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

Michael Berger, Michèle Fraatz, Katrin Auweiler, 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 food availability. However, can internal energy storage influence the type of memory that is formed? We show that the duration of starvation determines whether *Drosophila melanogaster* forms appetitive short-term or longer-lasting intermediate memories. The internal glycogen storage in the muscles and adipose tissue influences how intensely sucrose associated information is stored. Insulin-like signaling in octopaminergic reward neurons integrates internal energy storage into memory formation. Octopamine, in turn, suppresses the formation of long-term memory. Octopamine is not required for short-term memory, because octopamine-deficient mutants can form appetitive short-term memory for sucrose and to other nutrients depending on the internal energy status. The reduced positive reinforcing effect of sucrose at high internal glycogen levels combined with the increased stability of food-related memories due to prolonged periods of starvation could lead to increased food intake.

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

Introduction

To ensure survival, the internal energy status of an animal needs to be adjusted to the energy expenditure of the organism and the availability of external food. Increased storage of energy correlates with increased food intake in the past. Dysregulation of food intake can lead to 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, primarily in the liver and muscles. Elevated 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 its primary energy source and stores glycogen primarily in the muscles—the main 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).

In *Drosophila*, the monoamine octopamine - functionally related to noradrenalin in vertebrates - is involved in the regulation of energy homeostasis. In tyramine-β-hydroxylase (*Tβh*) mutants lacking the neurotransmitter octopamine, the glucose and trehalose concentrations in the hemolymph change less upon starvation, and the life span is extended (Damrau et al., 2017; Li et al., 2016). *Tβh* 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 responsiveness to sucrose correlates with a reduced sucrose intake in *Tβh* 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 *Tβh* mutants (Scheiner et al., 2014). In addition to their changes in simple forms of neuronal plasticity, *Tβh* 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 Tβh 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). The question arises how the internal energy supply is integrated into the formation of food related memories and feeding behavior in *Tβh* mutants.

The peptide hormone insulin regulates glycose homeostasis at the cellular level (Saltiel and Kahn, 2001). Insulin and its *Drosophila* 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 ,* 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.

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

102 there is a correlation between the perceptions of the food reward, food-related
103 memories and food intake are not clear.
104
105
106 ***The duration of hunger influences the strength and stability of memories***
107 To investigate whether changes in internal energy storage influence food-related
108 memories, we used the olfactory associative learning and memory paradigm using
109 sucrose as a food-related reinforcer (Schwaerzel et al., 2003). In the paradigm, flies
110 learn to associate an odorant with sucrose and are later tested on whether they
111 remember the association. A prerequisite for this association to be made is that the
112 animals are hungry. During the training, starved flies were exposed for 2 min to the
113 unconditioned odorant, 4-methylcyclohexanol (MCH), followed by a 2 min exposure to a
114 second odorant, 3-octanol (3-OCT), that was paired with the reward of 2 M sucrose.
115 After the training, flies were given a choice between the two odorants (Figure 1A).
116 Normally, flies prefer the rewarded odorant already 2 min after the training. To avoid a
117 bias towards one odorant, in a reciprocal experiment, the first odorant was rewarded.
118 The performance index presents the average positive association between the reward
119 and both odorants and describes whether information is learnt and remembered. To
120 ensure that observed differences in learning and memory were not due to changes in
121 odorant perception, odorant evaluation or sucrose sensitivity, different fly populations of
122 the same genotypes were tested for their odorant acuity, odorant and sucrose
123 preference (Table S1). The flies of the different genotypes sensed the odorants and
124 evaluated them as similar salient in comparison. This is important to a avoid bias where

125 flies have to choose between the two odorants after training. They also sensed sucrose.
126 We next determined whether differences in sucrose preference influence sucrose intake
127 during training. Therefore, we measured the intake of food-colored sucrose of starved
128 flies in the behavioral set up. After 2 min, we evaluated whether there was dyed food in
129 the abdomen of the fly (Table S1). Flies of both genotypes fed sucrose within 2 min and
130 there was no difference between w^{1118} and $T\beta h^{nM18}$ flies.

131 To alter the internal energy status, flies were starved either for 16 h or for 40 h
132 before the training (Figure 1). We used 3- to 5-day-old male flies to minimize differences
133 in body weight and to control for differences in food preferences. We included $T\beta h^{nM18}$
134 mutants lacking octopamine in the experiments, since they showed defects in energy
135 metabolism and sucrose reward learning (Li et al., 2016; Schwaerzel et al., 2003). Flies
136 that were starved for 16 h established a positive association between the odorant and
137 the reward. They remembered this association for 2 min, but not for 6 h or 24 h.
138 Consistent with previous results, learning and memory performance was significantly
139 improved by prolonged starvation (Colomb et al., 2009). The memory was also
140 remembered for longer, since it was still detectable 6 h after training. The $T\beta h^{nM18}$
141 mutants starved for 16 h did not show 2 min memory but emerged memory 6 h later that
142 lasted up to 24 h (Das et al., 2014; Schwaerzel et al., 2003). In contrast, $T\beta h^{nM18}$
143 mutants remembered the rewarded odorant after only 2 min following starvation
144 extended to 40 h. The memory was still detectable after 6 h and 24 h (Figure 1B). We
145 repeated similar experiments using a lower concentration of 0.15 M sucrose as
146 reinforcer (Figure 1C). In contrast to the experiments using 2 M sucrose as a reward, 16
147 h starved $T\beta h^{nM18}$ mutants form a negative association with 0.15 M sucrose directly after

148 the training, that turns into a positive association when the flies were starved longer 40
149 h. Again, prolonged starvation significantly increased learning and memory
150 performance in controls and *Tβh*^{nM18} mutants. Thus, the length of starvation and the
151 reinforcer strength influence appetitive memory strength.
152 Next, we investigated how starvation affects the stability of memory. Protein
153 synthesis-dependent long-term memory is still labile immediately after training and can
154 be blocked by 4°C cold-shock anesthesia immediately after training. After consolidation,
155 long-term memory is cold shock resistant and insensitive to a cold shock 1 h before the
156 test (Krashes and Waddell, 2008). To investigate how hunger influences the stability of
157 food-related memories, we administered cold shock anesthesia immediately after the
158 training and 1 h before the test and analyzed the effect on memory formation in
159 differently starved control and *Tβh*^{nM18} flies (Figure 2A). We reduced the time for the
160 memory test after training from 6 h to 3 h to be able to detect memory in 16 h starved
161 flies. The memory was completely abolished by a cold shock directly after training or
162 shortly before the test. Prolonging the starvation period resulted in memory that was
163 sensitive to cold-shock directly after training but not shortly before the test. In contrast,
164 16 h starved *Tβh*^{nM18} mutants developed memory that was sensitive to cold shock
165 directly after training and cold shock insensitive shortly before the test. The results
166 together with the observed memory 24 h later of *Tβh*^{nM18} after the same training (Figure
167 1B) support that *Tβh*^{nM18} mutant develop long-term memory. Longer periods of
168 starvation in *Tβh*^{nM18} mutants resulted in anesthesia-resistant long-term memory.
169

170 To further confirm that the observed memory in 16 h starved *Tβh*^{nM18} is indeed
171 long-term memory, we wanted selectively inhibit long-term memory in *Tβh*^{nM18} mutants
172 and analyze their food-related memory (Figure 2B). We analyzed memory performance
173 6 h after training, as control flies show no memory at this time point (Figure 1B, Figure
174 2B). The R15A04-Gal4 driver targets dopaminergic neurons of the PAM cluster, which
175 are specifically required for appetitive long-term memory but not short-term memory
176 (Yamagata et al., 2015). Blocking the function of these dopaminergic neurons
177 immediately after training using a temperature-sensitive *shibire* transgene (UAS-shi^{ts})
178 and a 30-min heat pulse of 31°C resulted in loss of memory in *Tβh*^{nM18} mutants (Figure
179 2B). Control flies carrying one copy of the Gal4 transgene showed no memory. Without
180 heat shock, the *Tβh*^{nM18} mutants carrying the UAS-shi^{ts}, the R15A04-Gal4 or both
181 transgenes developed memory (Figure S1). Thus, although they do not show memory
182 immediately after training, *Tβh*^{nM18} mutants that were briefly starved develop long-term
183 memory.
184 These results, together with the course of memory loss, show that lightly hungry
185 flies form a memory that lasts up to 3 h and is sensitive to anesthesia (Figure 2D). Since
186 no memory can be observed 24 h later, this indicates that it is an anesthesia-sensitive
187 intermediate memory (ITM). Prolonged starvation results in anesthesia-resistant ITM
188 (Figure 2D). In contrast, mildly starved *Tβh*^{nM18} mutants form a long-term memory that is
189 sensitive to anesthesia-sensitive and detectable 24 h after the training. Prolonged
190 starvation in *Tβh*^{nM18} mutants leads to anesthesia-resistant long-term memory (Figure
191 2D).

192 Thus, the longer the starvation lasts, the more stable the memory becomes.
193 Depending on the duration of starvation after the same training phase, the animals first
194 form an STM memory, then anesthesia-sensitive or insensitive intermediate memory.
195 Mildly starved *Tβh*^{nM18} mutants do not form a short-term memory, but a long-term
196 memory and after prolonged starvation, short-term and anesthesia resistant memory.
197
198
199 ***Octopamine is a negative regulator of memory***
200 Since the *Tβh*^{nM18} mutants lack the neurotransmitter octopamine and form LTM,
201 we next investigated whether octopamine normally suppresses LTM. To do so, we first
202 blocked the function of octopamine receptors in controls immediately after the training
203 by feeding the octopamine antagonist epinastin for 1 h and analyzed memory 5 h later
204 (Figure 3A). If the octopamine receptor function is required for long-term memory,
205 prolonged memory should also occur in control flies that normally show no memory after
206 16 hours of starvation. This was the case. Consistent with the idea that octopamine is a
207 negative regulator of long-term memory, feeding octopamine to *Tβh*^{nM18} mutants
208 immediately after training blocked long-term memory (Figure 3A). To analyze whether
209 octopamine is also able to block STM, we fed octopamine prior to training to control flies
210 (Figure 3B). A short pulse of octopamine before the training inhibits STM.
211 We were wondering whether octopamine could reduce the appetite in starved
212 animals and thereby reducing the value of the food reward. To investigate this, we fed
213 starved flies with a short octopamine pulse and assayed their sucrose intake after 3 h
214 (Figure S2). Flies fed with octopamine showed a significant reduction of sucrose intake,

but not flies fed with a similar amount of tyramine or a 10-fold increase in tyramine concentration. Therefore, it is possible that increased octopamine content influences how food is evaluated. This is consistent with a function of octopamine as a signal for food or high internal energy levels. Taken together, octopamine is a negative regulator of appetitive long-term memory and STM (Figure 3C).

