Octopamine integrates the status of internal energy supply into the formation of food-related memories

Michael Berger, Katrin Auweiler, Michèle Tegtmeier, Katharina Dorn, Tanna El Khadrawe, Henrike Scholz*

Department of Biology, Institute for Zoology, University Köln, 50674-Köln, Germany

*Correspondence: henrike.scholz@uni-koeln.de

Summary

The brain regulates food intake in response to internal energy demands and the availability of food. However, can internal energy storage influence the kind of memory that is formed? We show that the duration of starvation determines whether *Drosophila melanogaster* forms appetitive short-term or long-term memory. The internal glycogen storage in the muscles and fat tissue influences how long sucrose is remembered. Insulin-like signaling in octopaminergic reward neurons integrates internal energy storage into memory formation. In turn, octopamine suppresses the formation of long-term memory. Octopamine is not required for short-term memory, since octopamine-deficient mutants form appetitive short-term memory to sucrose and to other nutrients depending on the internal energy status. The reduced positive reinforcing effect of sucrose in the presence of high internal glycogen levels combined with the increased stability of food-related memories due to increased starvation periods might drive increased food intake.

Keywords: *Drosophila*, stability of memory, starvation, glycogen, carbohydrates, octopamine, food intake, overeating

Introduction

The internal energy status of an animal needs to be adjusted to the energy expenditure of the organism and the availability of external food to ensure survival. Increased storage of energy correlates with increased food intake in the past. Dysregulation of food intake might result in diseases such as obesity and diabetes. Sucrose is a carbohydrate enriched in Western diets, and the breakdown product of sucrose - glucose - can be stored in the organism as glycogen mainly in the liver and muscles. Increased glycogen levels are a hallmark of glycogen storage diseases that are accompanied by defects in the liver, muscles and brain.(Ellingwood and Cheng, 2018) Similar to vertebrates, the fruit fly *Drosophila melanogaster* uses glucose as a primary energy source and stores glycogen mainly in the muscles—the major site of energy expenditure—and the fat body—the equivalent of the vertebrate liver.(Galikova and Klepsatel, 2023; Wigglesworth, 1949) As in vertebrates, glycogen levels are also found in the brain.(Yamada et al., 2018)

The peptide hormone insulin regulates glycose homeostasis at the cellular level.(Saltiel and Kahn, 2001) Insulin and its *Drosophila melanogaster* counterpart, insulin-like peptides, perform their functions through well-conserved signal transduction cascades.(Chatterjee and Perrimon, 2021; Inoue et al., 2018) In vertebrates, in addition to its function in fat tissue and muscles, the insulin receptor is broadly expressed in the brain and regulates neuronal plasticity.(Nakai et al., 2022) For example, in rats, reduced insulin receptor function in the hypothalamus results in loss of long-term potentiation and impaired spatial memory.(Grillo et al., 2015) In *Drosophila melanogaster,* the insulin receptor substrate Chico is required for the development of mushroom bodies, a brain

region required for learning and memory, and loss of Chico results in learning defects.(Naganos et al., 2012) Disrupting the function of the insulin receptor in the mushroom bodies also results in learning defects, whereas the insulin receptor and Chico are required in the ellipsoid body for protein biosynthesis-dependent long-term memory.(Chambers et al., 2015) While there is evidence that insulin receptor signaling regulates cellular physiology, it is not clear how the internal energy supply of the animal influences the formation of food-related memories.

In *Drosophila melanogaster*, the monoamine octopamine - functionally related to noradrenalin in vertebrates - is involved in the regulation of energy homeostasis. Upon starvation, the glucose and trehalose concentrations in the hemolymph change less in tyramine-β-hydroxylase (*Tbh*) mutants lacking the neurotransmitter octopamine, and in addition, the life span of *Tbh* mutants is extended.(Damrau et al., 2017; Li et al., 2016) *Tbh* mutants show an increased threshold to respond to sucrose with the extension of their proboscis when sucrose is offered to their tarsi – a structure that contains gustatory receptor neurons. The reduced response threshold to sucrose correlates with a reduced sucrose intake in *Tbh* mutants.(Li et al., 2016; Scheiner et al., 2014) Octopaminergic neurons potentiate the response of sugar-sensing gustatory receptor neurons in satiated flies, suggesting that octopaminergic neurons regulate feeding behavior by changing the sensitivity of taste receptor neurons.(Youn et al., 2018) The reduced responsiveness to sucrose is also thought to be responsible for defects observed in habituation in *Tbh* mutants.(Scheiner et al., 2014) In addition to their changes in simple forms of neuronal plasticity, *Tbh* mutants fail to form a positive association with a food reward in a classical olfactory conditioning paradigm. Flies

quickly learn to associate an odorant with sucrose, and Tbh mutants do not show this positive association directly after learning.(Schwaerzel et al., 2003) However, when a longer time after training has elapsed, memory appears.(Das et al., 2014) The release of octopamine mediates the reinforcing effect of sweet taste in short-term memory and the anesthesia-resistant form of long-term memory.(Burke et al., 2012; Huetteroth et al., 2015; Wu et al., 2013)

To analyze the relationship between energy status, the evaluation of a food reward and the formation of memory and food intake, we used the olfactory associative learning and memory paradigm. Using nutrients as a positive reward allows for investigating how the internal energy status influences the evaluation of the reinforcer and in turn learning and memory. As a step to understand the interconnection between reward evaluation, nutrient intake and the formation of food-related memories, we analyzed the function of octopamine in the regulation of internal energy homeostasis, learning and memory and food intake.

Results

Starvation increases learning and memory performance when using a food-related reward (Colomb et al., 2009). However, how the internal energy status is integrated into the evaluation of the reward and the stability of food-related memories and whether there is a correlation between the perceptions of the food reward, food-related memories and food intake are not clear.

The duration of starvation influences the strength and stability of memories

To investigate whether changes in internal energy storage influence food-related memories, we used the olfactory associative learning and memory paradigm using sucrose as a food-related reinforcer.(Schwaerzel et al., 2003) In the paradigm, flies learn to associate an odorant with sucrose and are later tested on whether they remember the association. Starved flies were exposed for 2 min to an unconditioned odorant, 4-methylcyclohexanol (MCH), followed by a 2 min exposure to a second odorant, 3-octanol (3-OCT), that was paired with the reward of 2 M sucrose. After the training, flies were given a choice between the two odorants (Figure 1A). Normally, flies have formed a positive association between the reward and the odorant within 2 min after the training. In a reciprocal experiment, the second odorant was rewarded. The learning index presents the average positive association between the reward and both odorants. Before the experiments, flies were tested to determine whether they perceived the odorants, preferred one odorant over other and responded to the reward similarly to ensure that the observed differences in behavior were not due to changes in odorant perception or sucrose sensitivity (Table S1). To alter the internal energy status, flies were starved either for 16 h or for 40 h before the training (Figure 1). We used 3- to 5-day-old male flies to minimize differences in body weight and to control for differences in food preferences. We included $T\beta h^{nM18}$ mutants lacking octopamine in the experiments, since they showed defects in energy metabolism and sucrose reward learning.(Li et al., 2016; Schwaerzel et al., 2003) Flies that were starved for 16 h formed a positive association between the rewarded odorants and remembered this association for 2 min but not for 6 h. Consistent with previous results, learning and memory performance was significantly improved by prolonged starvation.(Colomb et al., 2009) In

addition, 16 h starved *Tβh*^{nM18} mutants did not show 2 min memory but emerged memory 6 h after training.(Das et al., 2014; Schwaerzel et al., 2003) In contrast, after 40 h of starvation, *Tβh*^{nM18} mutants already remembered the sucrose paired odorant 2 min after training to a similar extent as after 6 h (Figure 2B). We repeated similar experiments using a lower concentration of 0.15 M sucrose as a reinforcer (Figure S1). Here, prolonged starvation significantly increased learning and memory performance in controls and *Tβh*^{nM18} mutants. In contrast to the experiments using 2 M sucrose as a reward, 16 h starved *Tβh*^{nM18} mutants form a negative association with 0.15 M sucrose 2 min after training, but when starved for 40 h, form a positive 2 min memory. Starvation and reinforcer strength regulate appetitive memory strength.

Next, we analyzed how starvation influences the stability of memory. Protein synthesis-dependent long-term memory is still labile directly after training and can be blocked by 4°C cold-shock anesthesia directly after training.(Krashes and Waddell, 2008) After consolidation, long-term memory is cold-shock resistant and insensitive to a cold shock 1 h before the test. In addition, a second form of longer lasting memory exists, which is directly after training cold-shock insensitive (Figure 1C). To investigate how starvation influences the stability of appetitive memory, we applied cold-shock anesthesia at different time points after training and analyzed the influence on memory formation in control and *Tβh*^{nM18} mutant flies (Figure 1C). As expected 3 h after training, memory was still present in 16 h starved flies and was completely abolished by a cold shock directly after training or shortly before the test. Increased starvation resulted in memory that was sensitive to cold shock directly after training but not shortly before the test. Thus, an extended period of starvation resulted in long-term memory in control

flies. In contrast to 16 h starved controls, 16 h starved *Tβh*^{nM18} mutants developed long-term memory that was sensitive to cold shock directly after training and cold shock insensitive shortly before the test. Longer periods of starvation resulted in *Tβh*^{nM18} mutants in anesthesia-resistant memory. Thus, with increasing length of starvation, memory becomes more stable. Depending on the duration of starvation, animals first form STM memory, then cold-shock-sensitive LTM and later ARM. The *Tβh*^{nM18} mutants that were starved for 16 h formed similar memories to the 40 h starved control flies.

