UNIVERSITY OF CALIFORNIA SAN DIEGO

Neuromodulation of Mushroom Body Output Neurons in *Drosophila* Regulates Hunger and Satiety

A Thesis submitted in partial satisfaction of the requirements for the degree
Master of Science

in

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by

Joseph George Ayoub

Committee in charge:

Professor Jing Wang, Chair
Professor Yishi Jin
Professor Chih-Ying Su

2018

The Thesis of Joseph George Ayoub is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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University of California San Diego

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All material is currently being prepared for submission for publication. Deshou Cao and Joseph Ayoub were the primary investigators and authors of this material. Cao, Deshou; Ayoub, Joseph; Wang, Jing. “Mild and Severe Starvation Sequentially Recruits Peripheral and Central Olfactory Circuits for Food Search Behavior”.

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ABSTRACT OF THE THESIS

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by

Joseph George Ayoub

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Professor Jing Wang, Chair

When faced with starvation, animals become more aware of food-related stimuli, at the expense of other behaviors such as courtship. Underlying this heightened stimulus perception is the altered activity of the sensory systems, particularly the olfactory system. To study this, the model organism *Drosophila* was used due to its simple anatomical olfactory and neural systems, and its genetic tractability. We used a high-throughput single-fly assay to measure the effect of starvation duration on food search behavior. We found mild and severe starvation conditions recruit differential neural substrates for odor search behavior. A signaling pathway for short Neuropeptide F (sNPF) in the mushroom body, particularly in the γ lobe, is shown to be required for this severe starvation-induced food search behavior, where sNPF receptors are localized on

the γ1 and γ2 MBONs. Lastly, the increased excitability of the γ1 and γ2 mushroom body output neurons (MBONs) was able to induce severe starvation behavior in mildly starved flies reinforcing the state-dependent neural plasticity of *Drosophila*. These results suggest a sNPF-signaling pathway that modulates the activity of the mushroom body in order to increase the food search behavior of *Drosophila* as starvation progresses.

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Introduction

Animals, including humans, have evolved robust innate behaviors such as fighting, fleeing, courtship, and food search. Within a particular species, innate behaviors can be observed consistently across different individuals and can also be prompted in response to external stimuli even without any prior experience. Innate behaviors are genetically programmed (Bargmann and Marder 2013; Manoli et al. 2006). For instance, the innate courtship behavior of male fruit flies is hindered when the function of a fruitless (*fru*) gene is disrupted because the gene plays an important role in the wiring of the neural circuit controlling sexual behaviors (Demir and Dickson 2005; Hall 1994; Kimura et al. 2005; Manoli et al. 2005; Ryner et al. 1996; Stockinger et al. 2005; Yamamoto and Koganezawa 2013). Examples like this highlight the fact that certain genes lead to the formation of neural circuits, which further underlie the reproducible innate behaviors that are conserved across a species. Our study seeks to understand the causal relationship between genes and the specific innate behavior called food search by taking a closer look into the neural circuitry that mediates this behavior.

Foraging is a critical innate behavior and a means for animals to obtain food for survival. Foraging is often prioritized over other behaviors such as courtship under conditions of limited food resources; this trade-off is crucial for an organism's immediate survival (McFarland 1977). Starvation changes many metabolic signals, for example, it causes a decline of insulin levels and an increase in ghrelin levels in mammals (Hosoda and Kangawa 2008). Depending on food availability, these different metabolic signals are integrated in the hypothalamus to control the central circuit regulating feeding behavior in mammals (Friedman and Halaas 1998). In *Drosophila*, it has been shown that insulin signaling reconfigures sensory processing of food odors under starved conditions (Ko et al. 2015). These neural circuits can enhance stimuli

perception and behavior that are reoriented toward finding food odor–related stimuli for survival (Ko et al. 2015; Perisse et al. 2016). Of these sensory systems, olfaction is the principal mode of sensation relied upon by *Drosophila* when searching for food (Pool and Scott 2014; Shaver et al. 1998; Su and Wang 2014). Our lab uses the model organism *Drosophila* to study the effects of starvation-induced neural circuitry changes, specifically occurring in the olfaction system, on food search behavior. In *Drosophila*, food search behavior can be characterized by increased activity that results in the wide roaming of an area, with an enhanced perception of food odors (Bell 1985, 1990a,b; Murata et al. 2017). The observable foraging behavior, genetic manipulability and rather simple anatomical structure of *Drosophila* combined with the utilization of a single-fly behavior assay allow us to effectively assess and quantify the consequences of starvation on odor driven food search behavior (Ko et al. 2015; Root et al. 2011; Zaninovich et al. 2013).

Using this behavioral assay, we observed that different starvation durations caused flies to exhibit characteristic activity and path movements: fed flies were fairly inactive and had a dispersed roaming around the arena, mildly starved flies were slightly more active and spent more time near the food odor, and severely starved flies displayed more frequent movement, specifically focused around the food odor. We measured food search behavior as the percentage of flies that found the food odor, showing that food search behavior does indeed change with varying starvation durations; severely starved flies (48-hour starved) were more likely to find the food odor source, apple cider vinegar (ACV), compared to mildly starved flies (24-hour starved). This elevated food search behavior in mildly starved flies has been attributed to the increased excitability of a group of olfactory receptor neurons (ORNs) called Or42b (Root et al. 2011). However, the same excitability of Or42b was not able to explain the further success in food

search behavior seen in severely starved flies. The mechanism driving higher foraging behavior activity in severely starved flies is not as well studied and still remains a mystery. Thus, the goal of our study is to investigate what underlying neural circuitry changes in the olfaction system lead to a more intense food search behavior as starvation progresses.

To look into these neural mechanisms, we first used short neuropeptide F (sNPF) as a chief candidate substrate in modulating severe-starvation behavior; sNPF in the ORNs was found to be required for mild-starvation behavior, and is also highly expressed in the mushroom body (MB), a structure associated with olfaction learning and memory for *Drosophila* (Guven-Ozkan and Davis 2014; Heisenberg 2003; Knapek et al. 2013; Lee et al. 2004; Perisse et al. 2016; Root et al. 2011; Stopfer 2014). For these reasons, we decided to take a closer look into the role of sNPF in the mushroom body in order to grasp a better understanding of the molecular mechanisms underlying severe starvation food search behavior.

In this paper, we propose a pathway for sNPF signaling to modulate the excitability within the mushroom body, specifically the γ1 and γ2 mushroom body output neurons (MBONs); this pathway ultimately leads to the higher food search behavior seen in severely starved flies. We confirm that sNPF signaling in MB, and specifically the γ lobe, is required for this severe starvation-induced behavior, while other lobes such as α/β and α′/β′ do not contribute. We further demonstrate that sNPF receptors in the γ1 and γ2 MBONs are critical for modulating this severe starvation-induced food search behavior. Lastly, we show that increasing the excitability of both γ1 and γ2 mushroom body output neurons are sufficient enough to induce this severe starvation-induced behavior in mildly starved flies. Overall, our results suggest an essential role for sNPF signaling, that is recruited as part of a higher-ordered brain pathway, in specifically modulating the food search behavior as starvation progresses to a severe time-point.