Starvation influences sucrose consumption preference

Starvation reduces the internal energy storage. The reduction could lead to a reassessment of the external food cues and increased food consumption to restore the energy supplies. First, we investigated how starvation reduces the glycogen storage in whole animals (Figure 4A). In controls and *Tβh*^{nM18} mutants, starvation reduces the glycogen level. However, non-starved *Tβh*^{nM18} mutant males had significantly higher glycogen levels at baseline. After 40 h of starvation, the glycogen levels of *Tβh*^{nM18} mutants were still higher than those of controls that were starved for the amount of time. Adult *Tβh*^{nM18} mutants have a reduced sucrose intake (Li et al., 2016; Scheiner et al., 2014). The assessment of external food sources might be reflected in the choice of food. To examine whether hunger influences the evaluation of an external food stimulus, we starved flies and determined the preference for consuming sucrose over protein-enriched food using the capillary feeder assay (Figure 4B). Non-starved flies reared on standard fly food preferred sucrose over protein-enriched food, but *Tβh*^{nM18} mutants to a significantly lesser extent than controls.18 h of starvation resulted in a decreased sucrose preference in controls and *Tβh*^{nM18} mutants, but to a significantly

238 lower level in *Tβh*^{nM18} mutants. Extending the starvation period to 40 h resulted in a
239 similar sucrose consumption in control and mutant flies.
240 To further investigate whether *Tβh*^{nM18} mutants integrate their internal glycogen
241 storage into their feeding preference, we deprived male flies of sucrose- or protein-
242 enriched food by feeding them 5% sucrose, 5% yeast or standard fly food for 3 days.
243 The food preference was analyzed after deprivation (Figure 4C). As controls, we
244 included mated females in our analysis as they have different nutritional requirements
245 due to their mating status (Ribeiro and Dickson, 2010; Vargas et al., 2010). Male flies
246 fed standard food for 3 days strongly preferred sucrose over protein-enriched food, with
247 *Tβh*^{nM18} mutants showing a significant reduction. Yeast-deprived and sucrose-deprived
248 *Tβh*^{nM18} mutant showed a significantly reduced preference for sucrose. In mated
249 females, no differences were observed in food preference between controls and the
250 *Tβh*^{nM18} mutant. In conclusion, the reduced preference for sucrose consumption
251 correlates in *Tβh*^{nM18} with increased glycogen levels. Furthermore, *Tβh*^{nM18} mutants can
252 sense the reduction of specific internal energy stores and change their food preferences
253 accordingly.
254
255
256 ***Internal glycogen storage influences sucrose-related memories***
257 Since memory performance increases when the internal energy supply is
258 reduced by starvation, we wanted to investigate whether the internal energy supply
259 influences memory performance. In *Drosophila*, glycogen is mainly found in the fat
260 bodies - the major energy storage organ - and the muscles, a major site of energy

261 expenditure (Wigglesworth, 1949). Glycogen synthase and glycogen phosphorylase
262 control glycogen content in the body (Figure 5A). Knockdown of glycogen synthase
263 using *GlyS*^{HMS01279}*-RNAi* efficiently reduced glycogen levels in larvae, while knockdown
264 of glycogen phosphorylase using *GlyP*^{HMS00032}*-RNAi* efficiently increased glycogen
265 levels (Yamada et al., 2018). We altered glycogen levels in the muscles using the *mef2*-
266 Gal4 driver (Ranganayakulu et al., 1998) and/or the fat bodies using the FB-Gal4 driver
267 (Gronke et al., 2003) and analyzed the effect of altered glycogen levels on short-term
268 memory (Figure 5). We used PAS staining to confirm the down- or up-regulation of
269 glycogen in larval muscles or fat bodies, respectively (Figure 5) (Yamada et al., 2018).
270 In addition, we quantified the glycogen levels in the bodies of adult flies (Figure S3). As
271 we could not always detect differences in glycogen content in whole flies, we also
272 analyzed the glycogen level in the muscle-rich thorax or fat body-rich abdomen (Figure
273 S3). The knockdown of glycogen phosphorylase increased glycogen levels and the
274 knockdown of glycogen synthase reduced glycogen levels.
275 Increasing glycogen levels in the muscles had no effect on the short-term
276 memory of the 16 h starved flies, but decreasing glycogen levels significantly improved
277 memory performance (Figure 5B). Increasing or decreasing glycogen levels in the fat
278 bodies had no effect on memory performance (Figure 5C). When the muscles and fat
279 bodies glycogen levels were significantly increased , flies showed a reduced memory to
280 odorants paired with sucrose. An increase in memory performance was observed when
281 glycogen levels were significantly reduced in both tissues (Figure 5D). Recently, it has
282 been shown that energy metabolism in mushroom bodies is important for the formation
283 of LTM (Placais et al., 2017). To analyze the function of the mushroom bodies, the

284 expression of *GlyS*^{HMS01279}*-RNAi* under the control of the Mef2-Gal4 driver was
285 repressed in the mushroom bodies using the mb247-Gal80 driver (Krashes et al., 2007).
286 The memory was still increased (Figure S4). Conversely, the reduction of *GlyS*^{HMS01279}-
287 *RNAi* using the mb247-Gal4 driver targeting the mushroom bodies(Zars et al., 2000) did
288 not alter short-term memory (Figure S4). Thus, low levels of glycogen in the muscles
289 after starvation positively influence appetitive short-term memory, whereas high levels
290 of glycogen in the muscles and fat body decrease reduce short-term memory.
291
292
293 ***Internal glycogen levels reduce sucrose-related memories in Tβh mutants***
294 The increased glycogen levels in *Tβh*^{nM18} mutants could be responsible for the
295 reduced STM. To determine whether the reduction in glycogen levels in the muscles or
296 fat bodies restores STM, we expressed *GlyS*^{HMS01279}*-RNAi* under the control of the
297 *mef2*-Gal4 or *FB*-Gal4 driver in *Tβh*^{nM18} mutants and analyzed STM (Figure 6A). Neither
298 the reduction in the muscles nor the reduction in fat bodies of *Tβh*^{nM18} mutants improved
299 STM. Only when glycogen was reduced in both tissues did the *Tβh*^{nM18} mutants show
300 improved STM compared to controls. Thus, *Tβh*^{nM18} mutant flies can form appetitive
301 STM similar to controls when the energy storage is sufficiently reduced. Next, we
302 analyzed whether male *Tβh*^{nM18} mutants can form STM to other nutrients than
303 carbohydrates by using a protein-enriched diet in the form of 5% yeast as a positive
304 reinforcer (Figure 6B). To determine whether there is a difference in the evaluation of
305 protein as a food source between male flies and *Tβh*^{nM18} mutants, we measured yeast
306 intake in non-starved and starved flies (Figure S5). Non-starved *Tβh*^{nM18} mutant males

307 have a significantly higher protein intake than controls. However, after 16 h of
308 starvation, the protein intake was comparable to that of control flies. When 5% yeast
309 was used as a food reward, male *Tβh*^{nM18} mutants showed comparable STM levels
310 controls (Figure 6B).

311 To further analyze the influence of the internal energy status on memory
312 performance, we took advantage of the observation that female flies have different
313 nutritional requirements depending on their mating status (Ribeiro and Dickson, 2010;
314 Vargas et al., 2010). Virgin females showed a higher consumption preference for
315 sucrose than mated females when given the choice between yeast and sucrose (Figure
316 4C), demonstrating a difference in internal energy demands and suggesting a difference
317 in valence for different diets. We tested virgin and mated female flies of controls and
318 *Tβh*^{nM18} mutants for STM with 2 M sucrose as reinforcer (Figure 6C). Virgin females
319 remembered the odorant paired with sucrose significantly better than mated females.
320 This was also true for *Tβh*^{nM18} mutant females. Thus, the internal energy status
321 influences how the reward is evaluated in learning and memory.

322

323

324 ***Insulin-like signaling in octopaminergic neurons regulates STM***

325 How is the internal energy status integrated into the reward system? Insulin-like
326 signaling regulates glycogen levels in invertebrates and vertebrates (Semaniuk et al.,
327 2021). Loss of the insulin receptor results in more circulating sugar but not increased
328 glycogen levels (Shingleton et al., 2005). Thus, the insulin receptor might be a good
329 candidate that links the internal energy level to reinforcing neurons. First, we analyzed

330 whether the insulin receptor is expressed in octopaminergic reward neurons in the brain
331 (Figure 7A). To detect the expression of an activated insulin receptor, we used an
332 insulin antibody that recognizes the phosphorylated form of the insulin receptor (InR).
333 This region is highly conserved between humans and flies. First, we tested whether the
334 antibody indeed recognized the activated insulin receptor. Therefore, we overexpressed
335 the activated insulin receptor using the UAS-InR.A1325D transgene under the control of
336 the *dTdc2*-Gal4 driver (Figure S6). The InR.A1325D protein variant mimics the human
337 V938D protein variant, which is constitutively active,(Longo et al., 1992) and the *dTdc2*-
338 Gal4 driver targets octopaminergic reward neurons (Busch et al., 2009). The expression
339 of activated InR resulted in increased immunoreactivity (Figure S6). The InR antibody
340 therefore detects the activated InR. In the brain, activated InR is broadly expressed in a
341 punctate manner and more specifically in the soma of *dTdc2*-Gal4-targeted neurons
342 (Figure 7A). To uncouple energy sensing via the insulin receptor in reward neurons, we
343 expressed UAS-InR.K1409A under the control of the *dTdc2*-Gal4 driver. The transgene
344 encodes a dominant negative variant of the InR (InR^{DN}) and disrupts with InR function
345 (Wu et al., 2005). In animals starved for 16 h, the expression of InR^{DN} under the control
346 of the *dTdc2*-Gal4 driver did not alter STM, but uncoupling of InR-dependent energy
347 sensing in reward neurons in $T\beta h^{nM18}$ restored STM to normal levels (Figure 7B and C).
348 To investigate whether the improved STM also affects the emerging LTM of the
349 $T\beta h^{nM18}$ mutants, we performed cold shock experiments in $T\beta h^{nM18}$ mutants in which
350 InR^{DN} was expressed in octopaminergic reward neurons (Figure 7D). The 3 h memory
351 in controls is cold shock sensitive, but the memory in $T\beta h^{nM18}$ mutants is cold shock
352 insensitive, supporting the idea that the mutants formed anesthesia-resistant memory.

