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Downregulation of *vitellogenin* gene activity increases the gustatory responsiveness of honey bee workers (*Apis mellifera*)

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Abstract

In the honey bee (*Apis mellifera*), young workers usually perform tasks in the nest while older workers forage in the field. The behavioral shift from nest-task to foraging activity is accompanied by physiological and sensory changes so that foragers can be characterized by a higher juvenile hormone (JH) level, a lower vitellogenin protein titer, and an increased responsiveness to water and sucrose stimuli. JH was hypothesized to be the key mediator of behavioral development, physiology, and sensory sensitivity in honey bee workers. Recent research, however, has shown that JH is controlled by the hemolymph vitellogenin level, which implies that the fat body specific *vitellogenin* gene can be a key regulator of behavioral change. Here, we show that downregulation of *vitellogenin* activity by RNA interference (RNAi) causes an increase in the gustatory responsiveness of worker bees. Our observations suggest that vitellogenin is an important regulator of long-term changes in honey bee behavior.

Keywords

Honey bee worker; *Vitellogenin* gene; Juvenile hormone; Social behavior; Sensory sensitivity; Gustatory responsiveness

1. Introduction

Adult honey bee workers are functionally sterile females that go through a characteristic behavioral progression [1]. After emergence, worker bees first perform tasks within the protected nest, such as feeding larvae, and later they collect pollen, nectar, water, and propolis in the field. This shift from nursing to foraging behavior typically occurs after 18–28 days of adult life [1], and it is accompanied by distinctive changes of physiology (reviewed in ref. [2]) and sensory integration (reviewed in ref. [3]).

At the physiological level, changes at the onset of foraging include an increase in the juvenile hormone (JH) titer [4], and a decrease in the hemolymph vitellogenin level [5]. JH is produced by the corpora allata complex by the subesophageal ganglia, and is part of the hormonal control machinery of oogenesis in solitary insects. Vitellogenin is a conserved yolk precursor protein that it is synthesized by the fat body in invertebrates [6]. In many insect species, JH is involved in the regulation of reproductive behavior and sensory tuning (reviewed in ref. [7]). It was formerly suggested that JH has a behavioral control-function in worker bees that

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represents a heritage from the bee's solitary ancestry, or alternatively the function evolved de novo in the worker caste [8–10]. This framework proposed that JH is the director of the behavioral development of worker bees. However, it was recently shown that the JH titer of workers is a function of their hemolymph vitellogenin protein level, and that downregulation of *vitellogenin* gene expression by RNA interference (RNAi) causes an increase in the JH concentration of the hemolymph [11]. The dynamic dose–response of JH to *vitellogenin* knockdown mimics the actual transition state of decreasing vitellogenin protein titer and increasing JH level that is observed at onset of foraging. These data indicated for the first time that vitellogenin has regulatory properties, as proposed by Amdam and Omholt [12]. A prediction of this hypothesis is that vitellogenin depletion due to metabolic consumption [2, 13], or via developmental loss of *vitellogenin* gene activity (G.V. Amdam, K.-A. Nilsen, K. Hartfelder, and M.K. Fondrk, unpublished data), constitutes the upstream signal that paces behavioral development and triggers foraging onset in the bee. Consequently, honey bee *vitellogenin* should be a key regulator of changes in behavior.

One systemic change that characterizes the transition to foraging activity is sensory-response modulation. Compared to nurse bees (bees that feed larvae), foragers show higher responsiveness to water and sucrose stimuli that are applied to their antennae [14]. Foragers also perform better in associative tactile and olfactory learning [15,16] and this superior ability is related to their increased sensory responsiveness. Foragers and young nest bees, moreover, differ in their non-associative learning performance [3]. The physiological basis for these changes in sensory sensitivity and learning is not clear. JH may play a role since treatments with JH mimic that accelerate the onset of foraging [10] also increase gustatory responsiveness [17], but a causal association has not been demonstrated so far. Since vitellogenin and JH are interlinked, it is possible to ask if the *vitellogenin* gene, as an upstream controller of the physiological JH level, affects individual responsiveness.

