pratt GE, Farnsworth DE, Siegel NR, Fok FK, FryeReisen R (1989) Identification of an allatostatin from adult Dilpophila punctata. Biochem Biophys Res Commun 163(3): 1243–1247.

22. Fure M, Zhang JR, Parrtridge E, Nachman RJ, Orchard L, et al. (1999) Effects of an allatostatin and a myosuppressor on midgut carbohydrate enzyme activity in the cockroach Dilpophila punctata. J Comp Physiol B 169(11): 1253–1259.

23. Carelr RB, Beller M, Fellert S, Ramakrishnan H, Jackle H, et al. (2003) Control of fat storage by a Drosophila PAT domain protein. Current Biology 13(7): 609–606.

24. Anderl C, Farnsworth DE, Siegel NR, Fok FK, FryeReisen R (1989) Identification of an allatostatin from adult Dilpophila punctata. Biochem Biophys Res Commun 163(3): 1243–1247.

25. Fure M, Zhang JR, Parrtridge E, Nachman RJ, Orchard L, et al. (1999) Effects of an allatostatin and a myosuppressor on midgut carbohydrate enzyme activity in the cockroach Dilpophila punctata. J Comp Physiol B 169(11): 1253–1259.
31. Sokolowski MB (1980) Foraging strategies of *Drosophila melanogaster* - a chromosomal analysis. Behav Genet 10(3): 291–302.
32. Sokolowski MB (2001) *Drosophila*: Genetics meets behaviour. Nature Reviews Genetics 2(11): 879–890.
33. Ben-Shahar Y, Robichon A, Sokolowski MB, Robinson GE (2002) Influence of gene action across different time scales on behavior. Science 296(5568): 741–744.
34. Fujimura M, Sengupta P, McIntire SL (2002) Regulation of body size and behavioral state of *C. elegans* by sensory perception and the EGL-4 cGMP-dependent protein kinase. Neuron 36(6): 1091–1102.
35. Ingram KK, Oefner P, Gordon DM (2005) Task-specific expression of the foraging gene in harvester ants. Mol Ecol 14(3): 813–818.
36. Kaun KR, Riedl CAL, Chakaborty-Chatterjee M, Belay AT, Douglas SJ, et al. (2007) Natural variation in food acquisition mediated via a *Drosophila* cGMP-dependent protein kinase. J Exp Biol 210(20): 3547–3558.
37. Kaun KR, Sokolowski MB (2009) cGMP-dependent protein kinase: Linking foraging to energy homeostasis. Genome 52(1): 1–7.
38. Toth AL, Robinson GE (2005) Worker nutrition and division of labour in honeybees. Anim Behav 69(2): 427–435.
39. Wang C, Zhang J, Tobe SS, Bendena WG (2012) Defining the contribution of select neuropeptides and their receptors in regulating sesquiterpenoid biosynthesis by *Drosophila melanogaster* ring gland/corpus allatum through RNAi analysis. Gen Comp Endocrinol http://dx.doi.org/10.1016/j.ygcen.2011.12.039.