353 Given that octopamine is a negative regulator of memory and that it is still absent in
354 *Tβh*^{nM18} mutants with blocked insulin signaling on octopaminergic reward neurons, it is
355 not surprising that ARM is still observed in the mutants.
356 The results can be described with the following model (Figure 7E). Insulin receptor
357 signaling on reward neurons integrated the internal energy status into memory
358 formation. When energy levels are high enough due to increased glycogen levels or
359 food intake, the insulin pathway suppresses the formation of STM. In return, the system
360 releases octopamine to suppress the formation of food-related LTM. In mildly starved
361 *Tβh*^{nM18} mutants, the integration of the internal energy status into the reward neurons is
362 still intact. In addition, the level of the internal energy supply combined with the loss of
363 octopamine is still high enough to support the formation of protein biosynthesis-
364 dependent LTM. In *Tβh*^{nM18} mutants, two functionally different memory traces are
365 formed after training, an insulin receptor-sensitive appetitive short-term memory and a
366 long-term memory. In starved control animals, the glycogen levels are more severely
367 depleted and octopamine is still present. This supports the formation of STM and an
368 intermediate form of memory (ITM).
369
370
371 ***Increased starvation results in overconsumption in Tβh^{nM18} mutants***
372 To analyze whether the increased glycogen levels and the reduced sucrose
373 reward of *Tβh*^{nM18} mutants correlate with food consumption, the energy requirements in
374 flies under different starvation conditions were analyzed by measuring food intake
375 (Figure 8). Control flies starved for shorter or longer periods consumed similar amounts

376 of 5% sucrose. In contrast, *Tβh*^{nM18} mutants starved for 16 h consumed significantly less
377 sucrose than controls. Longer-starved mutants consumed approximately 34% more
378 sucrose (Figure 8B). After longer starvation, the glycogen levels in *Tβh*^{nM18} mutants
379 were still higher than those in controls (Figure 4A). Therefore, they overconsumed
380 sucrose. The overconsumption was independent of the diet, as they showed similar
381 overconsumption when fed with a solution containing 5% yeast and 5% sucrose (Figure
382 8B). To investigate whether the internal energy status is also integrated into the feeding
383 behavior by the octopaminergic reward neurons, we blocked insulin signaling in reward
384 neurons in 16 h-starved *Tβh*^{nM18} mutants and analyzed sucrose consumption (Figure
385 8C). The reduced sucrose consumption of *Tβh*^{nM18} mutants was significantly improved
386 compared to controls. Thus, the octopaminergic reward system integrates via insulin
387 receptor signaling the internal energy level into the regulation of food consumption. The
388 mechanism is still intact in *Tβh*^{nM18} mutants.
389
390
391 **Discussion**
392 Depending on the duration of starvation, flies develop STM and an anesthesia-
393 sensitive intermediate type of a food-related memory. The extending the hunger period,
394 memory formation shifts to an anesthesia-resistant intermediate memory. Presentation
395 of the reward leads to the release of octopamine and this the suppression of LTM. The
396 shifting of different memories phases in response to different energy supplies has been
397 also described for memories that are formed of aversive non-food-related memories.
398 Depending on the reduction of the internal energy supply, flies initially form protein-

399 dependent aversive olfactory LTM and later ARM, a protein synthesis-independent form
400 of memory (Wu et al., 2005). In times of energy shortage, the organism exchanges the
401 costly protein biosynthesis-dependent LTM for the “less costly” protein biosynthesis-
402 independent ARM (Mery and Kawecki, 2005). The effect of memory transition can also
403 be observed at the level of appetitive LTM. In *Tβh*^{nM18} mutants, mild starvation along
404 with elevated glycogen levels leads to the formation of LTM and prolonged starvation
405 leads to the formation of anesthesia-resistant LTM. Furthermore, *Tβh*^{nM18} mutants form
406 only anesthesia-sensitive aversive memory (Wu et al., 2013). In contrast, *Tβh*^{nM18}
407 mutants form cold shock-resistant LTM. Therefore, Tβh^{nM18} mutants are not defective in
408 appetitive anesthesia-resistant types of memories rather the internal energy status
409 defines how quickly anesthesia-resistant appetitive memories forms.
410
411
412 ***Octopamine gates memory formation***
413 What function does octopamine play in memory formation? Octopamine is a
414 negative regulator of LTM, as feeding octopamine receptor antagonists to control flies
415 results in memory, and emerging memory in *Tβh*^{nM18} mutants is suppressed by
416 octopamine after conditioning. Octopamine acts in the formation of LTM upstream of
417 dopaminergic neurons, as blocking neurotransmitter release of dopaminergic LTM-
418 mediating neurons immediately after conditioning blocks memory formation in *Tβh*^{nM18}
419 mutants. The results are consistent with the model describing that dopamine release is
420 not required during the pairing of the conditioned stimulus and the reward rather than as

421 gain control after the training (Adel and Griffith, 2021). The inhibitory function of
422 octopamine controls the gain.
423 During the acquisition of appetitive STM, octopamine acts upstream of
424 dopaminergic neurons M (Burke et al., 2012; Liu et al., 2012). However, at first glance,
425 the function of octopamine appears to differ in STM and LTM. Loss of octopamine in
426 *Tβh*^{nM18} mutants results in loss of STM, indicating that octopamine is required as a
427 positive regulator for appetitive STM (Schwaerzel et al., 2003). The loss of STM in
428 *Tβh*^{nM18} mutants has been attributed to the loss of labile STM which forms in response
429 to the sweetness of sucrose, but not due to defects in stable LTM, which forms in
430 response to the caloric value of sugar (Burke et al., 2012; Fujita and Tanimura, 2011).
431 Sweetness is sensed by taste receptor that change their sensitivity during hunger
432 (Inagaki et al., 2012; Marella et al., 2012) and thereby influencing the response to the
433 external reinforcer. The responsiveness could be regulated by the octopaminergic
434 system, as octopaminergic neurons regulate the sucrose sensitivity of gustatory
435 neurons in *Drosophila* (Youn et al., 2018). However, the reduced sensitivity of gustatory
436 receptors cannot explain why octopamine-deficient mutants do not form STM. The
437 *Tβh*^{nM18} mutants form aversive memory to lower concentrations of sucrose and the
438 differences in sucrose preference of *Tβh*^{nM18} do not translate to differences in food
439 intake during training.
440 The internal energy status influences how the external source of sucrose is
441 evaluated rather than octopamine being directly involved in the acquisition of appetitive
442 STM. Consistently, *Tβh*^{nM18} mutants can exhibit STM after prolonged periods of
443 starvation. In mildly starved *Tβh*^{nM18} flies that do not show STM, uncoupling of energy

444 sensing in reward neurons by blocking insulin receptor function leads to STM.
445 Furthermore, *Tβh*^{nM18} mutant females with different energy requirements form appetitive
446 STM, and a short pulse of octopamine before conditioning can even block STM. Thus,
447 octopamine is not required in the acquisition of appetitive STM but rather suppresses
448 LTM when enough energy is available. The function of octopamine as a suppressor of
449 different memory types allows for selecting the memory type depending the internal and
450 external information. This gating function in memory formation can also be observed in
451 other behaviors. For example, octopamine regulates the decision to approach or turn
452 away from a food source (Classen and Scholz, 2018). Taken together, the
453 octopaminergic system integrates internal energy demands and the evaluation of
454 external food supplies.
455
456
457 ***The internal energy level of the animal influences memory formation***
458 At the cellular level, insulin receptor signaling regulates energy metabolism
459 (Chatterjee and Perrimon, 2021). The broad expression pattern of the insulin receptor in
460 the *Drosophila* brain (Figure 7) indicates that every cell in the brain needs to regulate its
461 own energy homeostasis. However, the fly's entire energy resources and the evaluation
462 of the external food supply must also be integrated. The reward system evaluates the
463 external food supply. In mice, insulin receptor signaling in dopaminergic neurons
464 mediates food reward (Konner et al., 2011). Consistently, insulin receptor function in
465 octopaminergic reward neurons regulates the rewarding properties of sucrose in
466 appetitive STM memory (Figure 6A) and during food intake (Figure 7C).

467 The systemic metabolic rate is regulated by fat bodies. For example, after food
468 intake, the fat body secretes Unpaired 2 (Upd2), which in turn regulates the secretion of
469 Drosophila insulin-like peptides via GABAergic neurons (Rajan and Perrimon, 2012).
470 We show that the reduction of glycogen in the fat bodies is not sufficient to change
471 appetitive STM, but reduction in the muscles or both tissues is. The results support a
472 feedback mechanism between the assessment of glycogen levels in the muscles and
473 the brain. In addition, the muscles and the fat bodies communicate about the energy
474 content of both structures, and this information is signaled back to the brain. Such long-
475 range signals from the muscles exist. Skeletal muscles are secretory organs (Pedersen
476 and Febbraio, 2012). For example, the muscle-secreted Amyrel amylase reduces the
477 age-related accumulation of polyubiquitinated proteins in the brain (Rai et al., 2021).
478 Regardless of how the glycogen levels of both tissues are communicated to the brain,
479 they influence how the reinforcer is evaluated. The feedback between the energy levels
480 in both structures is still intact in octopamine-deficient mutants, as $T\beta h^{nM18}$ mutants still
481 form STM when the energy storage is sufficiently reduced.
482
483
484 ***Are food-related memories and internal glycogen levels predictive of food intake?***
485 The feeding behavior in hungry animals is regulated by various neural systems,
486 including networks that receive and process sensory information and networks that
487 assign reward properties to food (Berridge, 2009). Blocking insulin receptor function in
488 dopaminergic neurons results in increased food intake and weight gain in mice (Konner
489 et al., 2011). Similarly, blocking insulin receptor function in octopaminergic reward

490 neurons in flies with increased glycogen levels and reduced food intake increases food
491 intake. Thus, the octopaminergic system assigns reward properties to food. However,
492 blocking insulin receptor signaling on reward neurons does not result in extensive
493 overconsumption, supporting that the regulation of the food quantity is still intact and is
494 not due to insulin receptor resistance in reward neurons. Increased levels of internal
495 energy, e.g., glycogen, reduced the reward properties of food, resulting in a decreased
496 positive association of food-related memories (Figure 5D; Figure 7A) and decreased
497 food consumption (Figure 8). This is intended to prevent increased food intake when
498 there is excess food. Insulin resistance could contribute to weight gain, because in
499 addition to high internal levels of glycogen, the flies show normal food intake. However,
500 the overconsumption only becomes visible after a prolonged period of hunger, which
501 correlates with the emerging stable appetitive long-term memory. This suggests that the
502 stability of appetitive memory could lead to the re-evaluation of food and could trigger
503 the overconsumption of food.
504
505 In summary, the octopaminergic neurotransmitter system integrates several
506 aspects that influence the regulation of food memories. Octopamine modulates the
507 sensory perception of the conditioned stimulus and the sensory perception of the
508 reward. Here, we show that the evaluation of food reward in the context of the energy
509 storage is integrated by the octopaminergic system and influences the stability of food-
510 related memories. The function of octopamine as a negative regulator of various forms
511 of memories allows for selective regulation of food related behaviors such as food
512 intake, and the loss of this regulation could also promote dysregulation of food intake.