In this study, we addressed the proposition that the honey bee *vitellogenin* gene is a regulator of social behavior [11,12] by studying the relationship between the hemolymph vitellogenin level and the gustatory responsiveness of worker bees. Seven-day-old bees were used—first to compare workers with a *vitellogenin* knockdown phenotype induced by RNAi to control bees, and subsequently to test for covariance patterns in worker groups with naturally fluctuating hemolymph vitellogenin concentrations.

2. Materials and methods

2.1. Bees

The experiment was performed at the University of California, Davis. Approximately equal numbers of newly emerged worker bees from three source colonies were mixed together and divided into three groups that each were marked on the thorax with a separate color of paint (Testors™ Enamel). Bees from two of the groups were injected with different forms of double-stranded RNA (dsRNA, see the next section for further information) dorsally between the fifth and sixth abdominal segment using a micro-syringe (Hamilton) with a G30 needle (BD). The remaining group was left untreated (i.e., non-injected “NoI-Controls”). The three groups were subsequently mixed together, and the bees were introduced into host colonies. The workers were retrieved for sampling after 7 days.

2.2. Knockdown- and control phenotypes

Bees with *vitellogenin* knockdown phenotype were obtained with the RNAi protocol previously described by Amdam et al. [18]. The procedure was slightly modified so that our injected control group received dsRNA derived from green fluorescent protein (GFP) sequence instead of the vehicle used by Amdam et al. [18]. Thus, the two injected groups described in

Section 2.1 received 2 µl dsRNA solution (5 µg dsRNA per µl nuclease free water) against the *vitellogenin* gene (i.e., “Vg-RNAi” treatment) or derived from GFP sequence (i.e., “GFP-Controls”), respectively. GFP-derived dsRNA does not share close homology with *Apis mellifera* genes, and close homology is required to trigger RNAi [19–23]. Therefore, injections of GFP-derived dsRNA allowed us to control for effects stemming from the full injection procedure; including handling, wounding, and transfer of dsRNA template.

For dsRNA synthesis, primers were designed from the sequence of the *Apis mellifera vitellogenin* (GenBank number: AJ517411) cDNA clone *AP4a5*, and the GFP encoding sequence (GenBank number: AF324407) of the pGFP vector (Clontech). Primer sequences were fused with T7 promoter sequence (underlined): for clone *AP4a5*: 5'-TAATACGACTCACTATAGGGCGAACGACTCG-ACCAACGACTT-3' and 5'-TAATACGACTCACTATAGGGCGAAACGAAA-GGAACGGTCAATTCC-3'; and for pGFP: 5'-TAATACGACTCACTATAGGGCGATTCATGGCCAACACTTGTCC-3', and 5'-TAATACGACTCACTATAGGGCGATCAAGAAGGACCATGTGGTC-3'. PCR reactions were performed according to standard procedures using *AP4a5* and the pGFP vector as templates. Resulting products excluding the fused T7 promoters were 504 and 503 bp, respectively. These were purified using the QIAquick™ PCR purification kit (QIAGEN), and RNA was prepared using the Promega RiboMax™ T7 system (Promega). RNA was extracted by TRIzol LS reagent (Gibco-BRL), resuspended in nuclease free water, heated at 96 °C for 2 min in an Eppendorf Thermomixer (Brinkmann), and left to cool at room temperature for 20 min. The integrity of the dsRNA was tested using 1.5% agarose gels, and the products were diluted with nuclease free water (Qiagen) to the final concentration of 5 µg/µl.

2.3. Measuring gustatory responsiveness

The 7-day-old worker bees were collected in the morning and placed individually in glass vials. Each bee was stored at approximately 4 °C until it showed first signs of immobility. It was then mounted in a metal holder and fixed with one strip of adhesive tape between head and thorax and a second strip over the abdomen as described by [24]. Half an hour after mounting, gustatory responsiveness was tested using the proboscis extension response (PER). The investigator was blind to the treatment identity of the bees. Water was first presented to every bee by applying it to her antennae. Thereafter, the following 6 sucrose concentrations were applied: 0.1%, 0.3%, 1%, 3%, 10%, 30% (w/v). It was recorded which solution elicited proboscis extension. Between each test, a 2 min interval was inserted to ensure that we did not measure sensitization effects. The sum of the responses to water and the 6 sucrose concentrations represents the gustatory response score (GRS) of an individual and is a measure of its gustatory responsiveness [25]. Gustatory response scores ranged between 0 (no response to water or any of the sucrose solutions) and 7 (proboscis extension response to all solutions). After measuring gustatory responsiveness, the bees were prepared for hemolymph sampling.

