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Title: Identification of a neural pathway governing satiety in *Drosophila*

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Key words: Food intake, BW, MIP, satiety, anorexigenic pathway

Abbreviations: BW, body weight; MIP, myoinhibitory peptide; SPR, sex peptide receptor; PER, proboscis extension reflex

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Abstract
Satiety cues a feeding animal to cease further ingestion of food for protecting from excessive energy gain. Impaired control of satiety is often associated with feeding-related disorders such as obesity. In the present paper, we reported the identification of a neural pathway that expresses myoinhibitory peptide (MIP) critical for satiety responses in *Drosophila*. Targeted silencing of MIP neuron activity strikingly increased body weight (BW) through elevated food intake. Similarly, genetic disruption of the gene encoding MIP also elevated feeding and BW. Suppressing MIP pathway behaviorally transformed satiated flies to feed like the starved ones with augmented sensitivity to food. Conversely, temporal activation of MIP neuron markedly reduced food intake and BW, and blunted the sensitivity of starved flies to food as if they have been satiated. Shortly after termination of MIP neuron activation, the reduced BW was reverted to normal level with a strong feeding rebound. These results consistently suggest the switch-like role of MIP pathway in feeding by controlling satiety.

Main Text
Appropriate regulation of feeding behaviors is critical for survival in animal. Disruption of feeding regulation results in feeding-related disorders including obesity and hyper- or hypophagia. The feeding behaviors are primarily shaped by two motivational states, hunger and satiation. Hunger cues an animal to seek food sources and ingest nutritive food, while satiation signals a feeding animal to cease further ingestion of food to prevent animals from excessive energy gain.

Recent studies using *Drosophila* have shed much light on animal feeding behaviors specifically on hunger-driven behaviors. Several lines of evidence have suggested that hungry fruit flies are capable of evaluating caloric values of sugars and selecting nutritive sugars over zero-calorie ones. Dh44 (the homolog of cortisol releasing hormone; CRH)-expressing neurons are activated by nutritive sugars, but not by zero-calorie sugars, to promote feeding. Another group of neurons expressing the gustatory receptor 43 (Gr43a) in the brain detects hemolymph fructose levels after a meal to promote feeding in hungry flies. More recently, a subset of serotonergic neurons was shown to evoke several traits of hunger responses when activated, indicating that these neurons mediate the representation of hunger in *Drosophila*.

In mammals, feeding behaviors are regulated by anorexigenic proopiomelanocortin (POMC) neurons and orexigenic agouti-related peptide (AGRP) neurons in the hypothalamic arcuate nucleus. Genetic ablation of POMC markedly elevates both food intake and BW. Conversely, activation of AGRP neurons that also express neuropeptide Y (NPY) promotes feeding activity. As in mammals, neuropeptide F, an orthologue of mammalian NPY, has been shown to promote feeding activity and hunger-driven behaviors in *Drosophila*. Unlike POMC system in mammals some known anorexigenic neural pathways in *Drosophila* appear dispensable for BW homeostasis.

To identify an anorexigenic neural pathway that functions critically in BW regulation of *Drosophila*, we and our colleagues performed a genetic screen using the GAL4-UAS system. A collection of neuropeptide-GAL4 driver lines that label neuropeptide neurons was with a neural silencer line bearing *UAS-tetanus toxin* (*UAS-TNT*). In the screen, the progenies from the cross would harbor individual neuropeptide neurons selectively silenced depending on the driver. Our working hypothesis was that flies with silenced neuropeptide neurons critical for satiety control would show notable BW increase. Indeed, we were able to select a GAL4 driver line that elicited striking BW increase in both sexes when crossed to *UAS-TNT*. This GAL4 driver was composed of GAL4 fused with the 5' upstream regulatory sequence of the gene encoding MIP.