513 The close relationship between the octopaminergic neurotransmitter and the
514 noradrenergic neurotransmitter system suggests that the integrator function may be
515 conserved.
516
517
518 **Material and methods**
519
520 ***Drosophila melanogaster***
521 Flies were raised on an ethanol-free standard cornmeal-molasses food at 25°C and
522 60% relative humidity on a 12 h/12 h day-night cycle. The following lines were used:
523 *w*^{1118}; *w*^{1118}*,Tβh*^{nM18}(Monastirioti et al., 1996); *UAS-GlyS*^{HMS01279}*-RNAi* (BDSC #34930);
524 *UAS-GlyP*^{HMS00032}*-RNAi* (BDSC #33634)*;* *w*^{1118}; *UAS-shi*^{ts}(Kitamoto, 2001); w^{1118};
525 P{UAS-InR. K1409A} (BDSC#8253); *w*^{1118}*;FB-Gal4* (a generous gift from the Partridge
526 Lab); *mef2-Gal4* (Ranganayakulu et al., 1998); R15A04-Gal4 (BDSC #48671); *Tdc2-*
527 *Gal4* (Cole et al., 2005). All lines were backcrossed to *w*^{1118} (Scholz Lab) for at least five
528 generations to isogenize the genetic background. For behavioral experiments, three- to
529 five-day-old male flies were used, if not otherwise indicated. Male flies of the F1
530 generation carrying one copy of the transgene were used as controls for behavioral
531 experiments. Animal studies using *Drosophila melanogaster* were conducted in
532 agreement with the regulations of the DFG and the Land North Rhine-Westphalia.
533 Other ethics approval and informed consent statement are not applicable for research
534 using *Drosophila.*
535
536 ***Olfactory learning and memory***

537 Associative olfactory learning and memory was trained and tested with a modified
538 version of the Tully and Quinn olfactory conditioning apparatus (Schwaerzel et al., 2003;
539 Tully and Quinn, 1985). Approximately 70 one- to two-day-old male flies were collected
540 with CO_2 anesthesia and were kept for 2 days at 25°C to recover from CO_2 sedation.
541 Briefly, three- to five-day-old male flies were starved for 16 h or 40 h in vials with water-
542 soaked filter paper at the bottom. Flies were transferred to training tubes and exposed
543 to the first odorant for 2 min, either 3-octanol (3-OCT diluted 1:80 in paraffin oil) or 4-
544 methylcyclohexanol (MCH diluted 1:100 in paraffin oil). After that, they were transferred
545 to a second tube and exposed to the second odorant in the presence of filter paper
546 soaked with either 0.15 M sucrose, 2 M sucrose or 5% yeast. To analyze the abilities of
547 the flies to learn and remember the odorant paired with the reward, 2 min, 3 h, 6 h or 24
548 h after training, flies were given the choice between odorant 1 (CS+) and odorant 2 (CS-
549) for 2 min. The performance index (PI) was calculated as PI = (# (CS+) + # (CS-) / (total
550 # flies), where CS+ indicates the odorant associated with the appetitive reinforcer and
551 CS- indicates the odorant that was not associated with the reinforcer. To exclude non-
552 associative effects, each “n” consists of a reciprocally trained, independent group of
553 naïve flies. For pharmacological experiments, flies were fed water, 3 mM OA or 2 M
554 sucrose before training or with 3 mM OA or 3 mM epinastine between training and
555 testing. The assay was performed at RT with 60% relative humidity. For cold shock
556 experiments, flies in vials were placed in ice-cold water for 2 min.
557 Odorant acuity and odorant balance were tested with naïve flies that were not used later
558 for learning and memory experiments. For testing, the flies were placed into the Tully
559 Quinn setup. They chose between the odorant- or the solvent paraffin oil-containing

560 sides or between both odorants for 2 min. The sucrose and yeast preference was
561 determined by analyzing whether they preferred the side with a sucrose or yeast-
562 soaked filter paper over a water-soaked filter paper. To determine sucrose intake, flies
563 were fed with a food-safe colored sucrose solution and food intake was analyzed by the
564 color in the abdomen (Table S1).
565
566 ***Food intake***
567 To analyze the consumption of nutrients in flies, the capillary feeder (CaFe) assay was
568 performed (Diegelmann et al., 2017; (Ja et al., 2007)). Briefly, eight three- to five-day-
569 old male or female flies that were either non-starved or starved for 18 h or 40 h had
570 access to 4 capillaries filled with either 5% sucrose or 5% yeast or a mixture of both for
571 24 h at 25°C and 60% humidity. The solution was colored with red food dye. During
572 starvation, flies were kept in vials with wet filter paper at 25°C and 60% humidity. The
573 amount of consumed solution was determined using an electronic caliper. To account
574 for evaporation, the average evaporation of three CAFE setups without flies was
575 measured and used to normalize the average food intake per fly. To normalize food
576 intake to body weight, at least 5 times 100 male flies per genotype and condition were
577 weighed, and the average body weight of a single fly was determined. The total
578 consumption per µg fly was calculated by dividing the total consumption per fly by the
579 mean weight of a fly. N indicates the number of tested groups.
580
581 ***Glycogen content***

582 Whole body glycogen levels were determined with the Glucose (HK) Assay Kit (Sigma
583 Aldrich, #GAHK20-1KT) according to the protocol of (Tennessen et al., 2014). A group
584 of five male flies that were either sated or starved for 16 h or 40 h were homogenized in
585 100 µl ice-cold 1x PBS. To reduce enzymatic degradation, proteins and enzymes were
586 heat inactivated at 70°C for 10 min. The supernatant was removed and diluted 1:3 with
587 1x PBS. Twenty microliters of the samples were either added to 20 µl of 1x PBS or
588 PBS/Amyloglucosidase mix and incubated at 37°C for 60 min. Then, 100 µl of HK-
589 reagent was added to the sample or glucose standard and incubated for 15 min at RT.
590 Absorbance was measured at 340 nm. The glycogen content was calculated by
591 subtracting the total glucose concentration from 1x PBS-treated samples from the total
592 glucose of amyloglucosidase-treated samples.
593
594 ***Periodic acid staining***
595 To visualize glycogen levels in the fat bodies and muscle tissue of larvae and adult
596 *Drosophila*, PAS staining was performed after Yamada et al., 2018 (Yamada et al.,
597 2018) with a slight modification. The samples were fixed in 3.5% formaldehyde for 20
598 min and washed two times for 5 min with 1% BSA/PBS. Periodic acid solution was
599 added for 5 min, followed by two washes of 5 min with 1% BSA/PBS. Schiff's Reagent
600 was added for 5 min, followed by two washes of 5 min with 1% BSA/PBS. Tissue was
601 stored in 50% glycerol.
602
603 ***Immunohistochemistry***

604 For immunohistochemistry, antibodies raised in rabbits against the activated insulin-like
605 receptor (cell signaling technology #3021) were used at a dilution of 1:50 in 5% normal
606 goat serum in PBS with 0.1% Triton and incubated for two days. The brains were
607 washed with PBS with 0.3% Triton.
608
609 **Quantification and statistical analysis**
610 Food intake was displayed as the mean ± s.e.m. For learning and memory experiments,
611 the data were displayed as boxplot ± minimum (Q1–1.5*IQR) and maximum (Q1 +
612 1.5*IQR). We used the Shapiro-Wilk test (significance level $P < 0.05$) followed by a QQ-
613 Plot chart analysis to determine whether the data were normal distributed. For normal
614 distributed data, we used the Student's t test to compare differences between two
615 groups and the one-way ANOVA with Tukey's post hoc HSD test for differences
616 between more than two groups. For nonparametric data, we used the Mann-Whitney U
617 test for differences between two groups and for more than two groups the Kruskal-
618 Wallis test with post hoc Duenn analysis and Bonferroni correction. The nonparametric
619 one-sample sign test was used to analyze whether behavior was not based on random
620 choice and differed from zero ($P < 0.05$). The statistical data analysis was performed
621 using statskingdom (https://www.statskingdom.com).Boxplots were generated with
622 Microsoft Excel 2016 and GIMP 2.10.12.
623
624 **Data availability**
625 All data related to figures are included in the supplement Table S2.
626

Acknowledgments

We thank the Linda Partridge lab and the Bloomington *Drosophila* Stock Center for providing *Drosophila* fly lines. H.S. was supported by SFB1340, DFG-Scho656-10-1 and the Hetzler award.

Author contributions

H.S. initiated the project. M.B., H.S. and K.D. designed the experiments. M.B., K.A., K. D., M.F. and T.E.K. performed the experiments and, together with H.S., analyzed the data. M.B, M.F. 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.

641 **Figure legends**

642

643 **Figure 1. Starvation influences the strength of memory**

644 **A**, Appetitive olfactory learning and memory paradigm. **B**, Appetitive 2 min STM, 6 h
645 and 24 h memory of w^{1118} or $T\beta h^{nM18}$ that were starved either for 16 h (white bars) or 40
646 h (dark gray bars) before the training. 2 M sucrose was used as reward. **C**, 0.15 M
647 sucrose was used as reinforcer. Prolonged starvation increases memory performance.
648 Prolonged starvation from 16 h to 40 h leads to the formation of STM in $T\beta h^{nM18}$.
649 Independent of the duration of starvation, 6 h after training, memory appeared in the
650 mutants. **C,** $T\beta h^{nM18}$ mutants starved for 16 h developed an aversive STM memory to
651 0.15 M sucrose. After 40 h of starvation, the mutants developed an appetitive STM.
652 Numbers below box plots indicate pairs of reciprocally trained independent groups of
653 male flies. The letter "a" marks a significant difference from random choice as
654 determined by a one-sample sign test ($P < 0.05$). The Student's t tests were used to
655 determine differences between two groups. For differences between more than two
656 groups one-way ANOVA with Tukey's post hoc HSD test was used. ($P^* < 0.05$; $P^{**} <$
657 0.01; $P^{***} < 0.001$).