Octopamine is a negative regulator of memory

Starvation induces the formation of protein synthesis-dependent long-term memory.(Krashes and Waddell, 2008) Thus, it is likely that the emerging memory 6 h after training in *Tβh*^{nM18} mutants is long-term memory. To address this, we abolished a mechanism specifically required for long-term memory in *Tβh*^{nM18} mutants and analyzed whether this interferes with emerging memory (Figure 2). The R15A04-Gal4 driver targets dopaminergic neurons of the PAM cluster specifically required for appetitive long-term memory but not short-term memory.(Yamagata et al., 2015) Blocking the function of these dopaminergic neurons directly after training using a temperature-sensitive *shibire* transgene (UAS-shi^{ts}) and a 30 min long heat pulse of 31°C resulted in loss of memory in *Tβh*^{nM18} mutants (Figure 2A). This is consistent with the fact that emerging memory is long-term memory. Since the *Tβh*^{nM18} mutants lack the neurotransmitter octopamine, we next investigated whether octopamine normally suppresses LTM. To address this, we first blocked the function of octopamine receptors in controls directly after the training by feeding the octopamine antagonist epinastin for 1

h and analyzed memory 5 h later (Figure 2B). If the function of the octopamine receptor is required for long-term memory, longer lasting memory should also appear in control flies that are starved for 16 h prior to the training, which indeed was the case. Consistent with the idea that octopamine is a negative regulator of long-term memory, feeding octopamine to *Tβh*^{nM18} mutants directly after training blocked long-term memory (Figure 2B). To analyze whether octopamine is also able to block STM, we fed octopamine prior to training to control flies (Figure 2C). A short pulse of octopamine before the training inhibits the STM. Thus, octopamine is a negative regulator of appetitive dopaminergic neuron-dependent long-term memory and can block STM.

Starvation influences sucrose consumption preference

Starvation reduces internal energy storage. The reduction might result in reevaluation of external food cues and increased food consumption to restore the energy supply. To investigate whether starvation depletes glycogen storage, we measured glycogen levels in whole animals (Figure 3A). In controls and *Tβh*^{nM18} mutants, starvation reduces glycogen levels; however, non-starved *Tβh*^{nM18} mutant males started out with significantly higher glycogen levels. After 40 h of starvation, the glycogen levels of *Tβh*^{nM18} mutants were still higher than those of controls that were starved for the same amount of time. The reevaluation of external food cues might be reflected in the choice of food. Adult *Tβh*^{nM18} mutants have a reduced sucrose intake.(Li et al., 2016; Scheiner et al., 2014) To investigate whether starvation influences the reallocation of an external food cue, we starved flies and determined the preference to consume sucrose to protein-enriched food using the capillary feeder assay (CAFE(Ja et al., 2007)). After

starvation, control flies and *Tβh*^{nM18} mutants chose between 5% sucrose and 5% yeast (Figure 3B). Prolonged starvation resulted in a decreased sucrose preference in controls and *Tβh*^{nM18} mutants, but *Tβh*^{nM18} mutants started with a lower sucrose preference. Only after 40 h of starvation did *Tβh*^{nM18} mutants show a similar preference to control sucrose consumption. To further investigate whether *Tβh*^{nM18} mutants have defects in the regulation of internal sucrose homeostasis, we deprived male flies of sucrose- or protein-enriched food by letting them feed on 5% sucrose, 5% yeast or standard food for 3 days and analyzed their food preference after deprivation (Figure 3C). As controls, we included mated females in our analysis since they have different nutritional requirements due to their mating status.(Ribeiro and Dickson, 2010; Vargas et al., 2010) Flies fed standard food for 3 days strongly preferred sucrose to protein-enriched food, with *Tβh*^{nM18} mutants showing a significant reduction. The yeast-deprived *Tβh*^{nM18} mutant showed a significantly reduced preference for sucrose, and sucrose-deprived mutants had sucrose preferences similar to those of controls. In mated females, no differences in food preference between controls and the *Tβh*^{nM18} mutant were observed. In summary, the reduced preference to consume sucrose correlated in *Tβh*^{nM18} with increased glycogen levels. In addition, *Tβh*^{nM18} mutants can sense the reduction in specific internal energy supplies and change their food preferences accordingly.

Internal glycogen storage influences sucrose-related memories

Since memory performance increases upon reduction of the internal energy supplies due to starvation, we wanted to investigate whether the internal energy supply

influences memory performance. In *Drosophila*, glycogen is mainly found in the fat bodies - the major energy storage organ - and the muscles, a major site of energy expenditure.(Wigglesworth, 1949) Glycogen synthase and glycogen phosphorylase control the levels of glycogen in the body (Figure 4A). Knockdown of glycogen synthase using *GlyS*^{HMS01279}*-RNAi* efficiently reduced glycogen levels in larvae, whereas knockdown of glycogen phosphorylase using *GlyP*^{HMS00032}*-RNAi* efficiently increased glycogen levels.(Yamada et al., 2018) We altered glycogen levels in the muscles using the *mef2*-Gal4 driver(Ranganayakulu et al., 1998) and/or the fat bodies using the FB-Gal4 driver(Gronke et al., 2003) and analyzed the effect of changed glycogen levels on short-term memory (Figure 4). We used PAS staining to confirm the down- or upregulation of glycogen in larval muscles or fat bodies, respectively (Figure 4).(Yamada et al., 2018) Additionally, we quantified the glycogen levels in the body of adult flies (Figure S2).

Increasing glycogen levels in the muscles did not change short-term memory in 16 h starved flies, but the reduction in glycogen significantly improved memory strength (Figure 4B). Increasing or decreasing glycogen levels in the fat bodies had no effect on memory performance (Figure 4C). When the glycogen levels were significantly increased in the muscles and fat bodies, flies showed a reduced memory to odorants paired with sucrose. An increase in memory performance was observed when glycogen levels were significantly reduced in both tissues (Figure 4D). Recently, it has been shown that energy metabolism in mushroom bodies is important for the formation of LTM.(Placais et al., 2017) To analyze the function of the mushroom bodies, the expression of *GlyS*^{HMS01279}*-RNAi* under the control of the Mef2-Gal4 driver was

repressed in the mushroom bodies using the mb247-Gal80 driver.(Krashes et al., 2007) The memory was still increased (Figure S3). Reciprocally, the reduction of *GlyS*^{HMS01279}-*RNAi* using the mb247-Gal4 driver targeting the mushroom bodies(Zars et al., 2000) did not change short-term memory (Figure S3). Thus, low levels of glycogen in the muscles upon starvation positively influence appetitive short-term memory, while high levels of glycogen in the muscles and fat body reduce short-term memory.

Internal glycogen levels reduce sucrose-related memories in Tβh mutants

The elevated glycogen levels in *Tβh*^{nM18} mutants might be responsible for the reduced STM. To determine whether the reduction in glycogen levels in the muscles or fat bodies restores STM, we expressed *GlyS*^{HMS01279}*-RNAi* under the control of the *mef2*-Gal4 or *FB*-Gal4 driver in *Tβh*^{nM18} mutants and analyzed STM (Figure 5A). Neither the reduction in the muscles nor the reduction in fat bodies of *Tβh*^{nM18} mutants improved STM. Only when glycogen was reduced in both tissues did the *Tβh*^{nM18} mutants show improved STM compared to controls. Thus, *Tβh*^{nM18} mutant flies can form appetitive STM similar to controls when energy storage is sufficiently reduced. Next, we analyzed whether male *Tβh*^{nM18} mutants can form STM to other nutrients than carbohydrates by using a protein-enriched diet in the form of 5% yeast as a positive reinforcer (Figure 5B). To evaluate whether there is a difference in the evaluation of protein as a food source between male flies and *Tβh*^{nM18} mutants, we determined yeast intake in non-starved and starved flies (Figure S4). Non-starved *Tβh*^{nM18} mutant males have a significantly higher protein intake than controls. However, after 16 h of starvation, the level of protein intake was comparable to that of the controls. Using 5% yeast as a food

reward, male *Tβh*^{nM18} mutants showed comparable levels of STM to controls (Figure 5B).

To further analyze the influence of the internal energy status on memory performance, we took advantage of the observation that female flies have different nutritional requirements depending on their mating status.(Ribeiro and Dickson, 2010; Vargas et al., 2010) Virgin females showed a higher consumption preference for sucrose than mated females when given the choice between yeast and sucrose (Figure 3C), supporting a difference in internal energy demands and suggesting a difference in valence for different diets. We tested virgin and mated female flies of controls and *Tβh*^{nM18} mutants for STM (Figure 5B). Virgin females remembered sucrose significantly better than mated females. This was also true for *Tβh*^{nM18} mutant females. Thus, the internal energy status influences how the reward is evaluated in learning and memory processing.

Insulin-like signaling in octopaminergic neurons regulates STM

How is the internal energy status integrated into the reward system? Insulin-like signaling regulates glycogen levels in invertebrates and vertebrates.(Semaniuk et al., 2021) Loss of the insulin receptor results in more circulating sugar but not increased glycogen levels.(Shingleton et al., 2005) Thus, the insulin receptor might be a good candidate that links the internal energy level to reinforcing neurons. First, we analyzed whether the insulin receptor is expressed in octopaminergic reward neurons in the brain (Figure 6). To detect the expression of an activated insulin receptor, we used an insulin antibody that recognizes the phosphorylated form of the insulin receptor (InR). This

region is highly conserved between humans and flies. First, we tested whether the antibody indeed recognizes the activated insulin receptor. Therefore, we overexpressed the activated insulin receptor using the UAS-InR.A1325D transgene under the control of the *dTdc2*-Gal4 driver (Figure S5). The InR.A1325D protein variant mimics the human V938D protein variant that is constitutively active,(Longo et al., 1992) and the *dTdc2*-Gal4 driver targets octopaminergic reward neurons.(Busch et al., 2009) The expression of activated InR resulted in increased immune reactivity (Figure S5). Thus, the InR antibody detects the activated InR. In the brain, activated InR is broadly expressed in a punctate manner and more specifically in the soma of *dTdc2*-Gal4-targeted neurons (Figure 6A). To uncouple energy sensing via the insulin receptor in reward neurons, we expressed UAS-InR.K1409A under the control of the *dTdc2*-Gal4 driver. The transgene encodes a dominant negative variant of the InR (InR^{DN}) and interferes with InR function.(Wu et al., 2005) In 16 h starved animals, the expression of InR^{DN} under the control of the *dTdc2*-Gal4 driver did not change STM, but uncoupling of InR-dependent energy sensing in reward neurons in $T\beta h^{nM18}$ restored STM to normal levels (Figure 6B and C).