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Materials and Methods

Fly Strains

All *Gal4*- and *UAS*- control flies were crossed to w^{1118} fly strain. The following fly stocks were used: *Orco-Gal4* (Kreher et al. 2005); *c772-Gal4 (*Zars et al. 2000*)*; *R71G10-Gal4* (39604) was order2ed from the Bloomington Drosophila Stock Center; *NP3061-Gal4* provided by Dr. Kei Ito; *c739-Gal4* (obtained from K. Kaiser, Division of Molecular Genetics, University of Glasgow, Scotland); *MB463B-Gal4*, *MB112C-Gal4*, *MB077C-Gal4* (Aso et al. 2014); *UAS-sNPF-RNAi* (Lee et al. 2008*)*, *UAS-sNPFR1-RNAi* (661) was ordered from the Vienna Drosophila Resource Center; *UAS-NaChBac* (Nitabach et al. 2006).

Behavioral Olfaction Food Search Assay

A high-throughput single-fly assay was used to quantify the food search behavior. Female flies aged 3-5 days were used in all experiments because their food search behavior is more robust than that of male flies (Root et al. 2011). 27 female flies and 4 male flies were collected from the crosses and placed in a food vial together. After two days of collecting these flies, they were placed in starvation vials for either 24 hours or 48 hours with only a small damp towel available to them. The fly vials were stored in an incubator with conditions of 21°C.

During the behavioral experiment, 5µl of a food odor (1% apple cider vinegar in a 1% low melting temperature agarose solution) was placed in the center of a food arena that was 60mm in diameter and 6mm in height. The arena itself was then placed into an isolated cart and placed on top of a sieve with a tub of water below it. The temperature conditions of the cart were maintained around 20°C - 21°C and humidity conditions of 50-60%. An individual fly was then placed into the arena inside the cart, where a camera above the arena would track the fly's

movement for a 10-minute time-span. The experiments were conducted in a dark room so that the fly could only rely on its olfaction sense to find the food odor. In order to visualize the recording of the fly, a red light (660 nm) was illuminated in the cart, which is outside the visible spectrum of the fly. The data itself was analyzed on a program in Igor Pro where it noted that if the fly was within a 5 mm distance of the food odor source for at least 5 seconds, it was considered successful for finding the food source. The average speed of the fly within the first 50 seconds was also recorded. Flies that deviated from the standard velocity, were inactive for a certain period of time, or were not within the boundaries of the arena were eliminated from the data analysis.

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Results

Food search behavior varies with different stages of starvation.

Flies, like all animals, modify their behaviors depending on their food intake. We wondered whether the food search behavior of flies changes with starvation durations. Thus, we performed an experiment where we starved *UAS-sNPFi* control flies for different durations (fed, 12-hour, 24-hour, 36-hour, 48-hour, 60-hour) and then used a single-fly behavior assay to quantify their food search behavior. In this assay, we used a machine vision system to track the location of a fly in an arena containing a food odor in the center for a 10-minute timespan. First, we looked at their movement patterns and noted a general pattern of fed flies to be more dispersed around the food arena while starved flies are more concentrated in the middle around the food odor (Fig. 1A). Our results revealed that as the duration of starvation increased, so did the likelihood of the fly being able to find the food, with flies starved for 60 hours having the highest success rate of finding the food odor and fed flies having the lowest (Fig. 1B). However, some flies died after 60 hours of starvation. It is interesting to note that flies starved for an increment of 12 hours (12hr, 24hr, 36hr and 48hr) exhibited two different levels of attraction to the food odor. Therefore, for the rest of the experiments, we used 24-hour starvation as a time-point for mildly starved flies and 48-hour starvation as a time-point for severely starved flies. Our assay showed that severely starved flies had a distinctly higher success rate of finding food compared to mildly starved flies (Fig. 1C).

sNPF signaling in ORNs and mushroom body account for severe starvation behavior

Our next step was to determine a neural substrate that could be responsible for this elevated food search behavior seen in severely starved flies. From previous studies, it was found

that short Neuropeptide F (sNPF) in olfactory receptor neurons (ORNs) is required for mild starvation behavior (Ko et al. 2015; Root et al. 2011). sNPF is the fly homolog of mammalian Neuropeptide Y; both neuropeptides, in elevated levels, are known to promote hunger (Feng et al. 2003; Lee et al. 2004). We performed an experiment where we knocked down sNPF in ORNs using *Orco-Gal4* and *UAS-sNPF-RNAi* transgenes in mildly starved flies. Consistent with previous results, we saw a reduction in food search behavior to the same level as fed flies, attributing this mild starvation behavior to sNPF in the ORNs (Fig. 2A). However, knocking down sNPF in the ORNs had no effect on the severe starvation behavior, leading us to conclude that there must be another pathway responsible for this behavior (Fig. 2D).

In the olfactory system, sNPF is highly enriched in the mushroom body as well as the olfactory sensory neurons (Guven-Ozkan and Davis 2014; Heisenberg 2003; Knapek et al. 2013; Lee et al. 2004; Perisse et al. 2016; Root et al. 2011; Stopfer 2014). Given the fact that sNPF expression in ORNs is required for the change in food search behavior in mildly starved flies, we hypothesized that sNPF expression in the mushroom body is required for the further change in severely starved flies (Ko et al. 2015; Root et al. 2011). To test this hypothesis, we knocked down sNPF in the mushroom body (MB) of mildly starved flies with *c772-Gal4* and *UAS-sNPF-RNAi* transgenes. We saw no effect, indicating that sNPF in the mushroom body does not play a role in mild starvation behavior (Fig. 2B,C). However, when we repeated the same experiment with severely starved flies, we saw a dramatic reduction in food search behavior (Fig. 2E). This suggests that the elevated food search behavior seen in severely starved flies depends on sNPF signaling in the mushroom body. Together, these results show that sNPF in the ORNs and the MB accounts for the entirety of the heightened 48-hour starvation behavior (Fig. 2F).

sNPF signaling in the γ lobe, but not in the α/β lobe and in the α′/β′ lobe, is required for food search behavior under severe starvation.