Flies with silenced MIP neurons (*MIP>TNT*) indeed showed abnormal elevation of food intake accompanying marked BW increase. The increased BW of *MIP>TNT* flies was completely restored to normal level in a restricted feeding condition, indicating the strong correlation of feeding and BW regulation. Having shown that silencing MIP neurons increased BW through elevated food intake, we sought to artificially activate MIP neurons using a thermogenetic approach to examine whether BW and food intake conversely decreased upon MIP neuron activation. To do so, we employed TRPA1 that excites neurons in response to a warmth temperature at 30°C when it is ectopically expressed. Flies harboring *UAS-TRPA1* and *MIP-GAL4* (*MIP>TRPA1*) incubated at 30°C showed a dramatic decrease in BW and this BW decrease was completely reversed to normal level upon termination of neural activation by incubating *MIP>TRPA1* flies at 18°C. Similarly, *MIP>TRPA1* flies exhibited strongly suppressed food intake at 30°C, however the suppressed food intake became restored to normal level with a strong feeding rebound upon neural termination, indicating the switch-like role of MIP neurons in regulation of food intake. Together these results revealed a negative correlation between MIP neuron activity and food intake in BW control.

Next, we and our colleagues sought to visualize the MIP neurons using the *MIP-GAL4* transgene, and a highly specific anti-MIP antibody and MIP mRNA anti-sense probe. Using double-labeling experiments, we found 52 authentic MIP neurons positive for both anti-MIP antibody and MIP mRNA anti-sense probe in the central nerve system.

Notable innervations by the MIP neurons were observed in the primary brain structures including the antennal lobe and subesophageal zone important for olfactory and gustatory perception of food. Remarkably, expression of *Mip RNAi* driven by *MIP-GAL4* eliminated most of anti-MIP antibody signals and elicited BW increase, indicating that specific subsets of *MIP-GAL4* neurons expressing MIP are critical for BW control. To delicately define the neurons responsible for BW control, we employed subset-specific GAL80 (the suppressor of GAL4) lines and identified that *Cha-GAL80* fully suppressed the BW phenotype of *MIP>TN*T flies. This result indicates that *Cha-GAL80* labels the subset neurons important for BW control. By comparing the expression patterns of *Cha-GAL80* with other GAL80 lines, we were further able to identify a cluster of neurons in the brain presumed to be critical for *Drosophila* BW control.

The observation that the activity of MIP neurons controlled BW and food intake and MIP was expressed throughout the MIP neurons led us to expect the significant role of MIP in BW and food intake. However, MIP was previously reported as a potent ligand for sex peptide receptor (SPR), and thus its role in sexual behaviors has been anticipated. Furthermore, a recent study showed that the MIP-SPR pathway is involved in the maintenance of sleep behavior in *Drosophila.* In this study, we and our colleagues for the first time generated a null mutation for MIP and examined MIP's role in food intake and BW. Similar to *MIP>TNT* flies, the mutants lacking MIP expression showed significant increases in BW and food intake. These defects were rescued by genetic restoration of MIP expression in the mutants, but not by genetic manipulation of SPR gene expression. These results suggest that MIP in MIP neurons plays a critical role in BW regulation independently of SPR.

Finally, we and our colleagues attempted to address the physiological meaning of MIP pathway-mediated BW regulation in animal by testing the possibility that MIP pathway controls satiety. Satiety is characterized by animals' blunted peripheral sensitivity to food. For example, satiated flies exhibit decreased olfactory attraction to food odors and/or reduced proboscis extension reflex (PER) to sugars as satiety responses. Using these behavioral paradigms, we quantified satiety responses of flies and examined the role of MIP pathway in satiety control. Flies with silenced MIP neurons and/or MIP mutation showed significantly enhanced olfactory attraction to food odors. Consistent with this result, electrophysiological recordings on olfactory sensilla of *MIP>TNT* flies showed elevated neural responses to food odors. Likewise, suppression of MIP pathway in satiated flies greatly elevated PER responses to sucrose as if the flies had been starved. Conversely, activation of MIP neurons in starved flies completely blunted the olfactory and gustatory responses as if the flies had been satiated. These results strongly support that MIP pathway is required and sufficient for inducing satiety responses.

Based on these data, we proposed a model in which MIP neurons in the brain induce satiety and suppress food intake to maintain a constant BW in a MIP neuropeptide-dependent mechanism.

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