To investigate whether the improved STM also affects the emerging LTM of the $T\beta h^{nM18}$ mutants, we performed cold-shock experiments in $T\beta h^{nM18}$ mutants in which InR^{DN} was expressed in octopaminergic reward neurons (Figure 6D). The 3 h memory in controls is cold-shock sensitive, but the memory in $T\beta h^{nM18}$ mutants is cold-shock insensitive, supporting the idea that the mutants formed anesthesia-resistant memory. Given that octopamine is a negative regulator of memory and that it is still missing in $T\beta h^{nM18}$ mutants with blocked insulin signaling on octopaminergic reward neurons, it is

not surprising that ARM is still observed in the mutants. Thus, two functionally distinct memory traces were formed after training, an insulin receptor-sensitive appetitive short-term memory and a long-term memory that can be blocked by octopamine.

Increased starvation results in overconsumption in Tβh^{nM18} mutants

To analyze whether the increased glycogen levels and the reduced sucrose reward correlate with food consumption, the energy demands in flies under different starvation conditions were analyzed by measuring food intake (Figure 7). When control flies were starved for 16 h or 40 h, they consumed similar amounts of 5% sucrose. In contrast, 16 h-starved *Tβh*^{nM18} mutants consumed significantly less sucrose, but after 40 h of starvation, they consumed approximately 34% more sucrose (Figure 7B). After 40 h of starvation, the glycogen levels in *Tβh*^{nM18} mutants were still higher than those in controls (Figure 3A). Thus, they overconsumed sucrose. The overconsumption was independent of the diet, as they showed similar overconsumption when fed with a solution containing 5% yeast and 5% sucrose (Figure 7B). To investigate whether the integration of the internal energy status is also integrated into feed behavior by octopaminergic reward neurons, we blocked insulin signaling in reward neurons in 16 h-starved *Tβh*^{nM18} cells and analyzed sucrose consumption (Figure 7C). The reduced sucrose consumption of *Tβh*^{nM18} cells was significantly improved to control levels when insulin signaling was blocked in reward neurons. Thus, the regulation of food consumption also requires the integration of internal energy levels into the reward system via octopaminergic neurons.

Discussion

Depending on the internal energy level, flies form first protein-dependent aversive olfactory LTM and later ARM, a protein synthesis-independent form of memory.(Wu et al., 2005) In times of energy shortage, for the organism, costly protein biosynthesis-dependent LTM is exchanged against “less costly” protein biosynthesis-independent ARM.(Mery and Kawecki, 2005) Similarly, we observed that after shorter periods of starvation, appetitive LTM appeared, whereas after longer periods of starvation, ARM emerged (Figure 1E). The *Tβh*^{nM18} mutants form only anesthesia-sensitive aversive memory.(Wu et al., 2013) In contrast, depending on the duration of starvation, the emerging appetitive memory in *Tβh*^{nM18} mutants is cold-shock sensitive or cold-shock insensitive. Thus, Tβh^{nM18} mutants are not defective in appetitive ARM, and the internal energy status defines how quickly ARM is formed.

Octopamine gates memory formation

What is the function of octopamine in memory formation? Octopamine is a negative regulator of emerging LTM, as feeding octopamine receptor antagonists to control flies results in memory, and emerging memory in *Tβh*^{nM18} mutants is suppressed by octopamine after conditioning. In addition, octopamine acts upstream of dopaminergic neurons in the formation of LTM, since blocking neurotransmitter release of dopaminergic LTM-mediating neurons directly after conditioning blocks memory formation in *Tβh*^{nM18} mutants. The results are consistent with the model that dopamine release is not required during the pairing of the conditioned stimulus and the reward

rather than as gain control after the training.(Adel and Griffith, 2021) The gain can be controlled by the inhibitory function of octopamine.

Similar to the function of octopamine upstream of dopaminergic neurons in LTM, octopamine acts upstream of dopaminergic neurons during the acquisition of appetitive STM.(Burke et al., 2012; Liu et al., 2012) However, at first glance, the function of octopamine seems to differ in STM and LTM. Loss of octopamine in *Tβh*^{nM18} mutants results in loss of STM, supporting that octopamine is required as a positive regulator for appetitive STM.(Schwaerzel et al., 2003) The loss of STM in *Tβh*^{nM18} mutants has been attributed to the loss of labile STM that forms in response to the sweetness of sucrose but not due to defects in stable LTM that forms in response to the caloric value of sugar.(Burke et al., 2012; Fujita and Tanimura, 2011) Sweetness is sensed by gustatory receptor neurons that change their sensitivity upon starvation(Inagaki et al., 2012; Marella et al., 2012) and thereby influence responsiveness to the external reinforcer. Responsiveness might be regulated by the octopaminergic system, since octopaminergic neurons regulate the sucrose sensitivity of gustatory neurons in *Drosophila*.(Youn et al., 2018) However, the reduced sensitivity of gustatory sucrose receptors cannot explain why octopamine-deficient mutants do not form STM, since *Tβh*^{nM18} mutants form aversive memory to lower sucrose concentrations.

The internal energy status influences how the external source of sucrose is evaluated rather than octopamine being directly involved in the acquisition of appetitive STM. Consistently, *Tβh*^{nM18} is able to show similar levels of STM as controls after longer periods of starvation. This is further strengthened by the results showing that octopaminergic reward neurons that are uncoupled from energy sensing by inactivation

of insulin receptor signaling and reduced glycogen levels in *Tβh*^{nM18} flies also improve appetitive STM. In addition, *Tβh*^{nM18} mutant females with different energy requirements form appetitive STM, and a short pulse of octopamine before conditioning can even block STM. Thus, octopamine is not required in the acquisition of appetitive STM but rather suppresses LTM when enough energy is available. In addition, the function of OA as a suppressor of different forms of memory allows for differential selection of what kind of memory is formed depending on internal and external information. This gating function in memory formation by octopamine can also be observed in other behaviors. For example, octopamine regulates the decision to approach or avert a food source.(Classen and Scholz, 2018) Thus, the octopaminergic system integrates internal energy demands and the evaluation of external information.

The internal energy level of the animal influences memory formation

On the cellular level, insulin receptor signaling regulates energy metabolism.(Chatterjee and Perrimon, 2021) The broad expression pattern of the insulin receptor in the *Drosophila* brain (Figure 4) indicates that every cell in the brain needs to regulate its own energy homeostasis. However, the overall energy resources of the fly and the evaluation of the external food supply also need to be integrated into memory formation. The external food supply might be evaluated by the reward system. In mice, insulin receptor signaling in dopaminergic neurons mediates food reward.(Konner et al., 2011) Consistently, insulin receptor signaling in octopaminergic reward neurons regulates the rewarding properties of sucrose in appetitive STM memory (Figure 4C) and food intake (Figure 5C).

The systemic metabolic rate is regulated by fat bodies. For example, after food intake, the fat body secretes Unpaired 2 (Upd2), which in turn regulates the secretion of Dilps via GABergic neurons.(Rajan and Perrimon, 2012) Reduction of glycogen in the fat bodies is not sufficient to change appetitive STM, but reduction in the muscles or both tissues is. The results support a feedback mechanism between the evaluation of glycogen levels in the muscles and the brain. In addition, the muscles and the fat bodies communicate about the energy content of both structures, and this information is signaled back to the brain. Such long-range signals from muscles exist. Skeletal muscles are secretory organs.(Pedersen and Febbraio, 2012) For example, the muscle-secreted Amyrel amylase reduces the age-related accumulation of polyubiquitinated proteins in the brain.(Rai et al., 2021) Independent of how the glycogen levels of both tissues are communicated to the brain, they influence how the reinforcer is evaluated in learning and memory processing. Feedback between the energy levels in both structures is still intact in octopamine-deficient mutants, since $T\beta h^{nM18}$ mutants still form STM when the energy storage is sufficiently reduced.

Are food-related memories and internal glycogen levels predictive of food intake?

Feeding behaviors in hungry animals are regulated by different neural systems, including networks receiving and processing sensory information and networks assigning reward properties to food.(Berridge, 2009) Blocking insulin receptor function in dopaminergic neurons causes increased food intake and weight gain in mice.(Konner et al., 2011) Similarly, blocking insulin receptor function in octopaminergic reward neurons in flies with increased glycogen levels and reduced food intake increases food

intake. Thus, the octopaminergic system assigns reward properties to food. However, blocking insulin receptor signaling on reward neurons does not result in extensive overconsumption, supporting that the regulation of the food amount is still intact and is not due to insulin receptor resistance in reward neurons. Increased levels of internal energy, e.g., glycogen, reduced the reward properties of food, resulting in a decreased positive association of food-related memories and decreased food consumption. This should prevent increased food intake in the presence of excess food. Insulin resistance might contribute to weight gain, since in addition to high levels of glycogen, the flies show normal food intake. However, weight gain might be differentially regulated by overeating. The overconsumption is only visible after a prolonged phase of starvation that is also correlated with emerging stable appetitive long-term memory. This suggests that the stability of appetitive memory might result in the re-evaluation of food and might trigger the overconsumption of food.

