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Too Fat to Fly? New Brain Circuits Regulate Obesity in *Drosophila*

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In mammals, fat store levels are regulated by brain centers that control food intake and metabolism. A new study by Al-Anzi and colleagues in this issue of *Neuron* identifies neurons with similar functions in *Drosophila*, further establishing the fly as a legitimate model to study obesity.

In the face of a major health epidemic in eating-related disorders, such as obesity and diabetes, it is urgent to understand the complex neural and molecular networks that regulate energy homeostasis and feeding behavior. The need to acquire food and balance energy intake with expenditure is ubiquitous among animals. The nervous system plays a crucial role in regulating long-term energy balance. It evaluates the amount of available fuel and modulates food intake and energy expenditure accordingly. In mammals, research has focused on how the hypothalamus and brain stem regulate feeding and energy output (Gao and Horvath, 2007). Yet the question of how the brain modulates feeding and energy homeostasis is far from solved.

There is a surprising amount of overlap between the simple fly and more complex animals when it comes to metabolic regulation. Flies have discrete organ systems paralleling those in mammals that play key roles as metabolic regulators (Baker and Thummel, 2007). Digestion and nutrient absorption occur in the *Drosophila* midgut, and what does not get immediately used is stored in the fat body. The fat body acts like the mammalian liver and white adipose tissue, metabolizing nutrients and storing large reserves of glycogen and lipid. Specialized clusters of cells called oenocytes accumulate lipids during starvation and are proposed to perform hepatocyte-like functions in lipid processing. A humoral signal is then believed to inform the central nervous system (CNS) of the energy status of the organism. The CNS, in turn, regulates a plethora of physiological and behavioral outputs designed to maintain the organism in an optimal energetic state. Thus, flies have an energy homeostasis "circuit" that is similar to that of mammals. Importantly, they also use conserved signaling pathways to affect carbohydrate, lipid, and energy homeostasis, as well as food intake. For example, NPY (NPF), triacylglycerol lipase (*brummer*), neuromedin-U (*hugin*), perilipin (*lsd2*), FOXO and the insulin signaling pathway all seem to function similarly in flies and mammals (Bharucha, 2009). As more information is revealed in flies, clear links to mammalian physiology will undoubtedly be uncovered. In this issue of *Neuron*, Al-Anzi and colleagues (2009) add another important similarity between mammals and flies: brain centers that regulate fat storage via control of food intake and metabolism.

Al-Anzi et al. (2009) set out to identify populations of fly neurons that control fat storage and metabolism and may therefore be functionally equivalent to mammalian hypothalamic feeding centers. The key to their success was the development of a method for measuring fat content that allows quick and accurate quantification of triglycerides and was thus suitable for unbiased and large-scale screens. Using transgenic tools to manipulate neural activity, they searched for neurons that when silenced or hyperactivated would alter fat deposition in adult flies. After screening through a collection of 350 lines with distinct brain expression patterns, they found two, c673a and Fru, that had the desired effects. Silencing these neurons created obese flies, while hyperactivating them produced lean flies.

Having identified these neuronal populations, Al-Anzi et al. (2009) embarked on the search for a center controlling fat storage. Both c673a and Fru are broadly expressed in the brain. Al-Anzi et al. (2009) first assessed whether the obesity phenotype produced by either set of neurons was due to an overlapping set of neurons. While there was little overall overlap in neuronal expression between these lines, both were expressed in neurons that express key regulators of food-related behaviors and obesity in mammals: insulin (insulin-like peptides in flies), dopamine, and serotonin (Gao and Horvath, 2007; Tecott, 2007). Surprisingly, none of these promising subsets of cells turned out to be responsible for the obesity phenotype, as silencing insulin-producing cells (which reside primarily in the fly brain), dopaminergic and/or serotonergic neurons had no effect on fat storage. They conclude that the dramatic fat-storage changes seen upon silencing/activating c673a and Fru neurons were not due to manipulations of dopaminergic, serotonergic, or insulin-producing cells, and that the effects of the two lines were likely due to independent sets of neurons. These data are quite surprising in light of the evolutionarily conserved role of serotonin and insulin in feeding behavior (Tecott, 2007; Schlegel and Stainier, 2007). It is possible that these molecules play a role in motivated feeding behaviors, such as under conditions where food availability is either quantitatively and/or qualitatively altered (Wu et al., 2005).