Having found that sNPF in the mushroom body is required for this severe starvation behavior; we wondered whether sNPF was expressed in a certain region of the MB and how this played a role in modulating the behavior. There are three lobes in the mushroom body (γ, α/β, α′/β′) and sNPF is known to be expressed specifically in the γ and α/β lobes but not in the α′/β′ lobe (Aso et al. 2014; Nassel et al. 2008). Using *R71G10-Gal4* and *c739-Gal4* with *UAS-sNPF-RNAi* transgenes, we knocked down sNPF in the γ lobe and the α/β lobe, respectively. Only when levels of sNPF were knocked down in the γ lobe in severely starved flies did we see a reduction in the food search behavior; while the reduced levels of sNPF in the α/β lobe had no effect on the severe starvation behavior (Fig. 3A,B). A different driver line, *NP3061-Gal4*, was used to knock down sNPF expression in the α/β lobe. Again, we did not see any effect on the food search behavior of severely starved flies (Fig. 4). These results suggest that sNPF signaling in the γ lobe, but not in the α/β lobe, promotes food search behavior in severely starved flies.

Next, we looked into the role of sNPF signaling in the α′/β′ lobe. Although sNPF is not expressed in the α′/β′ lobe, we wanted to ensure that there was no contribution from this lobe in severe starvation-induced food search behavior. Therefore, we used *UAS-sNPF-RNAi* and *MB463B-Gal4* transgenes to knockdown sNPF in the α′/β′ lobe. The results confirmed our expectations where we observed no behavioral difference between the transgenic and control flies (Fig. 5). Overall, these results indicate that while sNPF in the α/β lobe and the α′/β′ lobe does not contribute to the severe starvation food search behavior, sNPF in the γ lobe is necessary for this severe starvation behavior.

Expression of sNPF receptors in both γ1 and γ2 MBONs is required for severe starvation-induced behavior.

Knowing that sNPF signaling in the γ lobe is responsible for this severe starvation-induced behavior, we decided to take a closer look into the anatomy of the γ lobe. We know that Kenyon cells run through the entirety of the gamma lobe, which is crucial for processing olfactory information entering the mushroom body (Aso et al. 2014; Heisenberg 2003). The γ lobe itself can be separated into five compartments, γ1 to γ5, and each compartment receives input from dopaminergic neurons (DANs) and sends output to mushroom body output neurons (MBONs) (Aso et al. 2014; Guven-Ozkan and Davis 2014) Calcium imaging experiments have showed that the responses of the Kenyon cells in each of the γ lobes saturate at a 24-hour starvation point, suggesting that neural activity downstream of the MB γ lobes may be responsible for the further increase in food search intensity from mild to severe starvation (Deshou Cao, unpublished data),.

Previous opto-genetic experiments that activated the mushroom body output neurons (MBONs), specifically in γ1 and γ2, were sufficient to induce attraction in flies (Owald et al. 2015). These MBONs have been demonstrated by previous research to be associated with appetitive and aversive behavior (Perisse et al. 2016). Hence, we reasoned that the MBONs were a clear neuronal candidate to examine next. Furthermore, calcium-imaging experiments showed that the γ1 and γ2 MBONs exhibited a step-like increase in activity from fed to 24-hour to 48-hour starvation durations (Deshou Cao, unpublished data). Taken together, this information suggests that the γ1 and γ2 MBONs may be responsible for gating the activity of the Kenyon cell in mediating the change of food search behavior from mild to severe starvation.

Since γ1 and γ2 MBONs showed corresponding increases in activity responses after severe starvation, we reasoned that sNPF receptors might be expressed in the γ1 and γ2 MBONs. Therefore, we performed a behavioral assay in flies carrying *Gal4-MB112C* or *Gal4-MB077C* and *UAS-sNPFR-RNAi* transgenes to knockdown sNPF receptors in γ1 or γ2 MBONs, respectively. We found that neither affected the behavior of flies under severe starvation (Fig. 6A,B). However, knocking down sNPF receptors in both γ1 and γ2 MBONs significantly reduced food search behavior (Fig. 6C). Thus, we concluded that the expression of sNPF receptors in both γ1 and γ2 MBONs is required for severe-starvation behavior.

Excitability of γ1 and γ2 mushroom body output neurons are sufficient to induce severe starvation food search behavior in mildly starved flies.

We then asked whether increasing the excitability of the γ1 and γ2 MBONs would have an effect on the food search behavior of the fly at various starvation time-points. In order to test this, we expressed *UAS-NaChBac* in γ1 or γ2 MBONs by using either *MB112C-Gal4* or *MB077C-Gal4* drivers. NaChBac is a bacterial voltage-gated sodium channel that further increases neuronal excitability; in this case γ1 and γ2 MBONs would become more excitable (Nitabach et al. 2006; Ren et al. 2001). However, we did not see any behavioral differences when an individual γ lobe MBON was made more excitable (Fig. 7). Thus, we took another step in expressing *UAS-NaChBac* in both γ1 and γ2 MBONs, so that both neurons were simultaneously more active. Once this was done, we observed an increase in food search behavior from mildly starved flies, with activity that was comparable to severe starvation-induced behavior (Fig. 8B). This shows that the excitability of both the γ1 and γ2 MBONs are sufficient enough to induce this severe starvation food search behavior. Although we saw an increase in food search behavior

in mildly starved flies, we did not observe any further increase in the behavior of severely starved flies (Fig 8C). Together, these results suggest that sNPF receptor expression in the γ1 and γ2 MBONs controls their excitability and the two populations of neurons contribute to food search behavior in an additive manner.

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Discussion

Here we investigated the effect of starvation duration on food search behavior in *Drosophila melanogaster*. We found that severely starved flies (48-hour starvation) exhibit a more robust food search behavior than mildly starved flies (24-hour starvation). Mechanistically, the mushroom body, a central olfactory center, is recruited by severe starvation to elevate food search behavior to a higher level. Further genetic experiments suggest that the additional behavioral changes under severe starvation require sNPF expression in the MB Kenyon cells and sNPF receptor in the MB output neurons. Our work on different stages of starvation in the *Drosophila* olfactory system offers mechanistic insights into how olfactory processing contributes to food search behavior.

We have begun to investigate the neural substrate and molecular mechanisms underlying the increased drive in food search behavior after severe starvation. An earlier study from our lab found that sNPF signaling in the peripheral olfactory sensory neurons accounts for the observed behavior after mild starvation (Root et al. 2011). In the current study, we showed that sNPF signaling in the mushroom body is required for the increased drive in food search behavior after severe starvation. Specifically, sNPF signaling in the γ lobe of the mushroom body was found to be responsible for the further increase in food search behavior observed in severely starved flies. This aligned with previous studies that found the role of the γ lobe of the mushroom body to be involved with olfactory short-term learning and memory (Guven-Ozkan and Davis 2014; Heisenberg 2003; Stopfer 2014). It is interesting to note that sNPF signaling in the mushroom body is also required for the sugar-rewarded olfactory learning (Knapek et al. 2013). These results suggest that learning and innate behaviors share a common circuit and neuromodulation mechanism.