In summary, the octopaminergic neurotransmitter system integrates several aspects that influence the regulation of food memories. Octopamine modules the sensory perception of the conditioned stimulus and the sensory perception of the reward. Here, we show that the evaluation of food reward in the context of energy storage is integrated by the octopaminergic system and influences the stability of food-related memories. The function of octopamine as a negative regulator of different forms of memory allows for selective regulation of associated behaviors such as food intake, and the loss of this regulation might also promote dysregulation of food intake. The close relationship between the octopaminergic neurotransmitter and the noradrenergic neurotransmitter system suggests that integrator function might be conserved.

Material and methods

Drosophila melanogaster

Flies were raised on an ethanol-free standard cornmeal-molasses food at 25°C and 60% relative humidity on a 12 h/12 h day-night cycle. The following lines were used: w^{1118}; w^{1118},$T\beta h^{nM18}$;(Monastirioti et al., 1996) *UAS-GlyS*^{HMS01279}*-RNAi* (BDSC #34930); *UAS-GlyP*^{HMS00032}*-RNAi* (BDSC #33634); w^{1118}; *UAS-shi*^{ts};(Kitamoto, 2001) w^{1118}; P{UAS-InR. K1409A}, (BDSC#8253); w^{1118};*FB-Gal4* (a generous gift from the Partridge Lab); *mef2-Gal4*;(Ranganayakulu et al., 1998) R15A04-Gal4 (BDSC #48671); *Tdc2-Gal4*.(Cole et al., 2005) All lines were backcrossed to w^{1118} (Scholz Lab) for at least five generations to isogenize the genetic background. For behavioral experiments, three- to five-day-old male flies were used, if not otherwise indicated. Male flies of the F1 generation carrying one copy of the transgene were used as controls for behavioral experiments. Animal studies using *Drosophila melanogaster* were conducted in agreement with the regulations of the DFG and the Land North Rhine-Westphalia. Other ethics approval and informed consent statement are not applicable for research using *Drosophila melanogaster*.

Olfactory learning and memory

Associative olfactory learning and memory was trained and tested with a modified version of the Tully and Quinn olfactory conditioning apparatus.(Schwaerzel et al., 2003; Tully and Quinn, 1985) Approximately 70 one- to two-day-old male flies were collected with CO_2 anesthesia and were kept for 2 days at 25°C to recover from CO_2 sedation. Briefly, three- to five-day-old male flies were starved for 16 h or 40 h in vials with water-

soaked filter paper at the bottom. Flies were transferred to training tubes and exposed to the first odorant for 2 min, either 3-octanol (3-OCT diluted 1:80 in paraffin oil) or 4-methylcyclohexanol (MCH diluted 1:100 in paraffin oil). After that, they were transferred to a second tube and exposed to the second odorant in the presence of filter paper soaked with either 0.15 M sucrose, 2 M sucrose or 5% yeast. To analyze the abilities of the flies to learn and remember the odorant paired with the reward, 2 min, 3 h or 6 h after training, flies were given the choice between odorant 1 (CS+) and odorant 2 (CS-) for 2 min. The performance index (PI) was calculated as PI = (# (CS+) + # (CS-)/(total # flies), where CS+ indicates the odorant associated with the appetitive reinforcer and CS- indicates the odorant that was not associated with the reinforcer. To exclude nonassociative effects, each “n” consists of a reciprocally trained, independent group of naïve flies. For pharmacological experiments, flies were fed water, 3 mM OA or 2 M sucrose before training or with 3 mM OA or 3 mM Epinastine between training and testing. The assay was performed at RT with 60% relative humidity. For cold-shock experiments, flies in vials were placed in ice-cold water for 2 min. Odorant acuity and odorant balance were tested with naïve flies placed into the Tully Quinn setup. They chose for 2 min between odorant- or paraffin oil-containing sides or between both odorants. For sucrose and yeast preference, flies chose between sucrose- or yeast-soaked filter paper and water-soaked filter paper.

Food intake

To analyze the consumption of nutrients in flies, the capillary feeder (CaFe) assay was performed.(Diegelmann et al., 2017) Briefly, eight three- to five-day-old male or female

flies that were either nonstarved or starved for 18 h or 40 h had access to 4 capillaries filled with either 5% sucrose or 5% yeast or a mixture of both for 24 h at 25°C and 60% humidity. The solution was colored with red food dye. During starvation, flies were kept in vials with wet filter paper at 25°C and 60% humidity. The amount of consumed solution was determined using an electronic caliper. To account for evaporation, the average evaporation of three CAFE setups without flies was measured and used to normalize the average food intake per fly. To normalize food intake to body weight, at least 5 times 100 male flies per genotype and condition were weighed, and the average body weight of a single fly was determined. The total consumption per µg fly was calculated by dividing the total consumption per fly by the mean weight of a fly. N indicates the number of tested groups.

Glycogen content

Whole body glycogen levels were determined with the Glucose (HK) Assay Kit (Sigma Aldrich, #GAHK20-1KT) according to the protocol of.(Tennessen et al., 2014) A group of five male flies that were either sated or starved for 16 h or 40 h were homogenized in 100 µl ice-cold 1x PBS. To reduce enzymatic degradation, proteins and enzymes were heat inactivated at 70°C for 10 min. The supernatant was removed and diluted 1:3 with 1x PBS. Twenty microliters of the samples were either added to 20 µl of 1x PBS or PBS/Amyloglucosidase mix and incubated at 37°C for 60 min. Then, 100 µl of HK-reagent was added to the sample or glucose standard and incubated for 15 min at RT. Absorbance was measured at 340 nm. The glycogen content was calculated by

subtracting the total glucose concentration from 1x PBS-treated samples from the total glucose of amyloglucosidase-treated samples.

Periodic acid staining

To visualize glycogen levels in the fat bodies and muscle tissue of larvae and adult *Drosophila*, PAS staining was performed after Yamada et al.,(Yamada et al., 2018) with a slight modification. The samples were fixed in 3.5% formaldehyde for 20 min and washed two times for 5 min with 1% BSA/PBS. Periodic acid solution was added for 5 min, followed by two washes of 5 min with 1% BSA/PBS. Schiff's Reagent was added for 5 min, followed by two washes of 5 min with 1% BSA/PBS. Tissue was stored in 50% glycerol.

Immunohistochemistry

For immunohistochemistry, antibodies raised in rabbits against the activated insulin-like receptor (cell signaling technology #3021) were used at a dilution of 1:50 in 5% normal goat serum in PBS with 0.1% Triton and incubated for two days. The brains were washed with PBS with 0.3% Triton.

Quantification and statistical analysis

Food intake was displayed as the mean ± s.e.m. For learning and memory experiments, the data were displayed as boxplot ± minimum (Q1–1.5*IQR) and maximum (Q1 + 1.5*IQR).

The nonparametric one-sample sign test and the parametric one-sample t test were used to analyze whether behavior was based on random choice. Differences between two groups were determined with Student's t test, and more groups were compared with one-way ANOVA with Tukey's post hoc HSD test. Statistical analysis was performed with Statistica 9.1 (StatSoft, Tulsa, OK, USA). Boxplots were generated with Microsoft Excel 2016 and GIMP 2.10.12.

Data availability

All data related to figures are included in the supplement Table S2.

Acknowledgments

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Author contributions

H.S. initiated the project. M.B., H.S. and K.D. designed the experiments. M.B., K.A., K. D. and T.E.K. performed the experiments and, together with H.S., analyzed the data. M.B. and K.A. performed learning and memory tests. K.D. characterized insulin receptor expression. M.B. and T.E.K. performed food consumption assays. M.B. wrote a draft of the manuscript, and H.S. wrote the manuscript.

Declaration of interests

The authors declare no competing interests.

Figure titles and legends

Figure 1. Starvation influences the strength and stability of memory

A, Appetitive olfactory learning and memory paradigm. **B**, Appetitive 2 min STM and 6 h memory in w^{1118} and $T\beta h^{nM18}$ after 16 h (white bars) or 40 h starvation (dark gray bars) before the training using 2 M sucrose as reinforcer. Prolonged starvation increases memory performance. Prolonged starvation of $T\beta h^{nM18}$ for 40 h resulted in STM. Independent of the duration of starvation, 6 h after training, memory appeared in the mutants. **E**, Appetitive STM training with 2 M sucrose and cold shock directly or 2 h after training. Prolonged starvation shifts in control STM to LTM and in $T\beta h^{nM18}$ LTM to ARM. Numbers below box blots indicate pairs of reciprocally trained independent groups of male flies. Student's t tests were used to determine differences between two groups, and one-way ANOVA with Tukey's post hoc HSD test was used to determine differences between three groups. The letter "a" marks a significant difference from random choice as determined by a one-sample sign test ($P^* < 0.05$; $P^{**} < 0.01$).

Figure 2. Octopamine acts upstream of dopamine and negatively regulates memory

A, A 30 min block of dopaminergic PAM neurons required for LTM after training using a temperature-sensitive *shibire* transgene under the control of the R1504-Gal4 driver results in $T\beta h^{nM18}$ mutants losing LTM. **B**, Feeding 3 mM of the octopamine receptor antagonist Epinastine for 1 h directly after training resulted in w^{1118} flies in memory 6 h later. Feeding 3 mM octopamine for 6 h after training suppresses LTM in $T\beta h^{nM18}$. **C**, A 3 mM octopamine feeding pulse 30 min before training inhibits STM in $T\beta h^{nM18}$ mutants.