658

659

660 **Figure 2. Starvation influences the type of memory**

661 **A**, Appetitive STM training with 2 M sucrose and cold shock directly or 2 h after training.
662 Mildly starved control flies exhibit an appetitive memory sensitive to cold shock 3 h after
663 training. Severely starved control flies develop a memory that is initially sensitive to cold

664 shock, but becomes insensitive after 2 h. This phenotype is shared with mildly starved
665 *Tβh*^{nM18} mutants. Prolonged starvation in the mutants shifts memory to cold shock
666 insensitive memory. **B**, To block neuronal activity and the formation of LTM, a 30-min
667 heat shift was applied immediately after training to flies expressing a temperature-
668 sensitive *shibire* transgene under the control of the R1504-Gal4 driver. The block results
669 in *Tβh*^{nM18} mutants losing LTM. Numbers below box plots indicate pairs of reciprocally
670 trained independent groups of male flies. The letter "a" marks a significant difference
671 from random choice as determined by a one-sample sign test ($P < 0.05$). One-way
672 ANOVA with Tukey's post hoc HSD test or for data in **A**, and Kruskal-Wallis followed by
673 post-hoc Dunn's test and Bonferroni correction was used to determine differences in **B**.
674 ($P^* < 0.05$; $P^{**} < 0.01$). **C**, Model summarizing memory performance of control and
675 mutant flies, that were either starved for 16 h (blue line) or 40 h (orange line). The dots
676 present the average of the data presented in Figure 1 and Figure 2. Anesthesia
677 sensitive memory (ASM), anesthesia resistant memory (ARM), intermediate memory
678 (ITM) and long-term memory (LTM).
679
680
681 **Figure 3. Octopamine suppresses memory**
682 **A**, Feeding 3 mM of the octopamine receptor antagonist epinastine for 1 h after training
683 resulted in memory 6 h later in *w*^{1118} flies. Feeding 3 mM octopamine for 6 h after
684 training suppresses LTM in *Tβh*^{nM18}. **B**, A 3 mM octopamine feeding pulse 30 min
685 before training inhibits STM in *Tβh*^{nM18} mutants. Controls were water-fed. **A, B,** Flies
686 were starved for 16 h and 2 M sucrose was used as reinforcer. The letter "a" marks a

687 significant difference from random choice as determined by a one-sample sign test ($P <$
688 0.05). Student's t tests were used to determine differences between two groups. ($P^* <$
689 0.05). Numbers below box plots indicate pairs of reciprocally trained independent
690 groups of male flies. **C**, Model for memory suppression.
691
692
693 **Figure 4. Elevated glycogen levels correlate with reduced sucrose preference**
694 **A**, Analysis of whole-body glycogen levels in w^{1118} and $T\beta h^{nm18}$ flies. In $T\beta h^{nM18}$ flies,
695 glycogen content is significantly higher than in w^{1118} flies under similar starvation
696 conditions. N = 3 groups of 5 male flies. **B**, Flies were starved 18 h or 40 h before food
697 intake was measured for 24 h. Flies chose between 5% sucrose and 5% yeast. The
698 preference was determined. All flies showed a significant preference for sucrose
699 consumption. Starvation reduced the preference. $T\beta h^{nm18}$ showed a significantly
700 reduced preference for sucrose in comparison to control flies, but not after 40 h
701 starvation. N = 20 - 26 groups of eight flies. **C**, Feeding male flies for 3 days on standard
702 fly food (food), 5% sucrose or 5% yeast resulted in control flies preferring to consume
703 sucrose. Male $T\beta h^{nm18}$ mutants fed normal food, sucrose and yeast showed a significant
704 reduction in sucrose preference. Female flies of controls and mutants did not differ in
705 their preferences. N = 14 - 28 groups of eight flies. To determine differences between
706 two groups, the Mann-Whitney U test was used. $P^* < 0.05$; $P^{**} < 0.01$, $P^{***} < 0.001$.
707
708
709 **Figure 5. Carbohydrate storage influences appetitive STM**

710 **A**, Schemata of glycogen synthesis. The expression of *GlyP-RNAi* reduced glycogen
711 phosphorylase and increased glycogen levels, whereas *GlyS-RNAi* reduced glycogen
712 synthase and decreased glycogen levels in target tissues (Figure S3). **B** - **D**, PAS was
713 used to visualize glycogen levels in larval muscle or fat bodies. **B**, Increases in glycogen
714 in the muscles have no effect on STM, whereas reduced muscle glycogen increases
715 appetitive STM. **C**, Increased or decreased glycogen levels in the fat bodies did not
716 interfere with STM. **D**, A combined increase in glycogen in muscles and fat bodies
717 reduced STM, and a decrease in glycogen increased STM. Flies were starved for 16 h
718 and 2 M sucrose was used as reinforcer. Numbers below box plots indicate one pair of
719 reciprocally trained independent fly groups. The letter "a" marks a significant difference
720 from random choice as determined by a one-sample sign test ($P < 0.05$). The one-way
721 ANOVA with post hoc Tukey's HSD was used to determine differences between three
722 groups ($P^* < 0.05$; $P^{**} < 0.01$).
723
724
725 **Figure 6. Reducing glycogen in *Tβh^{nM18}* improves appetitive STM**
726 **A**, Decreasing glycogen concentration using UAS-*GlyS^{RNAi}* in the muscles or fat bodies
727 in *Tβh^{nM18}* mutants did not improve STM, but decreasing glycogen in both tissues
728 improved STM to control levels. Flies were starved for 16 h and 2 M sucrose was used
729 as reinforcer. **B**, 16 h starved *w^{1118}* and *Tβh^{nm18}* flies formed similar levels of appetitive
730 STM when 5% yeast was used as a reinforcer. **C**, 16 h starved virgin females of w1118
731 and *Tβh^{nm18}* displayed STM, whereas mated females of both genotypes did not.
732 Differences from random choice were determined using a one-sample sign test and

733 marked with the letter “a” ($P < 0.05$). Differences between two groups were determined
734 using Student’s t tests, and differences among four groups were determined with one-
735 way ANOVA with Tukey’s HSD post hoc test. $P^* < 0.05$; $P^{**} < 0.01$. Numbers below box
736 plots indicate one pair of reciprocally trained independent fly groups.
737
738
739 **Figure 7. Insulin signaling in reward neurons regulates STM**
740 **A**, The activated form of the InR is expressed in punctuate manner throughout the brain
741 (in magenta) and is also detected in octopaminergic reward neurons visualized by using
742 the UAS-mCD8::GFP transgene under the control of the *Tdc2*-Gal4 driver (in green). **B**,
743 Blocking InR signaling in *Tdc2*-Gal4-targeted octopaminergic neurons does not change
744 appetitive STM in 16 h starved flies using 2 M as reinforcer. **C**, Blocking InR signaling in
745 *Tdc2*-Gal4-targeted octopaminergic neurons in $T\beta h^{nM18}$ mutants restored STM to control
746 levels. **D**, A cold shock did not disrupt emerging memory in $T\beta h^{nM18}$ mutants with
747 blocked InR under the control of the Tdc2-Gal4 driver. Student’s t test was used to
748 determine differences between two groups, and one-way ANOVA with Tukey’s post hoc
749 HSD test to determine differences between three or more groups. n.s. is not significant;
750 $P^* < 0.05$; $P^{**} < 0.01$, $P^{***} < 0.01$. The letter “a” marks a significant difference from
751 random choice as determined by a one-sample sign test ($P < 0.05$). **E**, Model of
752 memory regulation in $T\beta h^{nM18}$ mutants. Long-term memory (LTM), intermediate-term
753 memory (ITM).
754
755

756 **Figure 8. Prolonged starvation results in rebound sucrose intake in**

757 **hyperglycemic *Tβh*^{nM18} mutants**

758 **A**, Capillary feeder assay used to determine food intake. **B**, Flies were starved 16 h or

759 40 h before 24 h food intake was measured. After 16 h of starvation, *Tβh*^{nM18} mutants

760 significantly consumed less 5% sucrose or 5% sucrose with 5% yeast. After 40 h of

761 starvation, *Tβh*^{nM18} mutants significantly consumed more sucrose and sucrose with

762 yeast. N = 20 - 26 groups of eight flies. **C**, Blocking InR signaling in *Tdc2*-Gal4-targeted

763 octopaminergic neurons in *Tβh*^{nM18} mutants significantly increased 5% sucrose

764 consumption. N = 20 - 28 groups of eight flies. To determine differences between two

765 groups, Student's t test was used, and to determine differences between three or more

766 groups, one-way ANOVA with post hoc Tukey's HSD was used. n.s. = non-significant;

767 $P^{*} < 0.05$; $P^{**} < 0.01$, $P^{***} < 0.001$.

768

769 **Supplemental information titles and legends**

770 Supplemental information includes 6 figures and two tables and can be found with this
771 article online.

772

773 **Figure S1 Controls for heat shift experiment in Figure 2B**

774 To control for memory performance without a heat-shift, flies expressing a temperature-
775 sensitive *shibire* transgene under the control of the R1504-Gal4 driver were trained and
776 tested at the permissive temperature and compared to the respective controls. The
777 tested groups showed memory 6 h after the training and did not differ from each other.
778 Flies were starved for 16 h and 2 M sucrose was used as reinforcer. The behavior
779 differed from random choice as determined using a one-sample sign test and marked
780 with the letter "a" ($P < 0.05$). The one-way ANOVA with post hoc Tukey's HSD was used
781 to analyze difference between groups. n.s = no significant differences.

782

783

784 **Figure S2. Octopamine but not tyramine suppresses food intake (related to Figure**
785 **3)**

786 The w^{1118} flies were starved for 17 h followed by feeding 1 h octopamine [10 mg/ml],
787 tyramine [10 or 100 mg/ml] or water. The intake of 5% sucrose after 3 h was determined
788 using the CAFÉ assay. Student's t tests were used to determine differences between
789 two groups and one-way ANOVA with Tukey's post hoc HSD test between three
790 groups. ($P^{***} < 0.001$; n.s. = non-significant). N = 13 – 20 groups of 20 male flies.