2.4. Quantification of vitellogenin titer

Drummond micropipettes were used to extract 1 µl hemolymph from an incision made between the second and third abdominal segment of each bee. Care was taken to avoid contaminants from tissues. Hemolymph was dissolved in 50 µl TRIS-buffer (20 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, pH 7.5, 1 mM phenylmethylsulfonyl fluoride, 5 mM benzamidin, 0.7 µM pepstatin, 8 µM chymostatin, 10 µM leupeptin, and 0.8 µM aprotinin, Sigma-Aldrich), before samples were separated by 7.5% SDS-polyacrylamide gel electrophoresis using standard methods [26]. A β-galactosidase standard (Sigma-Aldrich) was included to allow densitometrically quantification by the method of Lin et al. [27], in which vitellogenin is detected as a single band of 180 kDa [27,28]. The densitometrical analysis was performed by the Quantity One imaging software (Bio-Rad) after staining the gels with Commassie Brilliant Blue (Sigma-Aldrich). Gel-to-gel variation in staining intensity was controlled by background

correction and the β -galactosidase standard, which was loaded in equal dilution series on all gels [27].

2.5. Data analysis

Information on hemolymph vitellogenin level and gustatory responsiveness was obtained from 193 bees. Out of these workers, those with a GRS of zero ($n = 60$) were excluded from the analysis because their gustatory responsiveness could not be measured. The remaining sample sizes, by treatment, were: NoI-Controls, $n = 52$; GFP-Control, $n = 38$; and Vg-RNAi, $n = 43$. The datasets for the hemolymph vitellogenin titer and the gustatory responsiveness did not follow normal distributions, and therefore the non-parametric Kruskal–Wallis test was used to test for treatment effects. The two-tailed Kruskal–Wallis H -test was followed by the non-parametric Dunn's multiple comparisons test to determine the statistical relationship between the three treatments. A Spearman rank correlation was used to study the relationship between the vitellogenin titer and the responsiveness of the bees in the NoI-Control and GFP-Control groups.

3. Results

3.1. Vitellogenin titer

To validate the experimental protocol, we first established that we had a significant treatment effect on the vitellogenin level (KW = 44.71, $p < 0.0001$). The hemolymph titer of the Vg-RNAi group was significantly reduced (Fig. 1, rank sum difference: 52.15, $p < 0.001$, and rank sum difference: 37.09, $p < 0.001$) relative to the NoI-Control and the GFP-Control, respectively. The vitellogenin titers of the NoI-Control and the GFP-Control were not different (rank sum difference: 15.06, $p > 0.05$). These findings showed that the *vitellogenin* gene knockdown results in a lower circulating vitellogenin protein titer [18], and also confirmed that control injections of dsRNA derived from GFP, which presumably does not correspond to any native honey bee mRNA, do not affect the hemolymph vitellogenin level.

3.2. GRS

We found a significant effect of treatment on the GRS of the worker bees (KW = 9.89, $p < 0.01$). The post hoc multiple comparison tests revealed that the GRS of the NoI-Control and the GFP-Control did not differ (rank sum difference: 1.95, $p > 0.05$). Furthermore, the gustatory responsiveness of the Vg-RNAi group was significantly elevated (Fig. 2, rank sum difference: 22.94, $p < 0.05$, and rank sum difference: 20.99, $p < 0.05$) compared to the NoI-Control and the GFP-Control, respectively. This result demonstrated that downregulation of *vitellogenin* gene activity causes an increased gustatory responsiveness in 7-day-old bees.

3.3. Correlative association

We did not detect a significant relationship between the vitellogenin titer and the GRS in the data from the two control groups ($p > 0.05$, NoI-Control $\rho = 0.05$, GFP-Control $\rho = 0.13$, the two groups combined $\rho = 0.08$). This finding suggests that the range and distribution [2,11,12] of the hemolymph vitellogenin concentrations that were found in our experimental groups did not influence gustatory responsiveness.