585 Controls were water-fed. One-way ANOVA with Tukey's HSD post hoc test was used to
586 determine differences between three groups, and Student's t tests were used for two
587 groups. The letter "a" marks a significant difference from random choice as determined
588 by a one-sample sign test ($P^* < 0.05$; $P^{**} < 0.01$).
589
590 **Figure 3. Elevated glycogen levels correlate with reduced sucrose preference**
591 **A**, Analysis of whole-body glycogen levels in w^{1118} and $T\beta h^{nm18}$ flies. Glycogen levels in
592 $T\beta h^{nM18}$ flies are significantly higher than those in w^{1118} flies under similar starvation
593 conditions. N = 3 groups of 5 male flies. **B**, Flies were starved 18 h or 40 h before food
594 intake was measured for 24 h. Flies chose between 5% sucrose and 5% yeast. The
595 preference was determined. All flies showed a significant preference for sucrose
596 consumption. Starvation reduced the preference. $T\beta h^{nm18}$ showed a significantly
597 reduced preference for sucrose after 18 h. N = 20-26 groups of eight flies. **C**, Feeding
598 flies for 3 days on standard, 5% sucrose or 5% yeast resulted in control flies preferring
599 to consume sucrose. $T\beta h^{nm18}$ mutants fed normal food and sucrose showed a significant
600 reduction in sucrose preference but not when fed 5% yeast for three days. N = 20-28
601 groups of eight flies. To determine differences between two groups, Student's t test was
602 used. $P^* < 0.05$; $P^{**} < 0.01$, $P^{***} < 0.001$.
603
604 **Figure 4. Carbohydrate storage influences appetitive STM**
605 **A**, Schema of glycogen synthesis. The expression of *GlyP-RNAi* reduced glycogen
606 phosphorylase and increased glycogen levels, whereas *GlyS-RNAi* reduced glycogen
607 synthase and decreased glycogen levels in target tissues. **B**-**D**, PAS was used to

visualize glycogen levels in larval muscle or fat bodies. **B**, Increases in glycogen in the muscles have no effect on STM, whereas reduced muscle glycogen increases appetitive STM. **C**, Increased or decreased glycogen levels in the fat bodies did not interfere with STM. **D**, A combined increase in glycogen in muscles and fat bodies reduced STM, and a decrease in glycogen increased STM. Student's t tests were used to determine differences between two groups, and one-way ANOVA with post hoc Tukey's HSD was used to determine differences between three or more groups. The letter "a" marks a significant difference from random choice as determined by a one-sample sign test ($P^* < 0.05$; $P^{**} < 0.01$). Numbers below box blots indicate one pair of reciprocally trained independent fly groups.

Figure 5. Reducing glycogen in *Tβh*^{nm18} improves appetitive STM

A, Decreasing glycogen concentration using UAS-*GlyS*^{RNAi} in the muscles or fat bodies in *Tβh*^{nM18} mutants did not improve STM, but decreasing glycogen in both tissues improved STM to control levels. **B**, *w*^{1118} and *Tβh*^{nm18} flies formed similar levels of appetitive STM when 5% yeast was used as a reinforcer. **C**, Virgin females of w1118 and *Tβh*^{nm18} displayed STM, whereas mated females of both genotypes did not. Differences between two groups were determined using Student's t tests, and differences among more than two groups were determined with one-way ANOVA with Tukey's HSD post hoc test. Differences from random choice were determined using a one-sample sign test and marked with the letter "a". $P^* < 0.05$; $P^{**} < 0.01$. Numbers below box blots indicate one pair of reciprocally trained independent fly groups.

Figure 6. Insulin signaling in reward neurons regulates STM performance

A, The activated form of the InR is expressed in punctuate manner throughout the brain (in magenta) and is also detected in octopaminergic reward neurons visualized by using the UAS-mcd8::GFP transgene under the control of the *Tdc2*-Gal4 driver (in green). **B**, Blocking InR signaling in *Tdc2*-Gal4-targeted octopaminergic neurons does not change appetitive STM in 16 h starved flies. **C**, Blocking InR signaling in *Tdc2*-Gal4-targeted octopaminergic neurons in $T\beta h^{nM18}$ mutants restored STM to control levels. **D**, A cold shock did not disrupt emerging memory in $T\beta h^{nM18}$ mutants with blocked InR under the control of the Tdc2-Gal4 driver. Student's t test was used to determine differences between two groups, and one-way ANOVA with Tukey's post hoc HSD test was used to determine differences between three or more groups. The letter "a" marks a significant difference from random choice as determined by a one-sample sign test ($P < 0.05$). n.s. is not significant; $P^* < 0.05$; $P^{**} < 0.01$.

Figure 7. Prolonged starvation results in rebound sucrose intake in hyperglycemic $T\beta h^{nm18}$ mutants

A, Capillary feeder assay used to determine food intake. **B**, Flies were starved 16 h or 40 h before 24 h food intake was measured. After 16 h of starvation, $T\beta h^{nm18}$ mutants significantly consumed less 5% sucrose or 5% sucrose with 5% yeast, and after 40 h of starvation, $T\beta h^{nm18}$ mutants significantly consumed more sucrose. N = 20-26 groups of eight flies. **C**, Blocking InR signaling in *Tdc2*-Gal4-targeted octopaminergic neurons in $T\beta h^{nm18}$ mutants significantly increased 5% sucrose consumption. N = 20-28 groups of eight flies. To determine differences between two groups, Student's t test was used, and

to determine differences between three or more groups, one-way ANOVA with post hoc Tukey's HSD was used. $P^{*} < 0.05$; $P^{**} < 0.01$, $P^{***} < 0.001$.

Supplemental information titles and legends

Supplemental information includes 5 figures and two tables and can be found with this article online.

Figure S1. Starvation influences memory performance using 0.15 M sucrose as a reinforcer

A, Appetitive 2 min STM using 0.15 M sucrose as a reinforcer of w^{1118} and $T\beta h^{nM18}$ mutants starved for 16 h (white bars) or 40 h (gray bars) before the training. After 16 h of starvation, w^{1118} mutants formed STM, and prolonged starvation increased STM. After 16 h of starvation, $T\beta h^{nM18}$ mutants formed aversive STM to 0.15 M sucrose and prolonged starvation resulted in appetitive STM. Numbers below box blots indicate one pair of reciprocally trained independent groups of male flies. Student's t tests were used to determine differences between two groups, and one-way ANOVA with Tukey's post hoc HSD test was used to determine differences between three groups. The letter "a" marks a significant difference from random choice as determined by a one-sample sign test ($P^* < 0.05$; $P^{**} < 0.01$).

Figure S2. Glycogen level in adult flies with reduced GlyP and GlyS

The relative glycogen levels in flies with altered GlyP and GlyS in muscles, fat bodies or both. The expression of *GlyP-RNAi* and *GlyS-RNAi* under the control of *mef2*-Gal4 did not significantly change the relative glycogen level but did under the control of the FB-Gal4 driver. The expression of *GlyP-RNAi* in fat and muscle tissue resulted in significantly increased glycogen levels, and the expression of *GlyS-RNAi* significantly decreased glycogen levels. The glycogen was measured in 3 groups of 5 male flies and

normalized to the protein levels and to the glycogen levels of flies of the Gal4 driver. ANOVA with post hoc Tukey's HSD was used to determine differences between three groups. ($P^* < 0.05$; $P^{**} < 0.01$).

Figure S3. The glycogen level in the mushroom bodies does not influence appetitive STM

A, The expression of *GlyS-RNAi* under control of the mef2-Gal4; mb247-Gal4 drivers increased STM. **B**, The expression of *GlyS-RNAi* under control of the mb247-Gal4 driver did not change STM. One-way ANOVA with Tukey's HSD post hoc test was used to determine differences between the groups. The letter "a" marks a significant difference from random choice as determined by a one-sample sign test ($P^* < 0.05$; $P^{**} < 0.01$; n.s. = non-significant). Numbers below box blots indicate one pair of reciprocally trained independent fly groups. **C**, Sensory acuity tests for the genotypes used.

Figure S4. Starvation influences yeast consumption of *Tβh*^{nm18} flies

Non-starved *Tβh*^{nm18} male flies consumed significantly more yeast for 24 h. After 16 h of starvation, *Tβh*^{nm18} mutants consumed similar amounts of yeast within 24 h. N = 26 groups of eight flies. To determine differences between both groups, Student's t test was used. ***P< 0.001; n.s. = nonsignificant difference.

Figure S5. The antibody against phosphorylated InR recognizes activated InR

In the antennal lobes (AL) of the male adult brain, the immunoreactivity recognized by the anti-InR^{P} antibody is shown in magenta, and GFP expression is shown in green. **A**, **B**, Gal4 expression of the *Tdc2*-Gal4 driver targeting octopaminergic neurons is visualized using the UAS-mCD8::GFP transgene. **B**, The expression of the constitutively active insulin receptor in octopaminergic neurons results in increased immunoreactivity detected by the anti-InR^{P} antibody.