To further test the potential autonomy of the c673a and Fru neurons in regulating fat stores, the authors silenced or

activated the neurons and put the flies through a battery of behavioral, physiological, and molecular tests (Table 1). Manipulations of both c673a and Fru neurons led to predicted changes in lipid metabolism, quantified by CO_2 emissions and levels of an enzyme required for de novo fatty acid synthesis. However, different results were obtained upon measuring food intake and the metabolic fate of the radio-labeled ingested food. For example, while silencing c673a neurons caused an increase in food intake and an increase in lipid stores at the expense of carbohydrates, silencing Fru neurons reduced food intake and protein stores. Moreover, hyperactivation of Fru but not c673a neurons led to a massive increase in carbohydrate synthesis at the expense of proteins. These flies showed high levels of autophagy, a mechanism by which proteins and organelles are degraded to be used as an energy source. Flies with hyperactivated Fru neurons therefore appeared to be in a state of perceived energy deficit, a situation not seen upon hyperactivation of c673a neurons. The authors also showed that the expression of genes known to regulate energy homeostasis, such as the cytochrome P450 *cyp4g1* and the lipase *brummer* (Gutierrez et al., 2007; Gronke et al., 2005), was altered upon silencing and activating c673a and Fru neurons, although, again, there were differences between the two sets of neurons. Taken together, these data convincingly argue that the authors have found two brains circuits that mediate fat storage through overlapping as well as distinct mechanisms.

Table 1.

	Inhibition		Activation	
Phenotype	Fru	c673a	Fru	c673a
Fat Storage	↑	↑	↓	↓
Locomotor activity	–	–	–	–
Food consumption	↓	↑	–	–
CO_2 emissions	↓	↓	↑	↑
Lipolysis (upon starvation)	↓	↓		
bmm lipase (mRNA)	↓	–	↑	–
Cyp 4g1 (mRNA)	↓	–	↑	–
C_{14}-leucine to lipids	↑	↑	↓	↓
C_{14}-leucine to carbs	–	↓	↑	–
C_{14}-leucine to protein	↓	–	↓	–
Acetyl-CoA carboxylase (lipid biosynthesis)	↑	↑	↓	↓
Autophagy	–	–	↑	–

– unchanged ↑ increased ↓ decreased

In this issue of *Neuron*, Al-Anzi and colleagues identify and characterize two types of *Drosophila* neurons, c673a and Fru, that regulate food intake and metabolism. Summarized here are the results of the behavioral, physiological, and molecular tests following activation or inhibition of the c673a and Fru neurons (see main text for details). Increases or decreases are indicated by up or down arrows, respectively. Colored boxes indicate tests where results varied between the two neuron types.

To investigate the mechanisms through which c673a and Fru neurons regulate fat storage and energy homeostasis further, the authors investigated the role of molecular pathways well known to play these roles in mammals; the NPY and insulin pathways (Gao and Horvath, 2007). Perhaps unexpectedly, manipulations of either pathway produced no obvious change in fat content or modified the obesity phenotype of c673a- or Fru-silenced flies. Thus, c673a and Fru neurons appear to affect fat stores independently of known mammalian mechanisms. It should be noted, however, that flies with mutations in insulin signaling show altered lipid levels (Taguchi and White, 2008).

In humans, obesity is rarely a reversible condition (Aronne et al., 2009). However, the authors were able to reverse obesity in c673a- and Fru-silenced flies by simply restoring neural activity in those neurons. This resulted in a severe drop in food intake, thereby decreasing fat storage. This result indicates the existence of a signal, possibly emanating from fat cells, by which the fly brain detects the general status of energy stores in the body, thus allowing it to produce changes in feeding behavior that maintain a constant level of fat storage.