791

792 **Figure S3. Glycogen level in adult flies with reduced GlyP and GlyS (related to**
793 **Figure 5)**
794 The relative glycogen content in flies with altered GlyP and GlyS in muscles, fat bodies
795 or both. The expression of *GlyP-RNAi* and *GlyS-RNAi* under the control of *mef*2-Gal4
796 did not significantly change the relative glycogen level in whole animals, but in the
797 thorax. The expression of *GlyP-RNAi* under the control of *FB*-Gal4 increased
798 significantly glycogen levels in whole animals, and the expression of *GlyS-RNAi*
799 decreased glycogen in the abdomen. When both drivers are combined, the expression
800 of *GlyP-RNAi* increases glycogen in whole animals and the expression of *GlyS-RNAi*
801 decreases the glycogen levels. Glycogen was measured in 3 groups of 5 male flies and
802 normalized to the protein levels and to the glycogen levels of flies of the Gal4 driver.
803 ANOVA with post hoc Tukey's HSD was used to determine differences between three
804 groups. ($P^* < 0.05$; $P^{**} < 0.01$, $P^{***} < 0.001$).
805
806
807 **Figure S4. The glycogen level in the mushroom bodies does not influence**
808 **appetitive STM (related to Figure 5)**
809 **A**, The expression of *GlyS-RNAi* under control of the mef2-Gal4; mb247-Gal4 drivers
810 increased STM. **B**, The expression of *GlyS-RNAi* under control of the mb247-Gal4
811 driver did not change STM. Flies were starved for 16 h and 2 M sucrose was used as
812 reinforcer. One-way ANOVA with Tukey's HSD post hoc test was used to determine
813 differences between the groups. ($P^* < 0.05$; $P^{**} < 0.01$; n.s. = non-significant). The letter
814 "a" marks a significant difference from random choice as determined by a one-sample

815 sign test ($P < 0.05$). Numbers below box plots indicate one pair of reciprocally trained
816 independent fly groups. **C**, Sensory acuity tests for the genotypes used.
817
818
819 **Figure S5. Starvation influences yeast consumption of *Tβh*^{nm18} flies (related to**
820 **Figure 6)**
821 Non-starved *Tβh*^{nm18} male flies consumed significantly more yeast within 24 h. Starved
822 *Tβh*^{nm18} mutants consumed similar amounts of yeast. N = 26 groups of eight flies. To
823 determine differences between both groups, Student's t test was used. ***$P < 0.001$;
824 n.s. = non-significant difference.
825
826
827 **Figure S6. The antibody against phosphorylated InR recognizes activated InR**
828 **(related to Figure 7)**
829 **A**, **B**, In the antennal lobes (AL) of the male adult brain, the immunoreactivity
830 recognized by the anti-InR^{P} antibody is shown in magenta, and GFP expression is
831 shown in green. The Gal4 expression of the *Tdc2*-Gal4 driver targeting octopaminergic
832 neurons is visualized using the UAS-mCD8::GFP transgene. **B**, The expression of the
833 constitutively active insulin receptor results in increased immunoreactivity detected by
834 the anti-InR^{P} antibody.
835
836
837 **Table S1. Sensory acuity**

838 Odorant acuity and odorant balance were tested in independent fly groups of each
839 genotype used in the behavioral experiments. For testing, flies were placed in the
840 Tully Quinn setup. To analyze odorant preference and balance, they were allowed to
841 choose between the odorant- or paraffin oil-containing sides or between both odorants
842 for 2 min. Preference for sucrose and yeast was determined by analyzing whether flies
843 preferred the side with a sucrose- or yeast-soaked filter paper over a water-soaked filter
844 paper. Sucrose intake was analyzed by examining whether flies absorbed colored
845 sucrose solution into the abdomen.
846
847 **Table S2. Data related to Figures**

References

Adel, M., and Griffith, L.C. (2021). The role of dopamine in associative learning in *Drosophila*: an updated unified model. Neurosci Bull 37, 831–852.

Berridge, K.C. (2009). 'Liking' and 'wanting' food rewards: brain substrates and roles in eating disorders. Physiol Behav 97, 537–550.

Burke, C.J., Huetteroth, W., Owald, D., Perisse, E., Krashes, M.J., Das, G., Gohl, D., Silies, M., Certel, S., and Waddell, S. (2012). Layered reward signalling through octopamine and dopamine in *Drosophila*. Nature 492, 433–437.

Busch, S., Selcho, M., Ito, K., and Tanimoto, H. (2009). A map of octopaminergic neurons in the *Drosophila* brain. J Comp Neurol 513, 643–667.

Chambers, D.B., Androschuk, A., Rosenfelt, C., Langer, S., Harding, M., and Bolduc, F.V. (2015). Insulin signaling is acutely required for long-term memory in *Drosophila*. Front Neural Circuits 9, 8.

Chatterjee, N., and Perrimon, N. (2021). What fuels the fly: energy metabolism in *Drosophila* and its application to the study of obesity and diabetes. Sci Adv 7, eabg4336.

Classen, G., and Scholz, H. (2018). Octopamine shifts the behavioral response from indecision to approach or aversion in *Drosophila melanogaster*. Front Behav Neurosci 12, 131.

Cole, S.H., Carney, G.E., McClung, C.A., Willard, S.S., Taylor, B.J., and Hirsh, J. (2005). Two functional but noncomplementing *Drosophila* tyrosine decarboxylase genes: distinct roles for neural tyramine and octopamine in female fertility. J Biol Chem 280, 14948–14955.

Colomb, J., Kaiser, L., Chabaud, M.A., and Preat, T. (2009). Parametric and genetic analysis of *Drosophila* appetitive long-term memory and sugar motivation. Genes Brain Behav 8, 407–415.

Damrau, C., Toshima, N., Tanimura, T., Brembs, B., and Colomb, J. (2017). Octopamine and tyramine contribute separately to the counter-regulatory response to sugar deficit in *Drosophila*. Front Syst Neurosci 11, 100.

Das, G., Klappenbach, M., Vrontou, E., Perisse, E., Clark, C.M., Burke, C.J., and Waddell, S. (2014). *Drosophila* learn opposing components of a compound food stimulus. Curr Biol 24, 1723–1730.

Diegelmann, S., Jansen, A., Jois, S., Kastenholz, K., Escarcena, L.V., Strudthoff, N., and Scholz, H. (2017). The CApillary FEeder assay measures food intake in *Drosophila melanogaster*. J Vis Exp.

Ellingwood, S.S., and Cheng, A. (2018). Biochemical and clinical aspects of glycogen storage diseases. J Endocrinol 238, R131–R141.

Fujita, M., and Tanimura, T. (2011). *Drosophila* evaluates and learns the nutritional value of sugars. Curr Biol 21, 751–755.

Galikova, M., and Klepsatel, P. (2023). Endocrine control of glycogen and triacylglycerol breakdown in the fly model. Semin Cell Dev Biol 138, 104–116.

Grillo, C.A., Piroli, G.G., Lawrence, R.C., Wrighten, S.A., Green, A.J., Wilson, S.P., Sakai, R.R., Kelly, S.J., Wilson, M.A., Mott, D.D., and Reagan, L.P. (2015). Hippocampal insulin resistance impairs spatial learning and synaptic plasticity. Diabetes 64, 3927–3936.

891 Gronke, S., Beller, M., Fellert, S., Ramakrishnan, H., Jackle, H., and Kuhnlein, R.P.
892 (2003). Control of fat storage by a *Drosophila* PAT domain protein. Curr Biol 13, 603–
893 606.
894 Huetteroth, W., Perisse, E., Lin, S., Klappenbach, M., Burke, C., and Waddell, S. (2015).
895 Sweet taste and nutrient value subdivide rewarding dopaminergic neurons in *Drosophila*.
896 Curr Biol 25, 751–758.
897 Inagaki, H.K., Ben-Tabou de-Leon, S., Wong, A.M., Jagadish, S., Ishimoto, H., Barnea,
898 G., Kitamoto, T., Axel, R., and Anderson, D.J. (2012). Visualizing neuromodulation in vivo:
899 TANGO-mapping of dopamine signaling reveals appetite control of sugar sensing. Cell
900 148, 583–595.
901 Inoue, Y.H., Katsube, H., and Hinami, Y. (2018). *Drosophila* models to investigate insulin
902 action and mechanisms underlying human diabetes mellitus. Adv Exp Med Biol 1076,
903 235–256.
904 Ja, W.W., Carvalho, G.B., Mak, E.M., De la Rosa, N.N., Fang, A.Y., Liong, J.C., Brummel,
905 T., and Benzer, S. (2007). Prandiology of *Drosophila* and the CAFE assay. Proc Natl
906 Acad Sci U S A 104, 8253–8256.
907 Kitamoto, T. (2001). Conditional modification of behavior in *Drosophila* by targeted
908 expression of a temperature-sensitive shibire allele in defined neurons. J Neurobiol 47,
909 81–92.
910 Konner, A.C., Hess, S., Tovar, S., Mesaros, A., Sanchez-Lasheras, C., Evers, N.,
911 Verhagen, L.A., Bronneke, H.S., Kleinridders, A., Hampel, B.*, et al.* (2011). Role for
912 insulin signaling in catecholaminergic neurons in control of energy homeostasis. Cell
913 Metab 13, 720–728.
914 Krashes, M.J., Keene, A.C., Leung, B., Armstrong, J.D., and Waddell, S. (2007).
915 Sequential use of mushroom body neuron subsets during *Drosophila* odor memory
916 processing. Neuron 53, 103–115.
917 Krashes, M.J., and Waddell, S. (2008). Rapid consolidation to a radish and protein
918 synthesis-dependent long-term memory after single-session appetitive olfactory
919 conditioning in *Drosophila*. J Neurosci 28, 3103–3113.
920 Li, Y., Hoffmann, J., Li, Y., Stephano, F., Bruchhaus, I., Fink, C., and Roeder, T. (2016).
921 Octopamine controls starvation resistance, life span and metabolic traits in *Drosophila*.
922 Sci Rep 6, 35359.
923 Liu, C., Placais, P.Y., Yamagata, N., Pfeiffer, B.D., Aso, Y., Friedrich, A.B., Siwanowicz,
924 I., Rubin, G.M., Preat, T., and Tanimoto, H. (2012). A subset of dopamine neurons signals
925 reward for odour memory in *Drosophila*. Nature 488, 512–516.
926 Longo, N., Shuster, R.C., Griffin, L.D., Langley, S.D., and Elsas, L.J. (1992). Activation of
927 insulin receptor signaling by a single amino acid substitution in the transmembrane
928 domain. J Biol Chem 267, 12416–12419.
929 Marella, S., Mann, K., and Scott, K. (2012). Dopaminergic modulation of sucrose
930 acceptance behavior in *Drosophila*. Neuron 73, 941–950.
931 Mery, F., and Kawecki, T.J. (2005). A cost of long-term memory in *Drosophila*. Science
932 308, 1148.
933 Monastirioti, M., Linn Jr, C.E., and White, K. (1996). Characterization of *Drosophila*
934 tyramine beta-hydroxylase gene and isolation of mutant flies lacking octopamine. J
935 Neurosci 16, 3900–3911.