Table S1. Sensory acuity

Table S2. Data related to figures

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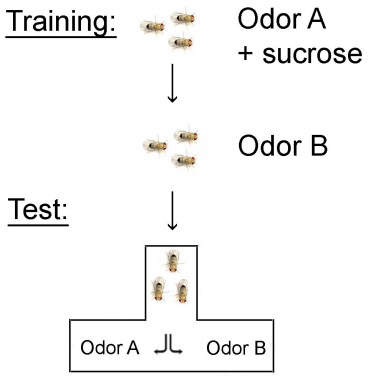
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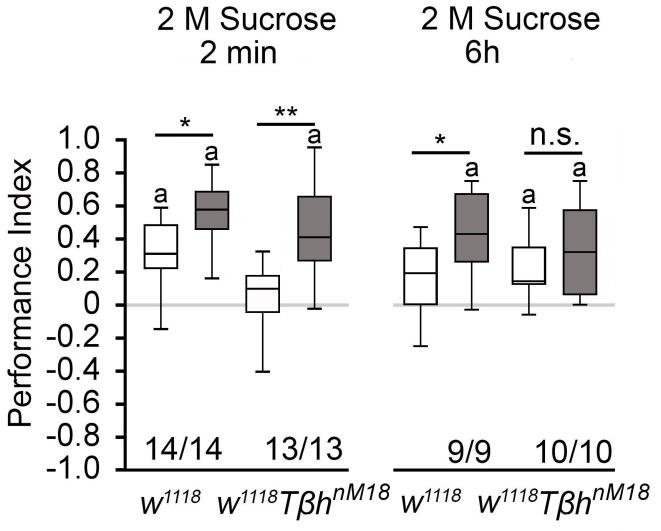
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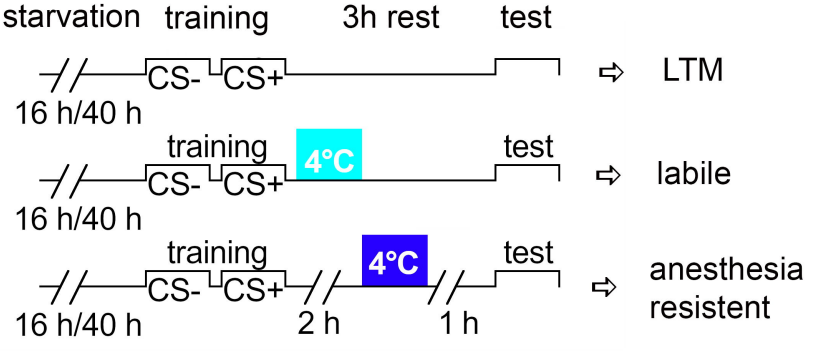
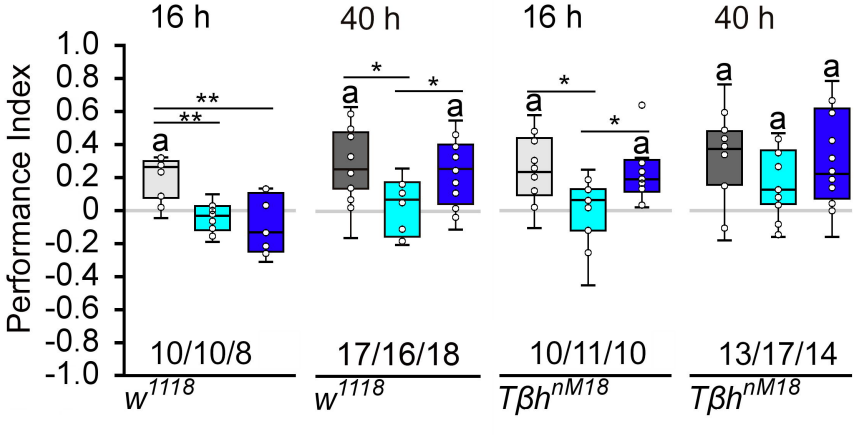
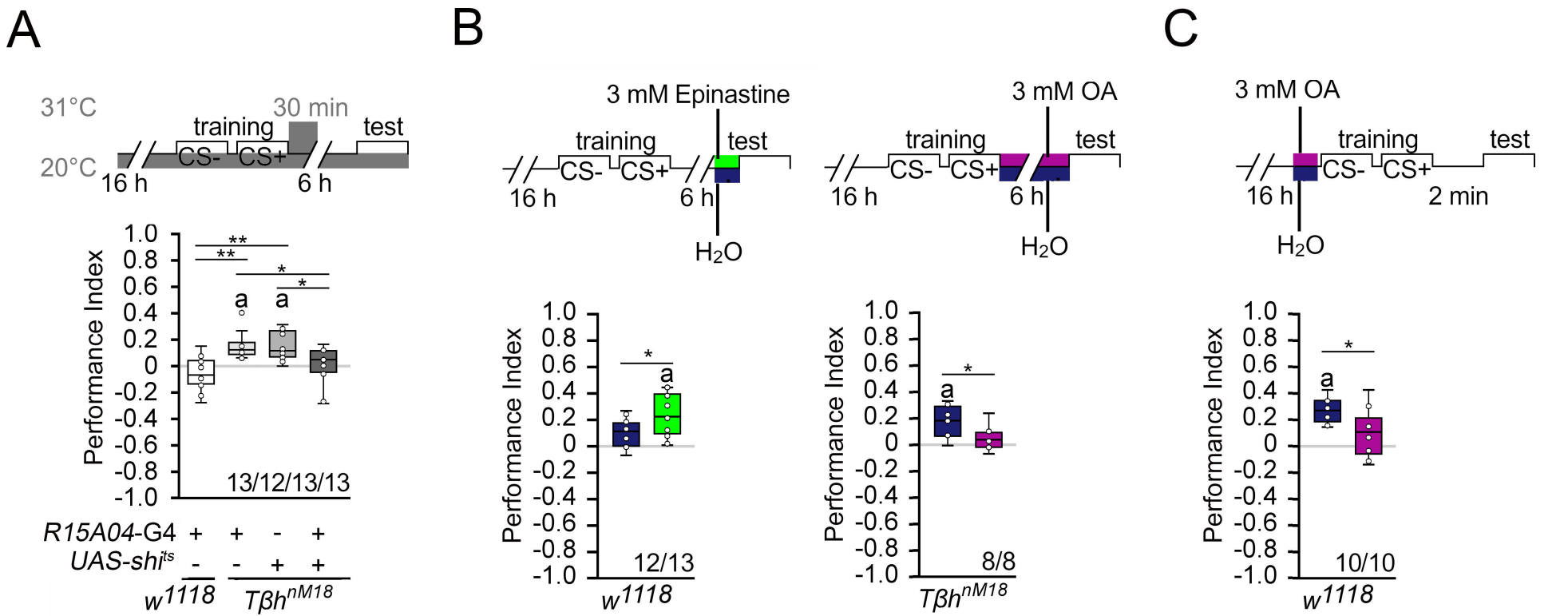
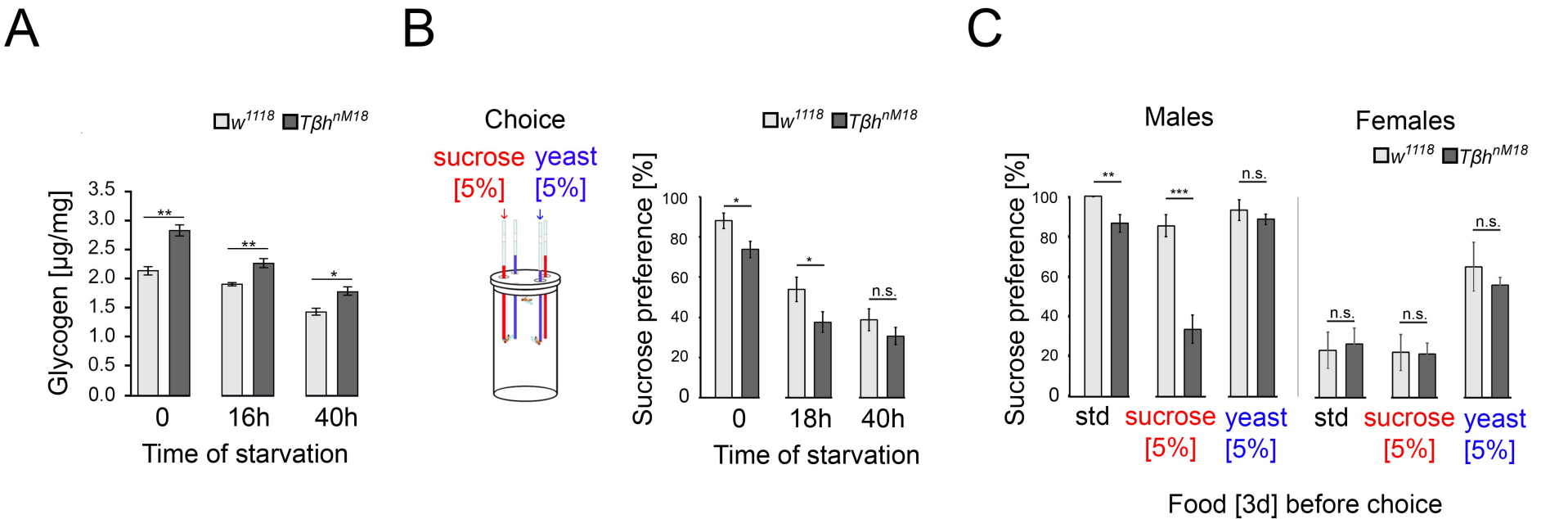
w1118Tβh nM18