4. Discussion

We have demonstrated that suppression of the hemolymph vitellogenin protein level by RNAi causes a significant increase in the gustatory responsiveness of 7-day-old worker bees. Seven-day-olds were chosen because the vitellogenin titer is higher in these bees than in younger

adults (0–3 or 5 days). The age group we tested was therefore more suitable for correlative analysis due to greater variance between individuals [11].

It has been shown previously that *vitellogenin* downregulation by RNAi results in an elevated JH level in 7-day-old bees [11]. Thus, our experimental Vg-RNAi group is characterized by a higher physiological JH titer than the controls in addition to its lower vitellogenin protein concentration. Experimental application of JH mimic triggers an increase in the bee's responsiveness to sucrose [17] but also reduces the rate of vitellogenin synthesis [29], possibly because of regulatory feedback between vitellogenin and JH [11,12]. It is unclear, however, if treatments with JH mimic result in circulating JH levels that are physiologically relevant. Thus, our results are important because they show that a gene knockdown that is characterized by an elevated physiological JH level is characterized also by increased gustatory responsiveness.

Our RNAi data cannot establish if higher GRS emerge via a hormonal regulatory cascade or other bioactive factors that are downstream of the *vitellogenin* gene, or if the low vitellogenin titer in the knockdown phenotype modulates responsiveness directly [12]. This question is addressed in part by our examination of the correlative relationship between the vitellogenin titer and the GRS of the control bees. Explicitly, the control groups exemplify intrinsic variation in vitellogenin concentration that emerges from naturally occurring fluctuations in vitellogenin synthesis, consumption, and breakdown [2,12]. Our finding that such variation is not correlated with GRS suggests that gustatory responsiveness is not modulated by the hemolymph vitellogenin level directly. Rather, the data suggest that elevated responsiveness is driven by a downstream cascade that is activated after a more extended period with a physiologically low vitellogenin titer. This proposition is supported by results of Amdam et al. [18], who found that *vitellogenin* dsRNA treatment at adult emergence suppresses *vitellogenin* mRNA expression in 5–15-day-old bees. Younger adults (i.e., 1–3 days old), which were not examined by Amdam et al. [18], are characterized by low vitellogenin titers also under natural conditions [30,31]. In sum, it is reasonable to infer that bees treated with *vitellogenin* dsRNA at adult emergence are characterized by low vitellogenin titers for a more extended period than controls. It may well be that JH, which in response to low vitellogenin levels may reinforce the shift to foraging behavior via further suppression of vitellogenin synthesis [12], is a key component in this pathway. A possible caveat, though, is that the vitellogenin level of our controls was somewhat lower than in a previous study where hemolymph was drawn shortly after the bees were collected from the hive [7]. This pattern may imply that workers metabolize vitellogenin, as proposed by Engels et al. [32], during the 2 h laboratory handling schedule that is required for the GRS assay. A direct relationship between vitellogenin titer and responsiveness may subsequently become difficult to detect.

Our findings that a prolonged suppression of the hemolymph vitellogenin level increases gustatory responsiveness, whereas gustatory responses appear to be independent of temporary fluctuations in the vitellogenin titer [2,12], has interesting implications. Our observations suggest that vitellogenin is an important regulator of long-term changes in honey bee behavior. This proposition arises from the fact that gustatory responsiveness correlates with associative and non-associative learning performance [3,25]. Differences in learning ability or learning performance, in turn, are correlated with patterns of behavioral development and division of labor [15,16]. Bees with high gustatory responsiveness are usually more sensitive to other stimulus modalities than bees with lower gustatory responsiveness [25], and individuals that display a high sensitivity for sucrose are also highly responsive to odors and to light [25]. Gustatory responsiveness increases with age in parallel with changes in worker behavior, and thus the tuning of the sensory system appears to be an element of the bee's age-related division of labor. Until recently, these associations were largely accredited to a systemic role of JH [33]. Instead, our data support the new view [11] that vitellogenin is a regulator of honey bee division of labor.