Although both mild and severe starvation conditions recruit sNPF signaling to alter neuronal sensitivity, the target of sNPF signaling is different between the peripheral and central circuits. For mild starvation, elevation in odor search behavior is mediated by an increased neurotransmission of the Or42b ORNs. This sensitization is mediated by sNPF in an autocrine manner. sNPF is released constitutively, whereas sNPF receptor expression in the ORNs is increased by starvation thereby sensitizing their olfactory response. The additional drive for odor search behavior under severe starvation is mediated by changes in the mushroom body. However, sNPF and its receptor are expressed in different populations of neurons in the mushroom body. sNPF is released by Kenyon cells, but detected by the output MBONs. We found that the sNPF receptors are localized on the γ1 and γ2 mushroom body output neurons and expression of these sNPF receptors in the γ1 and γ2 MBONs is required for the severe starvation-induced attraction behavior. Our results are consistent with the MBONs' role in inducing attraction with respect to odor-driven behavioral choice (Owald et al. 2015; Perisse et al. 2016). However, this process seems to be very intricate, as sNPF receptor knockdown in either lobe does not affect the severe starvation behavior.

Furthermore, we found that increasing the excitability of both γ1 and γ2 MBONs was able to induce the heightened severe starvation behavior in mildly starved flies. This proved that not only is this level of starvation behavior inducible, but it is also subject to physiological changes of neural circuits. As with the previous experiment, genetic manipulation in both γ lobe MBONs was necessary for a behavioral difference to be observed, indicating some sort of coordination occurring between both γ1 and γ2 MBONs. Additionally, we did not observe an increase in the activity of fed or severely starved flies when we performed this experiment. For fed flies, we propose that activation of the ORNs, which does not occur until mild starvation, is a

prerequisite for stimulating the Kenyon cells to provide input to the MBONs. For severely starved flies, we reasoned that the sNPF receptors were already saturated at that starvation duration, and increasing the excitability of these groups of neurons could have no further effect.

Although our study illustrates a pathway for which severe starvation leads to changes in neural activity in the γ1 and γ2 MBONs through sNPF signaling, the mechanism of how these groups of neurons are able to gate the activity of other neuronal structures is still unknown. It will be interesting to investigate the underlying molecular mechanism that facilitates the time-dependent coordination not only between the γ1 and γ2 MBONs but also between the Kenyon cells that provide input to the MBONs and the dopaminergic neurons that give input to the mushroom body (Aso et al. 2014; Guven-Ozkan and Davis 2014; Knapek et al. 2013).

These results further confirm that innate behaviors change in response to an organism's internal and external contexts (Tinbergen 1952). In the case of food search, the longer the fly was starved, the longer the organism would go without completing its innate behavior. This led to varied expression of the neuromodulator sNPF at different starvation durations and thus different behavioral outputs: sNPF in the ORNs was responsible for mild starvation behavior and then played a larger role in regulating severe starvation behavior in the mushroom body. This gives insight to a starvation state-dependent neuronal circuit that results in observable behavioral shifts. This starvation state-dependent neuronal plasticity has not only been shown in *Drosophila* but also in other organisms such as *C. elegans* where chemosensory neurons vary in responses depending on the organism's feeding state (Sengupta 2013). Hence, not only can the neuronal modulation of innate behaviors be observed across many species but it also shares a theme of activity-dependent neuronal plasticity dependent upon the completion of a behavior. Even in humans it has been noted that the internal state can affect perceptions of sensations (Cabanac

1971). Thus, it will be of even greater interest to understand how animals' behaviors change in response to state-dependent conditions mediated by external and internal stimuli.