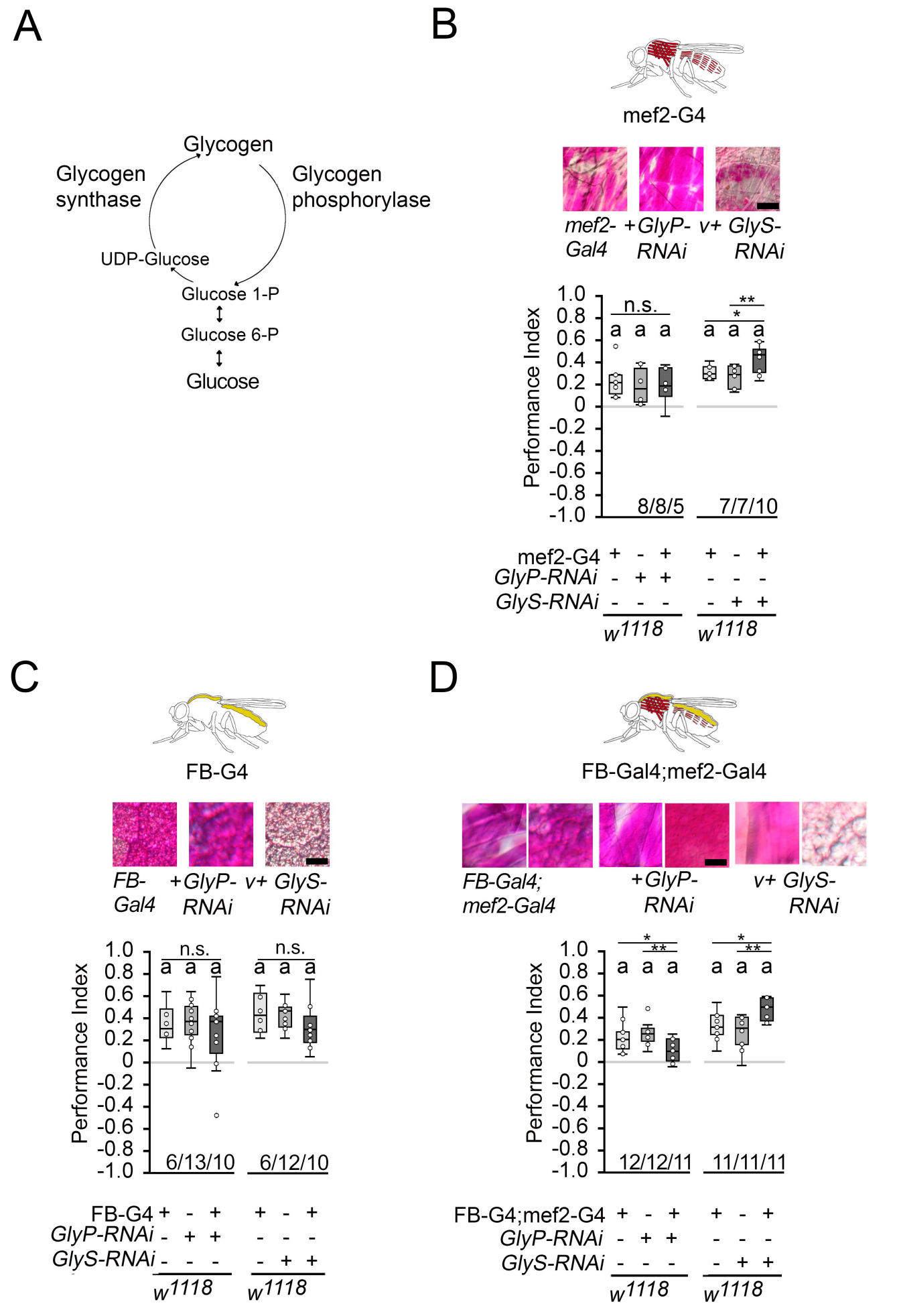
C


starvation
training
3h rest
test
CS-
CS+
16 h/40 h
LTM
training
4°C
test
CS-
CS+
16 h/40 h
labile
training
4°C
test
CS-
CS+
16 h/40 h
2 h
1 h
anesthesia
resistent

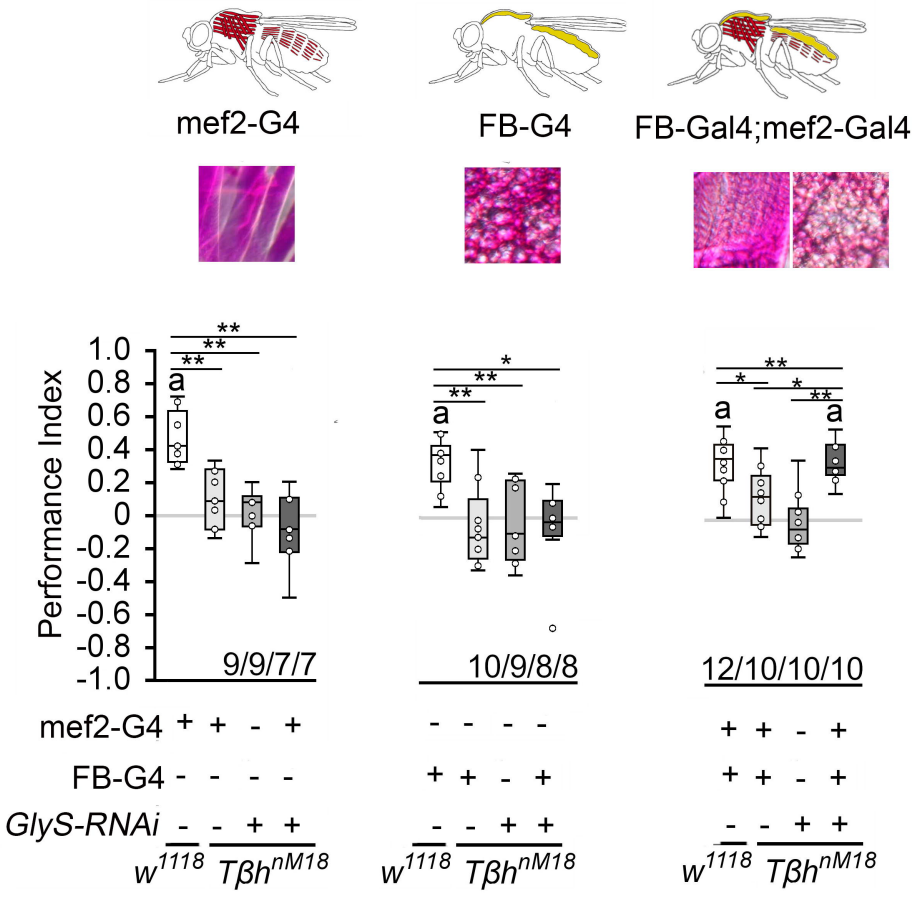

16 h
40 h
16 h
40 h
Performance Index
1.0
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1.0
**
*
a
10/10/8
17/16/18
10/11/10
13/17/14
w1118
w1118
Tβh nM18
Tβh nM18


A
31°C
30 min
training
test
20°C
CS-
CS+
16 h
6 h
Performance Index
13/12/13/13
R15A04-G4 + + - +
UAS-shi^ts - - + +
w1118
Tβh^nM18
B
3 mM Epinastine
training
test
CS-
CS+
16 h
6 h
H2O
12/13
w1118
3 mM OA
8/8
Tβh^nM18
C
3 mM OA
2 min
10/10
w1118

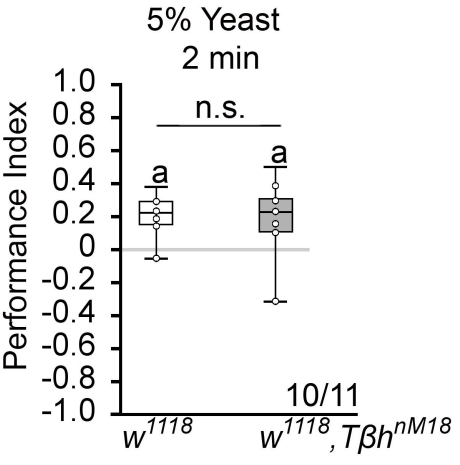

A
w^{1118} $T\beta h^{nM18}$
Glycogen [µg/mg]
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
**
**
*
0
16h
40h
Time of starvation
B
Choice
sucrose [5%]
yeast [5%]
w^{1118} $T\beta h^{nM18}$
Sucrose preference [%]
100
80
60
40
20
0
*
*
n.s.
0
18h
40h
Time of starvation
C
Males
Females
w^{1118} $T\beta h^{nM18}$
Sucrose preference [%]
100
80
60
40
20
0
**
***
n.s.
n.s.
n.s.
n.s.
std
sucrose [5%]
yeast [5%]
std
sucrose [5%]
yeast [5%]
Food [3d] before choice


A
Glycogen
Glycogen synthase
Glycogen phosphorylase
UDP-Glucose
Glucose 1-P
Glucose 6-P
Glucose
B
mef2-G4
mef2-Gal4
+GlyP-RNAi
v+ GlyS-RNAi
Performance Index
n.s.
**
*
a a a
a a a
8/8/5
7/7/10
mef2-G4 + - + + - +
GlyP-RNAi - + + - - -
GlyS-RNAi - - - - + +
w1118
w1118
C
FB-G4
FB-Gal4
+GlyP-RNAi
v+ GlyS-RNAi
Performance Index
n.s.
n.s.
a a a
a a a
6/13/10
6/12/10
FB-G4 + - + + - +
GlyP-RNAi - + + - - -
GlyS-RNAi - - - - + +
w1118
w1118
D
FB-Gal4;mef2-Gal4
FB-Gal4; mef2-Gal4
+GlyP-RNAi
v+ GlyS-RNAi
Performance Index
*
**
*
**
a a a
a a a
12/12/11
11/11/11
FB-G4;mef2-G4 + - + + - +
GlyP-RNAi - + + - - -
GlyS-RNAi - - - - + +
w1118
w1118