A question for further study is how levels of circulating vitellogenin are monitored physiologically so that prolonged suppression, and not fluctuations, can result in hormonal [11] and sensory (this paper) effects. One explanation is that vitellogenin binds with affinity to a censoring apparatus, or the allatoregulatory complex, as hypothesized by Amdam and Omholt [12]. In mathematical terms, this theoretical architecture produces a robust regulatory switch that is insensitive to short-term changes in the vitellogenin titer.

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References

1. Winston, ML. The biology of the honey bee. Cambridge: Massachusetts: Harvard University Press; 1987. p. 296
2. Amdam GV, Omholt SW. The regulatory anatomy of honeybee lifespan. *J Theor Biol* 2002;216:209–28. [PubMed: 12079372]
3. Scheiner R. Responsiveness to sucrose and habituation of the proboscis extension response in honey bees. *J Comp Physiol A* 2004;190:727–33.
4. Jaycox ER, Skowronek W, Guynn G. Behavioral changes in worker honey bees (*Apis mellifera*) induced by injections of a juvenile hormone mimic. *Ann Entomol Soc Am* 1974;67:529–34.
5. Engels W, Fahrenhorst H. Alters- und kastenspezifische Veränderungen der Haemolymph-Protein-Spektren bei *Apis mellifera*. *Roux's Arch* 1974;174:285–96.
6. Spieth J, Nettleton M, Zuckeraprisson E, Lea K, Blumenthal T. Vitellogenin motifs conserved in nematodes and vertebrates. *J Mol Evol* 1991;32:429–38. [PubMed: 1904098]
7. Amdam GV, Norberg K, Fondrk MK, Page RE. Reproductive ground plan may mediate colony-level selection effects on individual foraging behavior in honey bees. *Proc Natl Acad Sci USA* 2004;101:11350–5. [PubMed: 15277665]
8. Robinson GE. Regulation of division of labor in insect societies. *Annu Rev Entomol* 1992;37:637–65. [PubMed: 1539941]
9. Robinson GE, Vargo EL. Juvenile hormone in adult eusocial Hymenoptera: Gonadotropin and behavioral pacemaker. *Arch Insect Biochem Physiol* 1997;35:559–83. [PubMed: 9210289]
10. Bloch, G.; Wheeler, D.; Robinson, GE. Endocrine influences on the organization of insect societies. In: Pfaff, D.; Arnold, A.; Etgen, A.; Fahrenbach, S.; Moss, R.; Rubin, R., editors. *Hormones, brain and behavior*. 3. San Diego: Academic Press; 2002. p. 195–235.
11. Guidugli KR, Nascimento AM, Amdam GV, Barchuk AR, Angel R, Omholt SW, et al. Vitellogenin regulates hormonal dynamics in the worker caste of a eusocial insect. *FEBS Lett* 2005;579:4961–5. [PubMed: 16122739]
12. Amdam GV, Omholt SW. The hive bee to forager transition in honeybee colonies: the double repressor hypothesis. *J Theor Biol* 2003;223:451–64. [PubMed: 12875823]
13. Amdam GV, Norberg K, Hagen A, Omholt SW. Social exploitation of vitellogenin. *Proc Natl Acad Sci USA* 2003;100:1799–802. [PubMed: 12566563]
14. Pankiw T, Page RE. The effect of genotype, age, sex, and caste on response thresholds to sucrose and foraging behavior of honey bees (*Apis mellifera* L.). *J Comp Physiol A* 1999;185:207–13. [PubMed: 10488557]
15. Scheiner R, Page RE, Erber J. The effects of genotype, foraging role, and sucrose responsiveness on the tactile learning performance of honey bees (*Apis mellifera* L.). *Neurobiol Learn Mem* 2001;76:138–50. [PubMed: 11502146]
16. Scheiner R, Page RE, Erber J. Responsiveness to sucrose affects tactile and olfactory learning in preforaging honey bees of two genetic strains. *Behav Brain Res* 2001;120:67–73. [PubMed: 11173086]
17. Pankiw T, Page RE. Effect of pheromones, hormones, and handling on sucrose response thresholds of honey bees (*Apis mellifera* L.). *J Comp Physiol A* 2003;189:675–84.

