Comments to Recent Studies Showing Systemic Mechanisms Enabling *Drosophila* Larvae to Recover From Stress-Induced Damages

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ABSTRACT: Compensational recovery from the damage created by stressors is important for all animals. However, how organisms recover from stress-induced negative impacts has been poorly understood. An 1-hour exposure to heat stress at 35°C led to reduced feeding activity of *Drosophila melanogaster* larvae, which caused reduction in body weight 2 hours after the stress, but not at other times. Such weight losses seem to be rescued by following enhanced feeding activities. We investigated the mechanisms underlying the accelerated feeding activity after the stress-induced reduction in feeding behavior. Our data showed increased expression of sweet taste gustatory receptor genes (*Grs*) and concomitant decreased expression of bitter taste *Grs* in the mouth parts 2 to 4 hours after the heat treatment for 1 hour. However, nontypical taste *Gr* expression was not changed. Furthermore, integration of both messenger RNA and protein expression analysis revealed that expression levels of tropomyosin and ATP (adenosine triphosphate) synthase β subunit were significantly increased in their mouths 3 to 5 hours after the heat stress. The increased expression of these genes would contribute to accelerated muscular movement of the mouth hooks. This study indicated that *Drosophila* larvae possess an efficient systemic mechanism that enables them to recover from growth delay caused by stress conditions.

KEYWORDS: Stress, recovery, gustatory receptors, tropomyosin, ATP synthase ß subunit

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In natural environments, wild animals seldom experience fulfillment of all their biotic and abiotic requirements and are routinely exposed to a variety of environmental stressors. To combat these stressors, they have evolved highly successful strategies.[1,2] Much of the field of stress biology has focused on multiple physiological changes of test organisms to reveal such strategies that enable them to survive under stressful environments. It is becoming apparent that various cellular and molecular mechanisms essential for stress resistance and tolerance occur in organisms. A representative example of the research is the finding that heat shock proteins (Hsps) are conserved in a wide variety of organisms not only in the ways they are induced by stress but also in their primary structures.[3,4] A prolonged production of unfolded or misfolded proteins triggers the expression of Hsps, which represents an essential cellular strategy in all organisms to deal with stress conditions such as high temperature.[5,6] Despite substantial attention to stress resistance mechanisms, the processes of the recovery phase after stress have not been actively explored yet. It is therefore poorly understood whether animals possess a specific mechanism to recover from the damage done by environmental stresses.

We recently investigated the effects of acute heat stress on feeding activities and body weights of *Drosophila melanogaster* larvae of the y w strain during the poststress period to test our hypothesis that animals possess systemic mechanisms to cope with and survive the damage caused by environmental stressors.[7] Feeding rates of *Drosophila* larvae were shown to decrease soon after the stress at 35°C for 1 hour, which caused reduction in body weight 2 hours after the stress. Thereafter, feeding activities increased to higher levels compared with control larvae at 3 hours after the heat stress. This enhanced feeding action led to weight restoration by around 4 hours after heat stress. Those observations enabled us to surmise that *Drosophila* larvae possess a compensational recovery mechanism during poststress periods.

We examined the mechanism by which animals increase their feeding activity after the stress treatment (at 35°C for 1 hour). It is generally known that taste neurons expressing gustatory receptor genes (*Grs*) in insect gustatory organs detect tastants by direct interactions between Grs and ligands.[8,9] We expected that expression levels of Grs in mouths would be changed before and after the heat stress, which affects larval feeding activities. The results showed that expression of all sweet taste Grs such as *Gr5a, Gr64a, Gr64f*, and *Gr43a* was elevated at around 4 hours after the heat stress. By contrast, expression of bitter taste Grs such as *Gr66a* and *Gr33a* was depressed after the heat stress (Figure 1). No change was observed in expression of a nontypical taste Gr, *Gr68a*, sensing acoustic signals after the heat treatment[10] (Figure 1). Similar changes in expression levels of sweet taste Gr5a and bitter taste Gr66a genes were recorded in the mouths of test larvae after cold-stress treatment 4°C for 12 hours. We interpreted these results as an indication that enhanced expression of sweet taste Grs and depressed expression of bitter taste Grs would contribute to the increased feeding activities of *Drosophila* larvae.

Although we have not examined how expression of those *Grs* is controlled during the poststress period, we might take some hints from prior reports on starvation.[11] It is undoubted

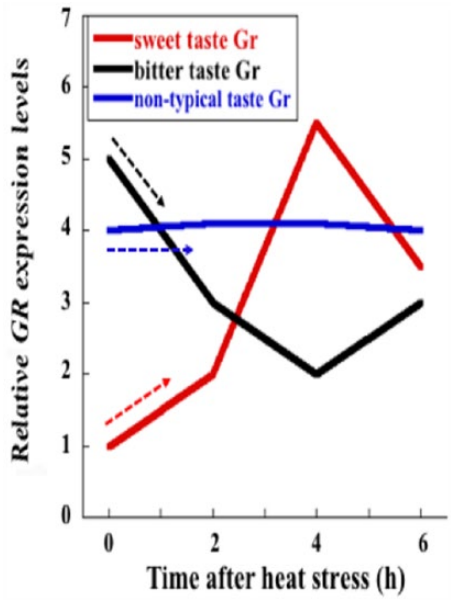

Figure 1. Tendency curves of sweet taste, bitter taste, and nontypical taste *Gr* expression changes after heat stress at 35°C for 1 hour.