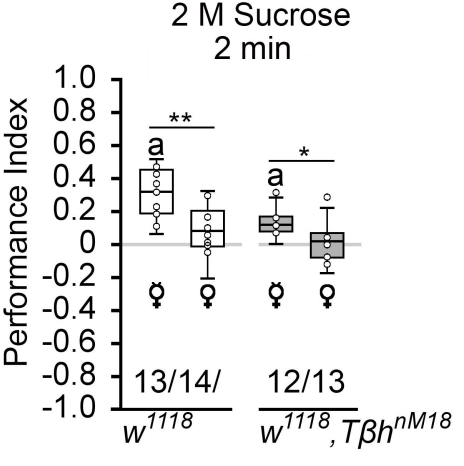
A

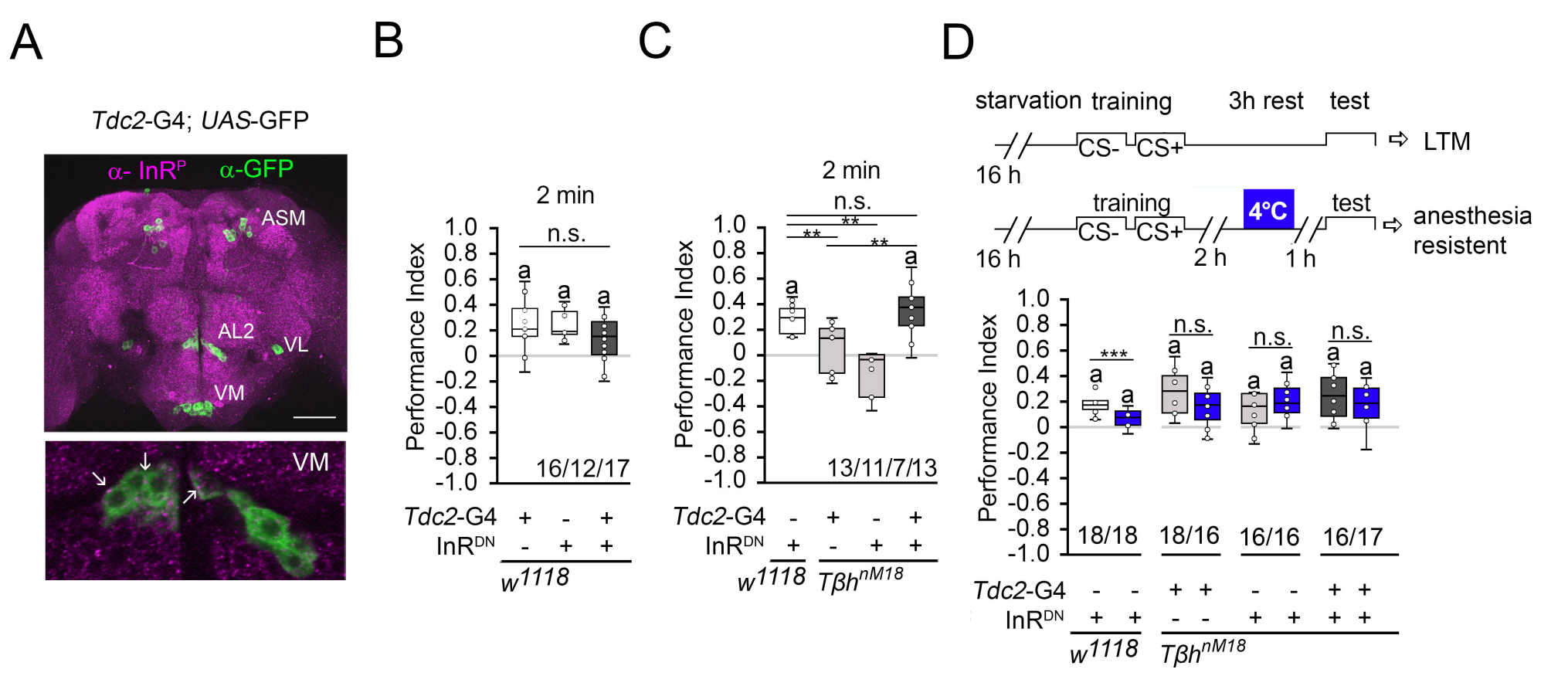

mef2-G4
FB-G4
FB-Gal4;mef2-Gal4
Performance Index
9/9/7/7
10/9/8/8
12/10/10/10
mef2-G4
FB-G4
GlyS-RNAi
w1118
Tβh nM18

B

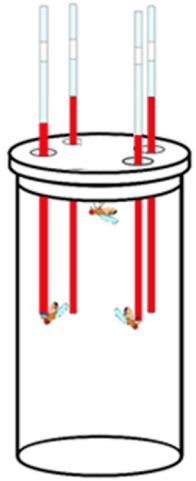

5% Yeast
2 min
n.s.
Performance Index
10/11
w1118
w1118, Tβh nM18

C

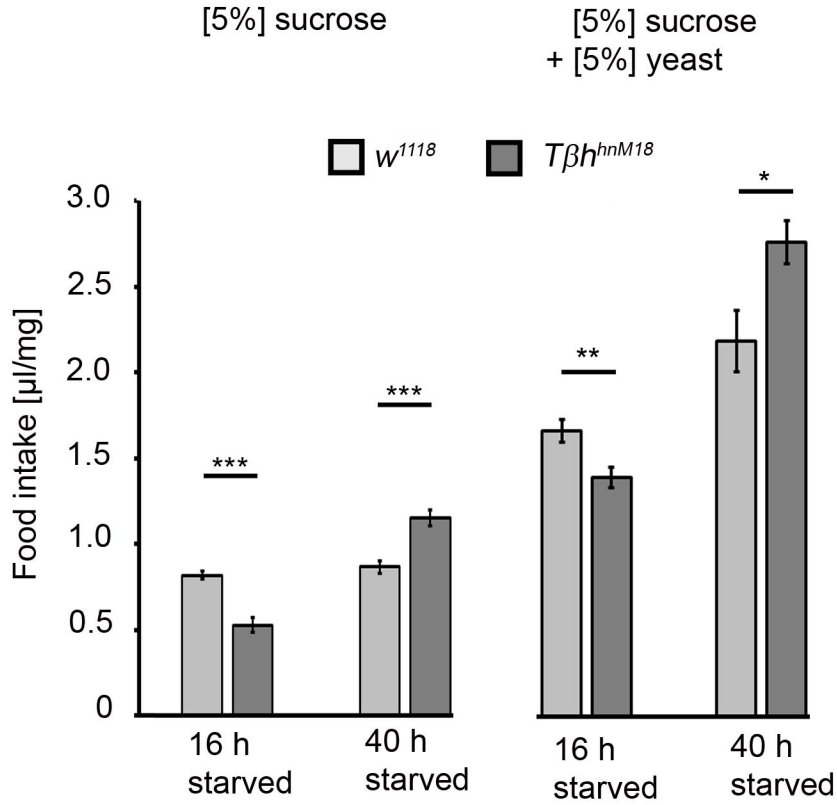

2 M Sucrose
2 min
Performance Index
13/14/
12/13
w1118
w1118, Tβh nM18


A
Tdc2-G4; UAS-GFP
α- InRP
α-GFP
ASM
AL2
VL
VM
VM
B
2 min
n.s.
Performance Index
16/12/17
Tdc2-G4 + - +
InRDN - + +
w1118
C
2 min
n.s.
**
**
**
Performance Index
13/11/7/13
Tdc2-G4 - + - +
InRDN + - + +
w1118
Tβh nM18
D
starvation training
3h rest
test
CS-
CS+
LTM
16 h
training
4°C
test
anesthesia resistent
16 h
2 h
1 h
Performance Index
***
n.s.
n.s.
n.s.
18/18
18/16
16/16
16/17
Tdc2-G4 - - + + - - + +
InRDN + + - - + + + +
w1118
Tβh nM18

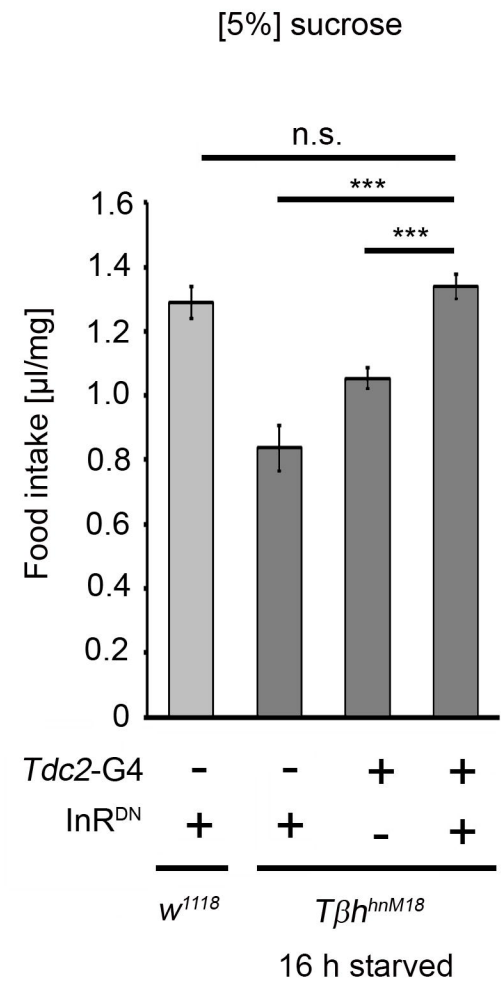
A



B


[5%] sucrose
[5%] sucrose
+ [5%] yeast
w1118
TβhhnM18
Food intake [µl/mg]
3.0
2.5
2.0
1.5
1.0
0.5
0
***
***
**
*
16 h
starved
40 h
starved
16 h
starved
40 h
starved

C


[5%] sucrose
n.s.
***
***
Food intake [µl/mg]
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Tdc2-G4 - - + +
InRDN + + - +
w1118
TβhhnM18
16 h starved