936 Naganos, S., Horiuchi, J., and Saitoe, M. (2012). Mutations in the *Drosophila* insulin
937 receptor substrate, CHICO, impair olfactory associative learning. Neurosci Res 73, 49–
938 55.
939 Nakai, J., Chikamoto, N., Fujimoto, K., Totani, Y., Hatakeyama, D., Dyakonova, V.E., and
940 Ito, E. (2022). Insulin and memory in invertebrates. Front Behav Neurosci 16, 882932.
941 Pedersen, B.K., and Febbraio, M.A. (2012). Muscles, exercise and obesity: skeletal
942 muscle as a secretory organ. Nat Rev Endocrinol 8, 457–465.
943 Placais, P.Y., De Tredern, E., Scheunemann, L., Trannoy, S., Goguel, V., Han, K.A.,
944 Isabel, G., and Preat, T. (2017). Upregulated energy metabolism in the *Drosophila*
945 mushroom body is the trigger for long-term memory. Nat Commun 8, 15510.
946 Rai, M., Coleman, Z., Curley, M., Nityanandam, A., Platt, A., Robles-Murguia, M., Jiao,
947 J., Finkelstein, D., Wang, Y.D., Xu, B*., et al.* (2021). Proteasome stress in skeletal muscle
948 mounts a long-range protective response that delays retinal and brain aging. Cell Metab
949 33, 1137–1154.e1139.
950 Rajan, A., and Perrimon, N. (2012). *Drosophila* cytokine unpaired 2 regulates
951 physiological homeostasis by remotely controlling insulin secretion. Cell 151, 123–137.
952 Ranganayakulu, G., Elliott, D.A., Harvey, R.P., and Olson, E.N. (1998). Divergent roles
953 for NK-2 class homeobox genes in cardiogenesis in flies and mice. Development 125,
954 3037–3048.
955 Ribeiro, C., and Dickson, B.J. (2010). Sex peptide receptor and neuronal TOR/S6K
956 signaling modulate nutrient balancing in *Drosophila*. Curr Biol 20, 1000–1005.
957 Saltiel, A.R., and Kahn, C.R. (2001). Insulin signalling and the regulation of glucose and
958 lipid metabolism. Nature 414, 799–806.
959 Scheiner, R., Steinbach, A., Classen, G., Strudthoff, N., and Scholz, H. (2014).
960 Octopamine indirectly affects proboscis extension response habituation in *Drosophila*
961 *melanogaster* by controlling sucrose responsiveness. J Insect Physiol 69, 107–117.
962 Schwaerzel, M., Monastirioti, M., Scholz, H., Friggi-Grelin, F., Birman, S., and
963 Heisenberg, M. (2003). Dopamine and octopamine differentiate between aversive and
964 appetitive olfactory memories in *Drosophila*. J Neurosci 23, 10495–10502.
965 Semaniuk, U., Strilbytska, O., Malinovska, K., Storey, K.B., Vaiserman, A., Lushchak, V.,
966 and Lushchak, O. (2021). Factors that regulate expression patterns of insulin-like
967 peptides and their association with physiological and metabolic traits in *Drosophila*. Insect
968 Biochem Mol Biol 135, 103609.
969 Shingleton, A.W., Das, J., Vinicius, L., and Stern, D.L. (2005). The temporal requirements
970 for insulin signaling during development in *Drosophila*. PLoS Biol 3, e289.
971 Tennessen, J.M., Barry, W.E., Cox, J., and Thummel, C.S. (2014). Methods for studying
972 metabolism in *Drosophila*. Methods 68, 105–115.
973 Tully, T., and Quinn, W.G. (1985). Classical conditioning and retention in normal and
974 mutant *Drosophila melanogaster*. J Comp Physiol A 157, 263–277.
975 Vargas, M.A., Luo, N., Yamaguchi, A., and Kapahi, P. (2010). A role for S6 kinase and
976 serotonin in postmating dietary switch and balance of nutrients in *D. melanogaster*. Curr
977 Biol 20, 1006–1011.
978 Wigglesworth, V.B. (1949). The utilization of reserve substances in *Drosophila* during
979 flight. J Exp Biol 26, 150–163, illust.

Wu, C.L., Shih, M.F., Lee, P.T., and Chiang, A.S. (2013). An octopamine-mushroom body circuit modulates the formation of anesthesia-resistant memory in *Drosophila*. Curr Biol 23, 2346–2354.

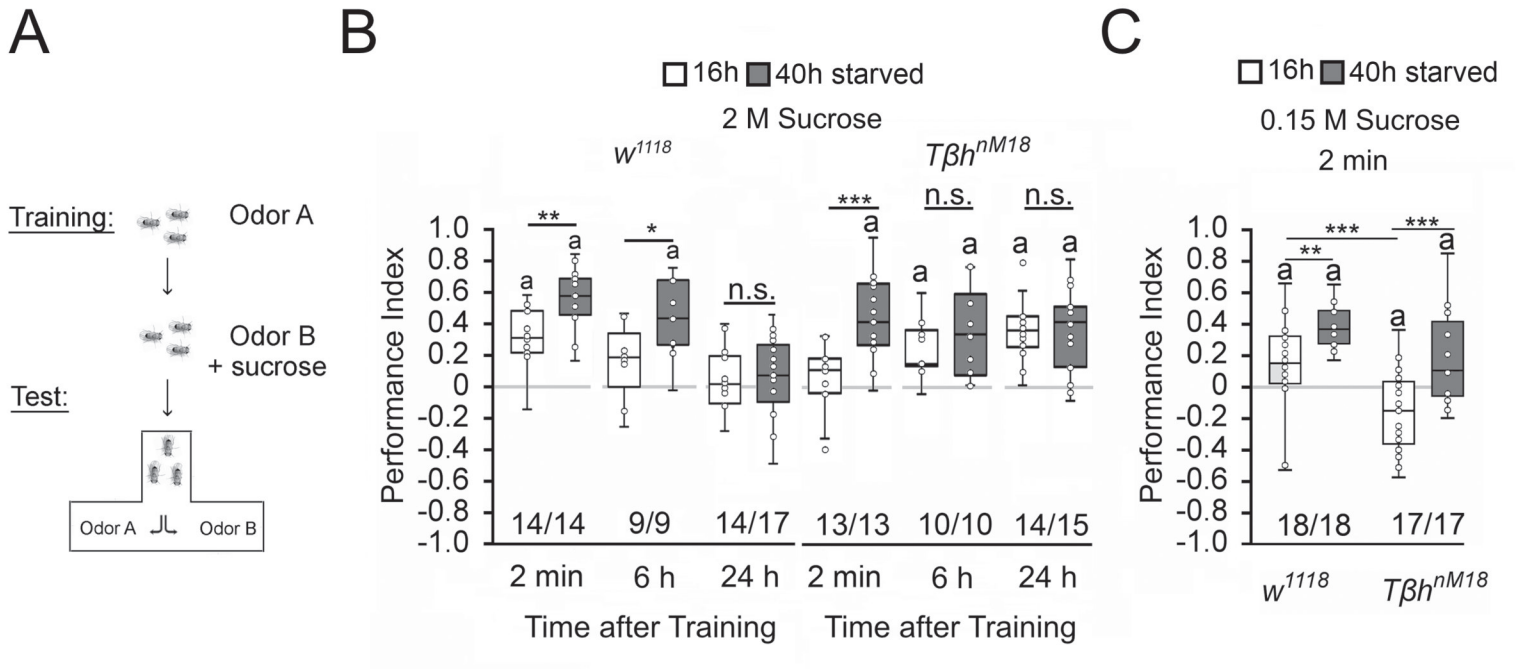
Wu, Q., Zhang, Y., Xu, J., and Shen, P. (2005). Regulation of hunger-driven behaviors by neural ribosomal S6 kinase in *Drosophila*. Proc Natl Acad Sci U S A 102, 13289–13294.