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Figures

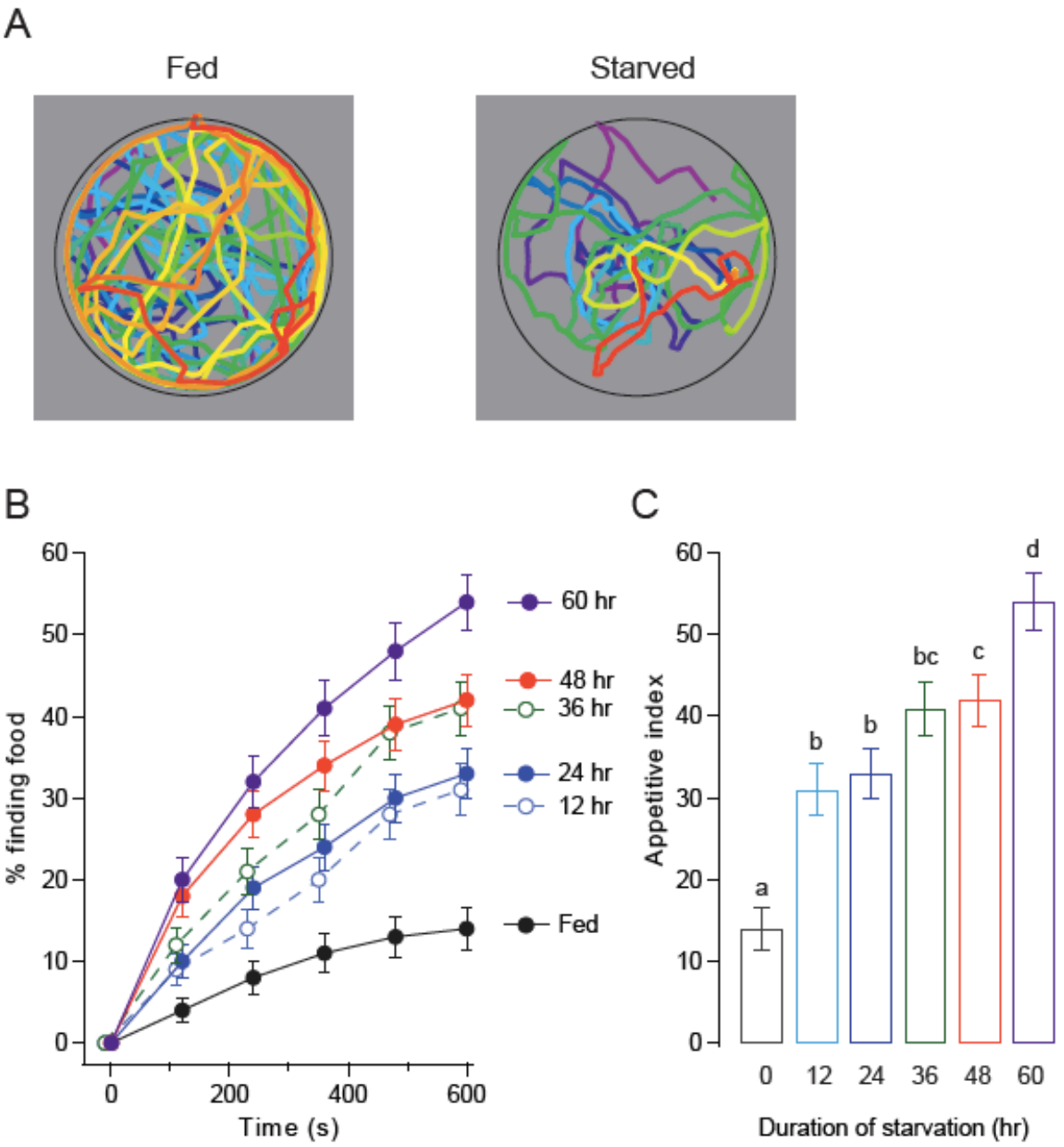

Figure 1: Food search behavior increases with the progression of starvation. UAS-sNPFi was used as a control fly for all starvation time points. Displayed are the typical behaviors of a fed and starved fly in an arena under the 10-minute time period of finding the food odor (A). As the progression of starvation increases, the percentage of flies that found the food odor increased as well (B). Severely starved flies (48-hour starvation) performed significantly better than mildly starved flies (24-hour starvation) (C). For each data point, n = 154 – 234.

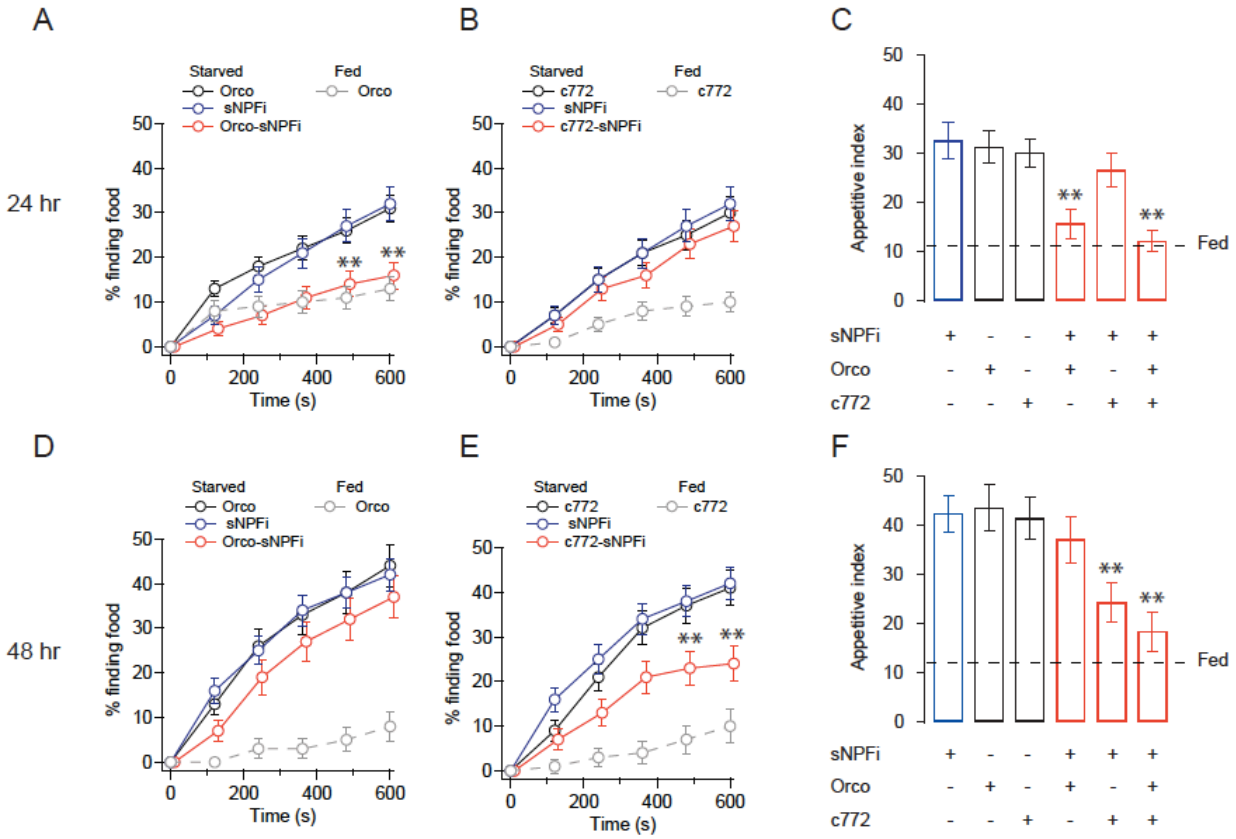

Figure 2: sNPF signaling in ORNs and MB accounts for nearly all of the severe starvation behavior. *Orco-Gal4* and *c772-Gal4* were used to knockdown sNPF expression in ORNs and MB, respectively. A significant reduction in food search behavior was seen when sNPF was knocked down in the olfactory receptor neurons (ORNs) at mild starvation (A), but not at severe starvation (D). sNPF knockdown in MB abolished food search behavior in severely starved flies (E), but not in mildly starved flies (B). n = 59-196. Error bars show s.e.m. *p < 0.05, **p < 0.01; z-test comparing knockdown flies to UAS-sNPFi and c772-Gal4 control groups in the starvation state (A, C, E, F).