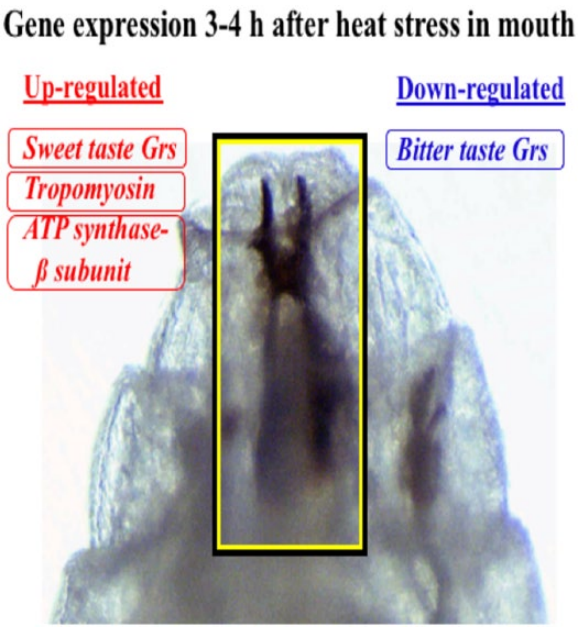

Figure 2. Graphic summary showing upregulated and downregulated expression of genes in the mouth part of *Drosophila* larvae after heat stress at 35°C for 1 hour.

that starved animals have increased appetite; at that time, they must become less selective in their food choices. Increasing their sweet taste sensitivity enhances their appetite.[12,13] In *Drosophila*, it has been reported that hunger elevates gustatory sensitivity to sweet, in part, via enhanced dopamine release in *Gr5a*-expressing neurons, which rises calcium responses to Gr activation.[14,15] Hunger also reduces sensitivity to inedible and toxic bitter compounds. It is possible that the upregulated sweet taste *Gr* expression and downregulated bitter taste Gr expression concomitantly occur in the gustatory organs of starved animals. We speculate that sugar-sensing neurons with increased sweet taste Gr expression and bitter-sensing neurons with decreased bitter taste Gr expression are, respectively, activated and inactivated via dopaminergic regulation in *Drosophila* larvae during the poststress phase just as in starved larvae.

Quantitative analysis of protein and messenger RNA species in the mouths of *Drosophila* larvae showed elevated expression of tropomyosin and ATP (adenosine triphosphate) synthase β subunit 3 to 5 hours after heat stress. In insects, feeding responses are physically controlled by synchronous muscle movement in which one nerve excitation leads to one muscle contraction. The muscle contraction unit structure is extremely conserved within the animal kingdom, together with a similar conservation of the major myofibrillar proteins chiefly consisted of actin, myosin, troponin, and tropomyosin.[16] Muscle tension is caused by interaction of myosin crossbridges on thick filaments with actin in thin filaments. Tropomyosin directly interacts with both actin and myosin and plays a pivotal role in regulating the actin-myosin interaction.[17] Therefore, elevated expression of tropomyosin in the mouth parts must enable *Drosophila* larvae to move the mouth hooks for acceleration of diet intake. Furthermore, enhanced expression of the ATP synthase β subunit would contribute to supplying ATP more efficiently because it has been reported that the ATP synthase β subunit serves as a key component in ATP synthesis in rat pancreatic β cells.[18,19] Therefore, it is reasonable to assume that both enhanced expressions of tropomyosin and ATP synthase β subunit are essential for *Drosophila* larvae to accelerate the diet intake by speedily moving the mouth hooks (Figure 2).

In summary, our recent study supported our hypothesis that insects have evolved a systemic mechanism that promotes their recovery from the damage done by stressors during poststress periods. The 2 new findings mentioned above, which are interesting in terms of physiological aspects, allow us to propose that this recovery system is composed of multiple functional components. First, we found the elevated taste-sensing capacities in test larvae after heat stress. Upregulation of sweet taste *Gr* expression and concomitant downregulation of bitter taste *Gr* expression during the accelerated feeding periods led us to propose that insect feeding activities would be controlled by expression patterns of sweet and bitter taste Gr genes. Although starvation has been found to facilitate odor representation in odor receptor–expressing neurons that process olfactory input,[20] it has not been reported any Gr expression changes in starved *Drosophila* larvae. Therefore, this must be the first observation implying that population of both sweet and bitter taste Grs in the mouths directly or indirectly controls feeding activities of insects. Second, we found the enhanced mouth movement ability of *Drosophila* larvae after heat stress. This is due to elevated expression of the mouth myofibrillar protein, tropomyosin, and the key enzyme in ATP synthesis, ATP synthase β subunit. Our data suggest that enhancement of feeding activity during

poststress periods would be supported by supplied mouth muscle fiber component together with the fuel ATP.

The fact that insects have evolved such a complicated and highly elaborate system is regarded as a testament to the importance of the mechanisms that overcome the damages caused by stressors. If the stress-induced damages significantly retard larval growth rates, their reproductive maturation and the synchronized emergence of matured male and female adults would be affected. Given that this is applied to animals with short life spans such as insects, it is reasonable to assume that the recovery mechanism characterized in this study crucially contributes to preventing their population from decreasing.

Author Contributions

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