In summary, in their attempt to find a fly feeding center comparable to the mammalian hypothalamus, the authors stumbled upon an exciting find: two distinct sets neurons that function through novel neural and (possibly) molecular mechanisms to regulate obesity. As with any new model, the one established by Al-Anzi et al. (2009) generates many interesting questions. How do c673a and Fru neurons sense the state of the fly's fat stores? What are the molecular pathways regulating their function? Does satiety and/or starvation alter the activity of c673a and Fru neurons? Which specific c673a and Fru neurons are crucial for their effects? What organs and cells send or receive input from these sets of neurons to modify behavior and physiology?

The growing availability of tools that allow the manipulation and visualization of functional neural circuits, together with assays to analyze energy homeostasis and feeding behavior, make the fly an efficient and promising system to answer these questions. In fact, *Drosophila* has been already provided new insights that were not evident from studies of the more complex vertebrate systems. These include novel regulators of the insulin and TOR signaling pathways and genes affecting fat deposition and storage (Baker and Thummel, 2007; Bharucha, 2009; Schlegel and Stainier, 2007). These discoveries in flies have contributed to the mechanisms mediating obesity in mammals.

The addition of Al-Anzi et al.'s discovery of neuronal populations that regulate fat storage, metabolism, and feeding behavior, further substantiates the use of *Drosophila* as a model to study obesity. Identification the molecular pathways mediating the effects of the c673a and Fru neurons should provide interesting, and perhaps novel, insights into the mechanism underlying fat storage and energy homeostasis.

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The Pre/Post LTP Debate

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The pre/post debate involves the question of whether long-term potentiation (LTP) is mediated by enhancement of release, enhancement of postsynaptic receptors, or both. Recent papers have presented evidence for purely postsynaptic or purely presynaptic changes, and a paper by Ahmed and Siegelbam (in this issue of *Neuron*) suggests a mechanism by which release is enhanced. This debate is increasingly constrained by technical advances that allow central synapses to be studied with increasing precision. A possible of way of reconciling conflicting evidence is suggested.

Could there be more disagreement than this? Roger Nicoll, a leading figure in the field of long-term potentiation (LTP), recently wrote a review (Kerchner and Nicoll, 2008) declaring victory for the postsynaptic hypothesis of LTP at hippocampal CA1 synapses. According to this hypothesis, the addition of AMPAR to the postsynaptic membrane makes the synapse more powerful, there being no significant role for presynaptic changes. But in a recent issue of this journal (Enoki et al., 2009), Alan Fine's group declared that LTP is due to increased release of vesicles from the presynaptic terminal, there being no significant postsynaptic changes. And in this issue of *Neuron* (Ahmed and Siegelbaum, 2009), Steven Siegelbaum's group provides evidence for a molecular mechanism by which LTP could enhance the release of vesicles.

The question of whether LTP is expressed presynaptically or postsynaptically has been pursued for over 20 years. How can such a seemingly simple question still be unanswered? One reason is that the field still lacks a clear picture of how central synapses work. Quantal analysis, a method that provided a straightforward way for dissecting presynaptic and postsynaptic processes at the neuromuscular junction (NMJ), has proven to be ambiguous at central synapses. At the NMJ, an increase in the probability of a quantal response implies a change in the presynaptic release machinery. Thus, when early studies on LTP showed a dramatic increase in the probability of response, the presynapticists declared victory. However, in a dramatic turnaround nicely described in Nicoll's review, it was then shown that the increase in probability could be due to postsynaptic changes, at least in the case of "silent synapses." At such synapses, there is initially no response at negative voltages (an NMDAR-mediated response is evident at positive voltages). After LTP, AMPARs are added to synapse, making the synapse responsive at negative voltages. Thus, the probability of response goes from zero to a finite value through a postsynaptic mechanism. As will be discussed later, another standard rule of quantal analysis at the NMJ, that addition of postsynaptic receptors increases quantal size, may not always be correct at central synapses.

Although quantal analysis has proven problematic, the pre/post debate has been exciting to watch because of the introduction of stunning new methods. Technical advances over the last few years now make it possible to study postsynaptic and presynaptic events with unprecedented precision. Thus, the debate between the presynapticists and postsynapticists is not just a rehash of the same old issues, but a debate in which each side is increasingly constrained by new findings.

Has Two-Photon Uncaging "Proven" Postsynaptic Involvement?

Kerchner and Nicoll start their review by summarizing classic data that pointed to a postsynaptic mechanism for LTP