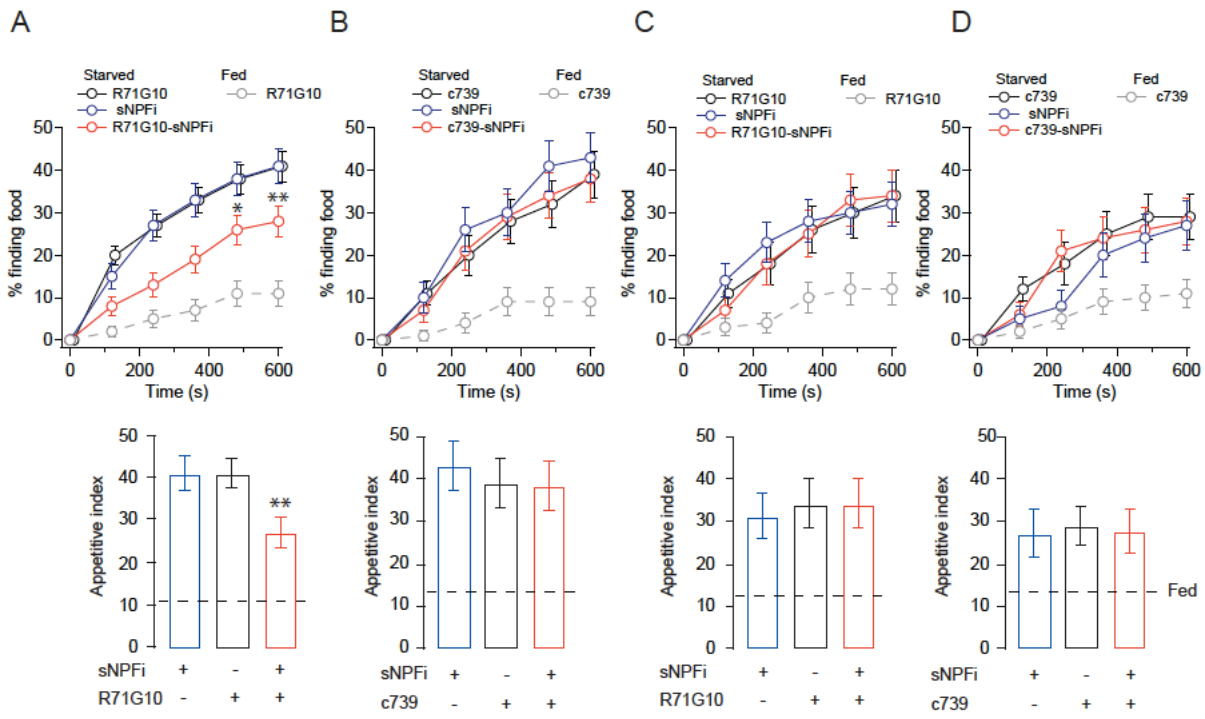

Figure 3: sNPF in the γ lobe is required for the severe starvation behavior. *R71G10-Gal4* and *c739-Gal4* were used to knockdown sNPF in the γ lobe and the α/β lobe, respectively. There was a significant reduction in food search behavior of severely starved flies observed when sNPF was knocked down in the γ lobe of the MB (A) but not in the α/β lobe (B). No effect was seen in food search behavior when sNPF was knocked down in the γ lobe and the α/β lobe at mild starvation (C, D). n = 59-196. Error bars show s.e.m. *$p < 0.05$, **$p < 0.01$; z-test comparing knockdown flies to UAS-sNPFi and R71G10-Gal4 control groups in the starvation state (A).

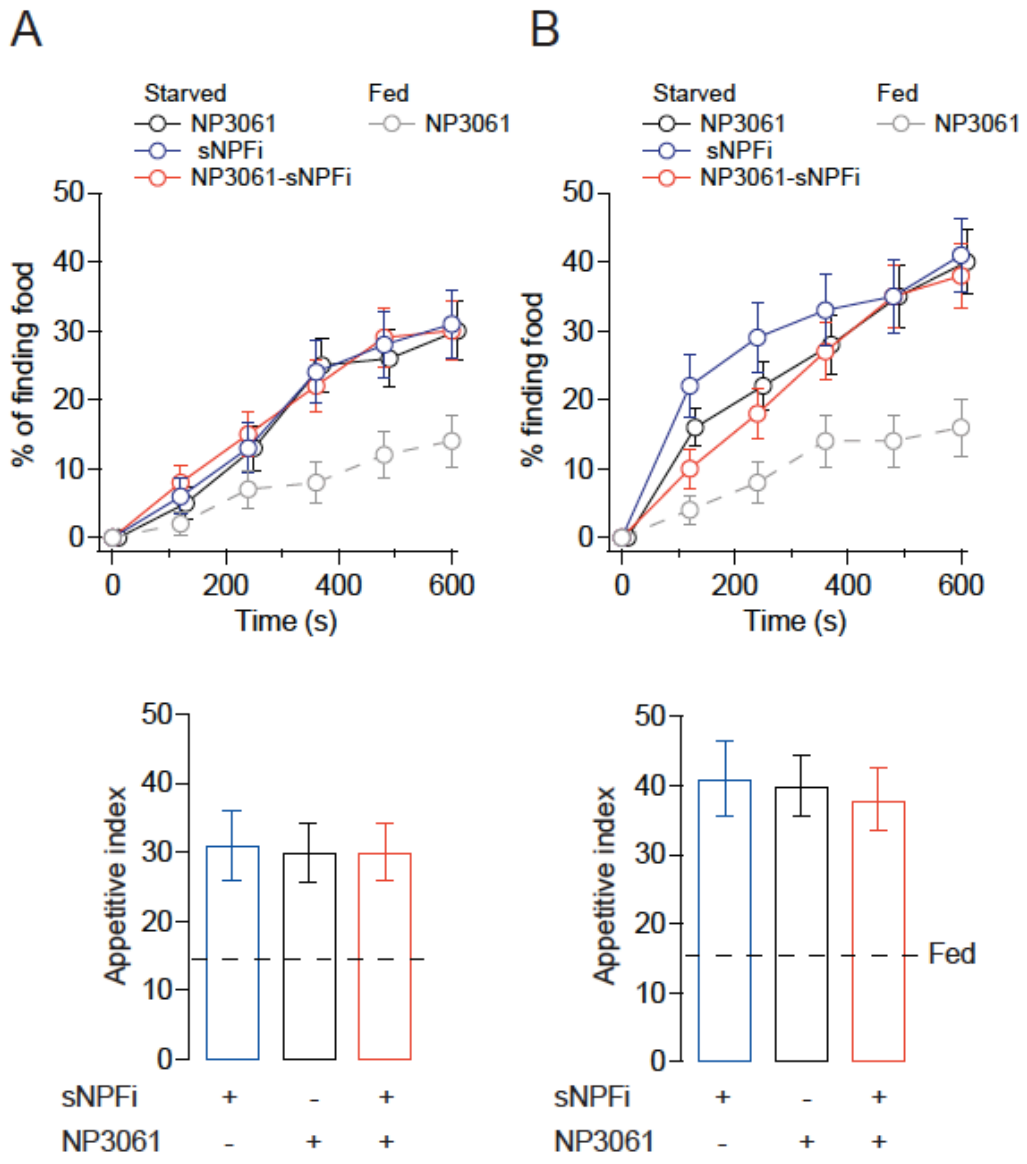

Figure 4: sNPF in the α/β does not contribute to the severe starvation behavior. No effect on food search behavior was seen when sNPF was knocked down in the α/β lobe in mildly and severely starved flies (A, B).