18. Amdam GV, Simões ZLP, Guidugli KR, Norberg K, Omholt SW. Disruption of vitellogenin gene function in adult honeybees by intra-abdominal injection of double-stranded RNA. *BMC Biotechnol* 2003;3:1–8. [PubMed: 12546706]
19. Beye M, Hartel S, Hagen A, Hasselmann M, Omholt SW. Specific developmental gene silencing in the honey bee using a homeobox motif. *Insect Mol Biol* 2002;11:527–32. [PubMed: 12421410]
20. Fire A. RNA-triggered gene silencing. *TIG* 1999;15:358–63. [PubMed: 10461204]
21. Parrish S, Fleenor J, Xu S, Mello C, Fire A. Functional anatomy of a dsRNA trigger: differential requirement for the two trigger strands in RNA interference. *Mol Cell* 2000;6:1077–87. [PubMed: 11106747]
22. Timmons L, Fire A. Specific interference by ingested dsRNA. *Nature* 1998;395:854. [PubMed: 9804418]
23. Fire A, Xu SQ, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 1998;391:806–11. [PubMed: 9486653]
24. Bitterman ME, Menzel R, Fietz A, Schäfer S. Classical conditioning of proboscis extension in honeybees (*Apis mellifera*). *J Comp Physiol* 1983;97:107–19.
25. Scheiner R, Page RE, Erber J. Sucrose responsiveness and behavioral plasticity in honey bee (*Apis mellifera*). *Apidologie* 2004;35:133–42.
26. Laemmli UK. Cleavage of structural proteins during assembly of head of bacteriophage-T4. *Nature* 1970;227:680–5. [PubMed: 5432063]
27. Lin H, Winston ML, Haunerland NH, Slessor KN. Influence of age and population size on ovarian development, and of trophallaxis on ovarian development and vitellogenin titres of queenless worker honey bee (Hymenoptera: Apidae). *Can Entomol* 1999;131:695–706.
28. Wheeler DE, Kawooya JK. Purification and characterization of honey bee vitellogenin. *Arch Insect Biochem Physiol* 1990;14:253–67. [PubMed: 2134180]
29. Pinto LZ, Bitondi MMG, Simões ZLP. Inhibition of vitellogenin synthesis in *Apis mellifera* workers by a juvenile hormone analogue, pyriproxyfen. *J Insect Physiol* 2000;46:153–60. [PubMed: 12770247]
30. Fluri P, Lüscher M, Wille H, Gerig L. Changes in weight of the pharyngeal gland and haemolymph titres of juvenile hormone, protein and vitellogenin in worker honey bees. *J Insect Physiol* 1982;28:61–8.
31. Amdam GV, Hartfelder K, Norberg K, Hagen A, Omholt SW. Altered physiology in worker honey bees (Hymenoptera: Apidae) infested by the mite *Varroa destructor* (Acari: Varroidae): a factor in colony loss during over-wintering? *J Econ Entomol* 2004;97:741–7. [PubMed: 15279246]
32. Engels, W.; Kaatz, H.; Zillikens, A.; Simões, ZLP.; Truve, A.; Braun, RP., et al. Honey bee reproduction: vitellogenin and caste-specific regulation of fertility. In: Hoshi, M.; Yamashita, O., editors. *Advances in invertebrate reproduction*. 5. Amsterdam: Elsevier Science Publishers B.V; 1990. p. 495-502.
33. Elekonich MM, Roberts SP. Honey bees as a model for understanding mechanisms of life history transitions. *Comp Biochem Physiol A Mol Integr Physiol* 2005;141:362–71. [PubMed: 15925525]

Fig. 1. Mean vitellogenin titer (Vg titer) and standard error of Vg-RNAi treated bees and the two controls. The *x*-axis shows the treatment. The *y*-axis displays the vitellogenin titer. Sample sizes: NoI-Control, $n = 52$; GFP-Control, $n = 38$; Vg-RNAi, $n = 43$. Vitellogenin is measured in µg/µl relative to a β -galactosidase standard.

Fig. 2. Mean gustatory response scores (GRS) and standard error of Vg-RNAi treated bees and the two controls. The *x*-axis shows the treatment. The *y*-axis displays the GRS. Sample sizes as in Fig. 1.