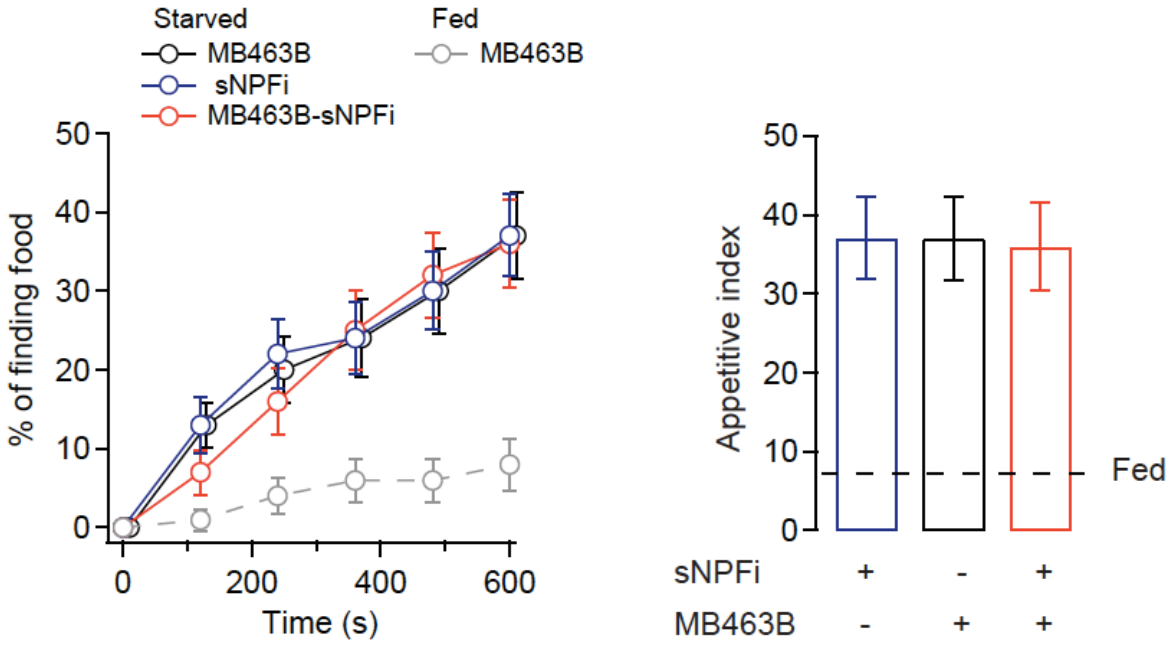

Figure 5: sNPF in the α′/β′ lobe does not contribute to the severe starvation behavior. *MB463B-Gal4* was used to knockdown sNPF in the α′/β′ lobe. Food search behavior was not affected when sNPF was knocked down in the α′/β′ lobe of severely starved flies.

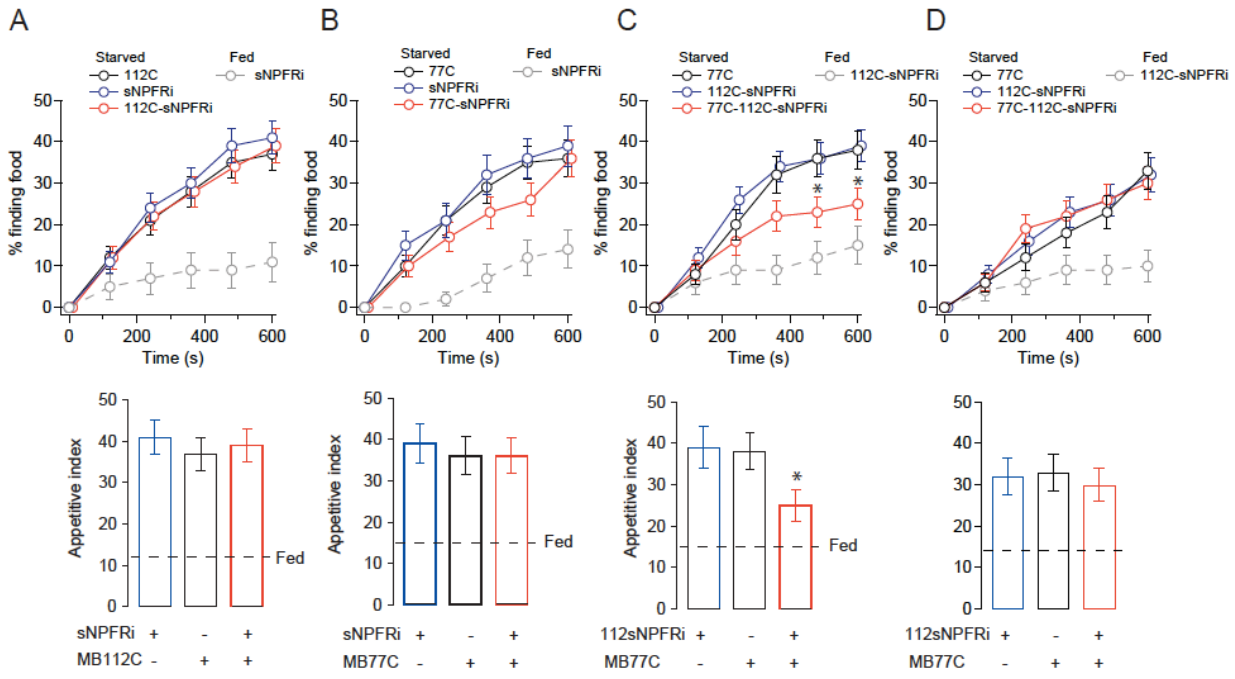

Figure 6: Expression of sNPF receptors in both γ1 and γ2 MBONs is required for the severe starvation-induced behavior. *MB112C-Gal4* and *MB77C-Gal4* were used to knockdown sNPF in the γ1 MBONs and the γ2 MBONs, respectively. No change was seen in food search behavior when sNPF receptors were knocked down in either the γ1 or the γ2 MBONs (A, B). Food search behavior was reduced when sNPF receptors were knocked down in both γ1 and γ2 MBONs at severe starvation (C), but not at mild starvation (D). n = 59-196. Error bars show s.e.m. *p < 0.05; z-tests comparing knockdown flies to MB112C-Gal4 and MB77C-Gal4 control groups in the starvation state (C).

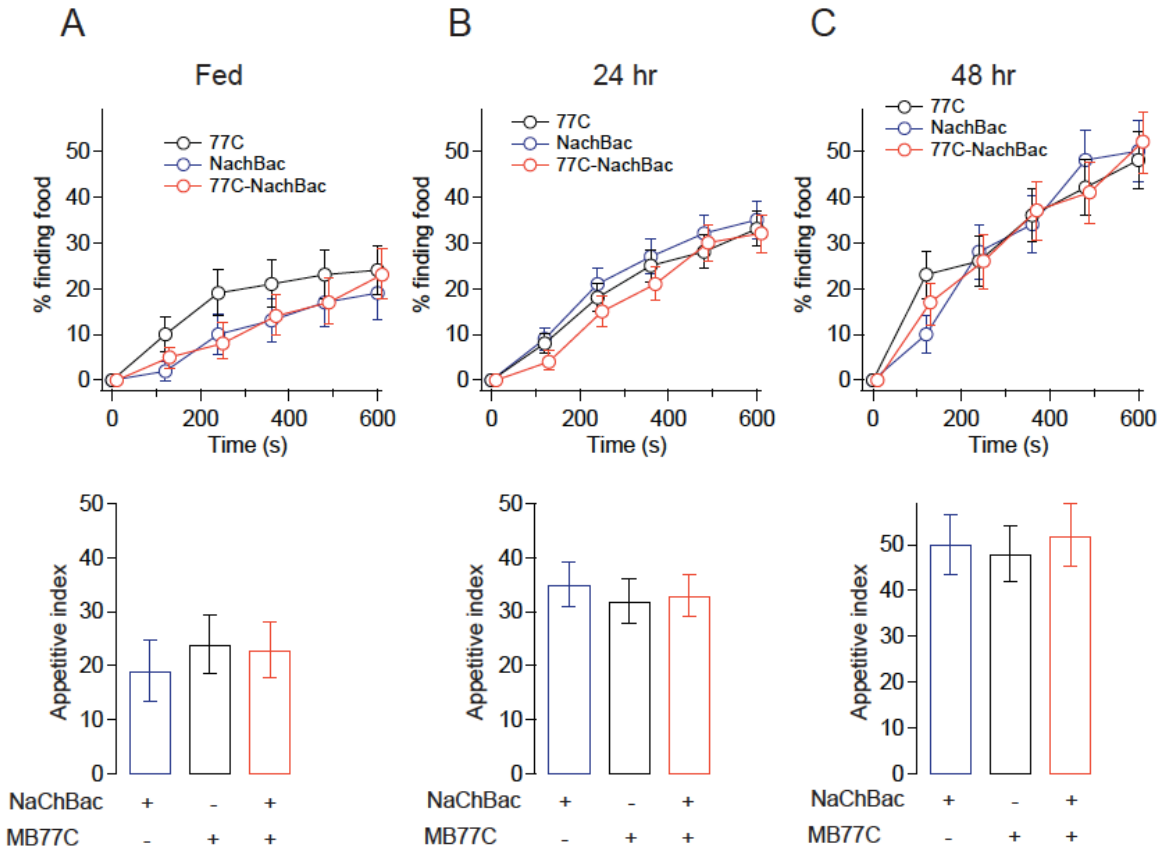

Figure 7: Excitability of γ2 MBONs is not sufficient for eliciting severe starvation behavior. The increased excitability of γ2 MBONs had no effect on food search behavior in fed flies (A), and in mildly starved (B), and severely starved flies (C).

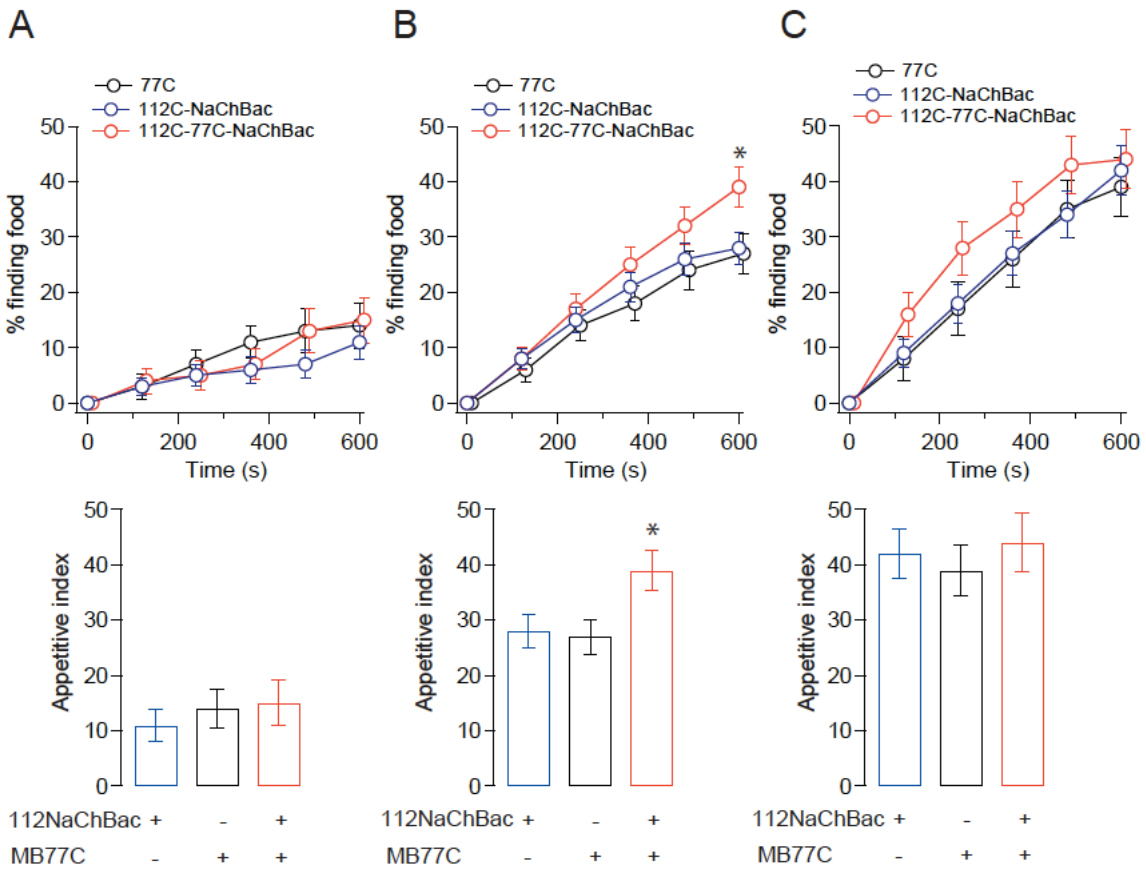

Figure 8: Excitability of both γ1 and γ2 MBONs is sufficient to induce severe starvation food search behavior in mildly starved flies. *MB112C-Gal4* and *MB77C-Gal4* were used to increase the excitability of the γ1 MBONs and the γ2 MBONs, respectively. Increasing the excitability of the γ1 and γ2 MBONs had no effect on the food search behavior of fed and severely starved flies (A, C). This increased excitability was able to induce severe starvation behavior in mildly starved flies (B). n = 59-196. Error bars show s.e.m. *p < 0.05; z-test comparing 112C-77C-NaChBac flies to 112C-Na control group in the starvation state (B).

All material is currently being prepared for submission for publication. Deshou Cao and Joseph Ayoub were the primary investigators and authors of this material. Cao, Deshou; Ayoub, Joseph; Wang, Jing. “Mild and Severe Starvation Sequentially Recruits Peripheral and Central Olfactory Circuits for Food Search Behavior”.

Results for data figures 1, 2 and 3 were primarily authored and investigated by co-author Dr. Deshou Cao. All remaining data was primarily researched and authored by Joseph Ayoub, the author of this thesis. Cao, Deshou; Ayoub, Joseph; Wang, Jing. “Mild and Severe Starvation Sequentially Recruits Peripheral and Central Olfactory Circuits for Food Search Behavior”.

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