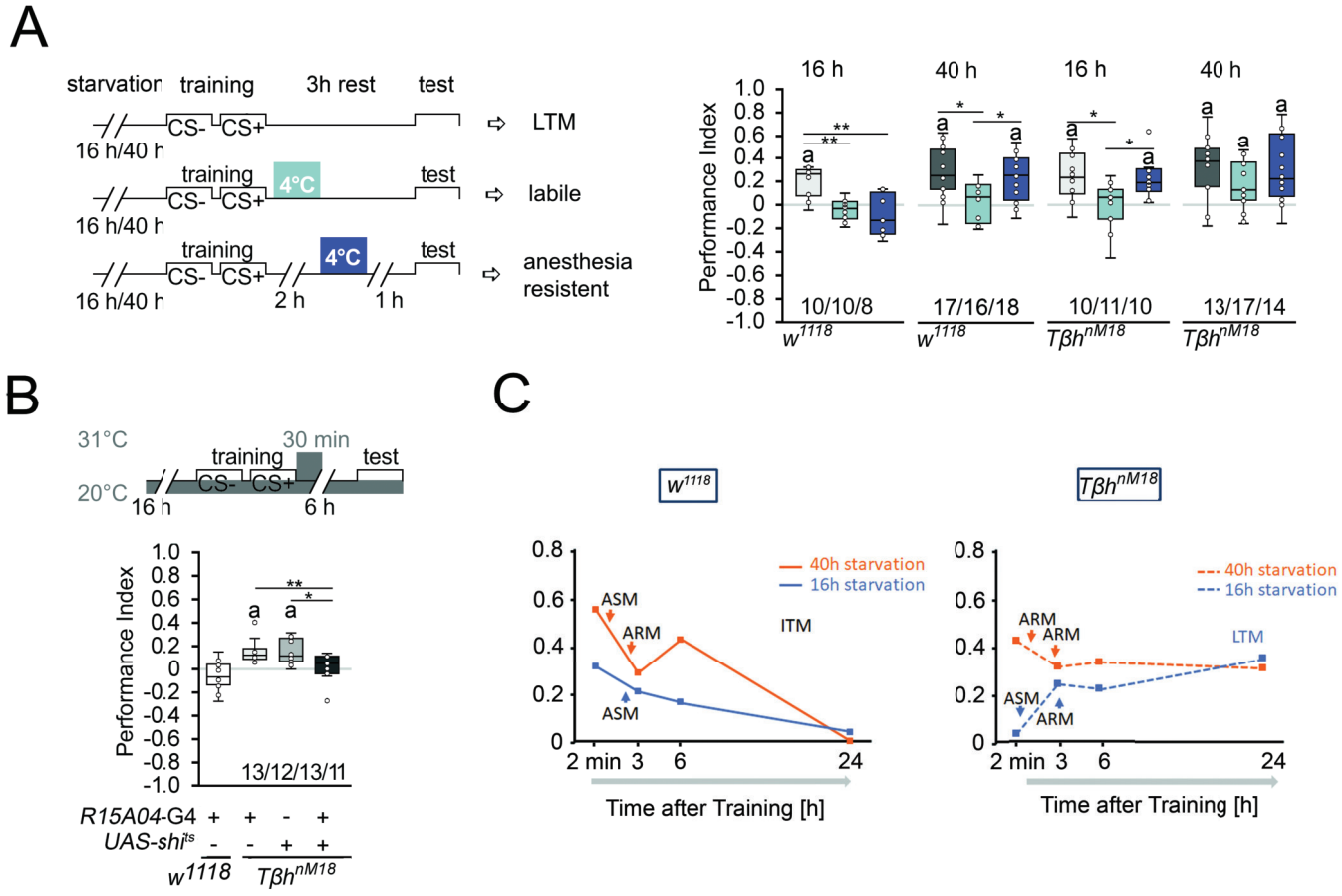
Yamada, T., Habara, O., Kubo, H., and Nishimura, T. (2018). Fat body glycogen serves as a metabolic safeguard for the maintenance of sugar levels in *Drosophila*. Development 145, dev158865.

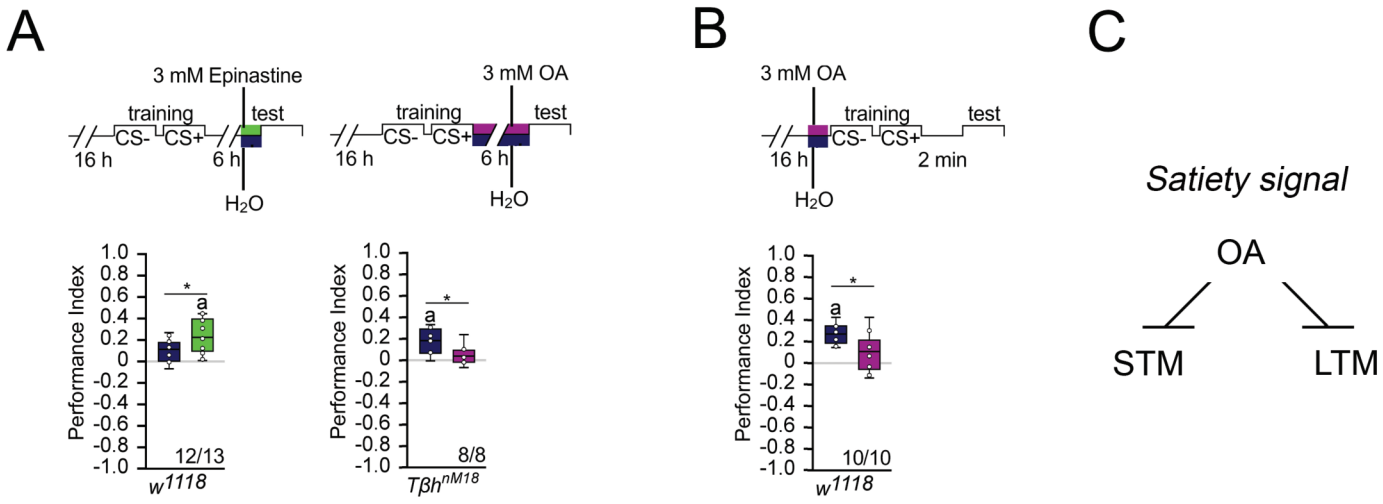
Yamagata, N., Ichinose, T., Aso, Y., Placais, P.Y., Friedrich, A.B., Sima, R.J., Preat, T., Rubin, G.M., and Tanimoto, H. (2015). Distinct dopamine neurons mediate reward signals for short- and long-term memories. Proc Natl Acad Sci U S A 112, 578–583.

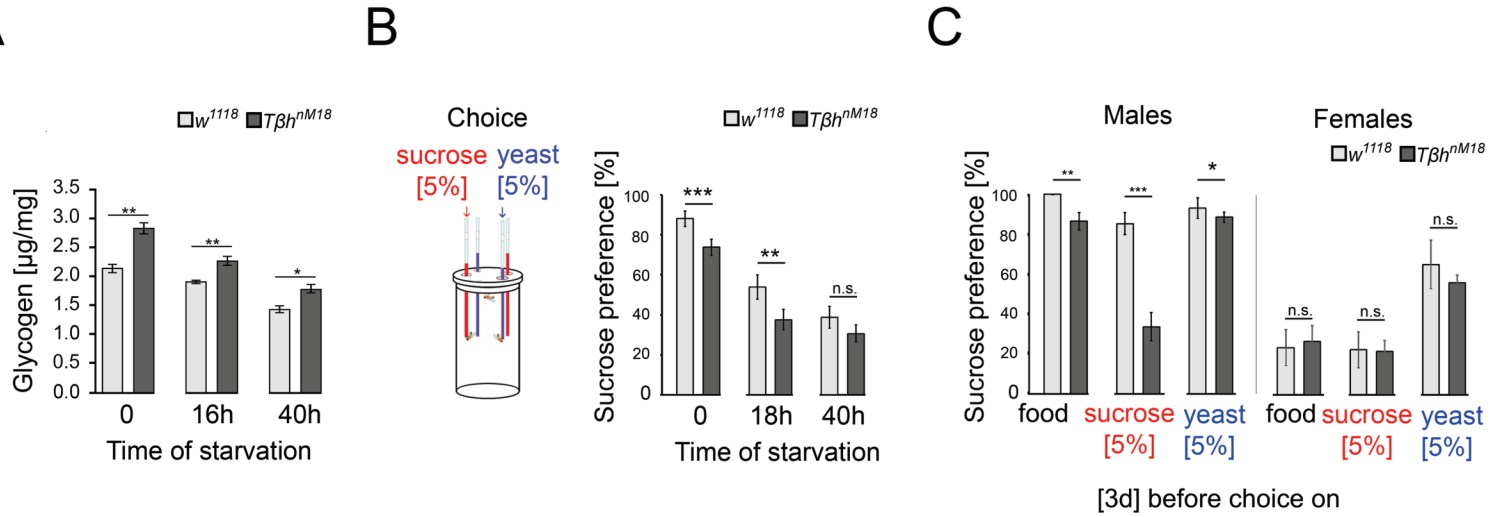
Youn, H., Kirkhart, C., Chia, J., and Scott, K. (2018). A subset of octopaminergic neurons that promotes feeding initiation in *Drosophila melanogaster*. PLoS One 13, e0198362.

Zars, T., Fischer, M., Schulz, R., and Heisenberg, M. (2000). Localization of a short-term memory in *Drosophila*. Science 288, 672–675.

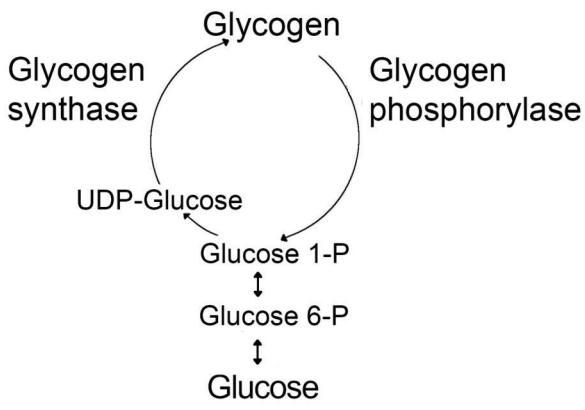

A
Training:
Odor A
Odor B
+ sucrose
Test:
Odor A
Odor B
B
16h
40h starved
2 M Sucrose
w1118
TβhnM18
Performance Index
1.0
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1.0
**
*
n.s.
***
n.s.
n.s.
a
14/14
9/9
14/17
13/13
10/10
14/15
2 min
6 h
24 h
2 min
6 h
24 h
Time after Training
Time after Training
C
16h
40h starved
0.15 M Sucrose
2 min
Performance Index
***
**
***
a
18/18
17/17
w1118
TβhnM18


A
starvation
training
3h rest
test
CS-
CS+
16 h/40 h
LTM
4°C
labile
2 h
1 h
anesthesia resistent
Performance Index
16 h
40 h
10/10/8
17/16/18
10/11/10
13/17/14
w1118
TβhnM18
B
31°C
20°C
30 min
16 h
6 h
13/12/13/11
R15A04-G4
UAS-shits
C
ASM
ARM
ITM
LTM
40h starvation
16h starvation
2 min
3
6
24
Time after Training [h]

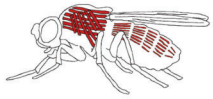

A
3 mM Epinastine
training
test
CS-
CS+
16 h
6 h
H2O
3 mM OA
training
test
CS-
CS+
16 h
6 h
H2O
Performance Index
1.0
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1.0
*
a
12/13
w1118
8/8
Tβh nM18
B
3 mM OA
training
test
CS-
CS+
16 h
2 min
H2O
10/10
w1118
C
Satiety signal
OA
STM
LTM


A
B
C
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
Choice
sucrose [5%]
yeast [5%]
Sucrose preference [%]
100
80
60
40
20
0
***
**
n.s.
0
18h
40h
Time of starvation
Males
Females
food
sucrose [5%]
yeast [5%]
food
sucrose [5%]
yeast [5%]
n.s.
[3d] before choice on

A

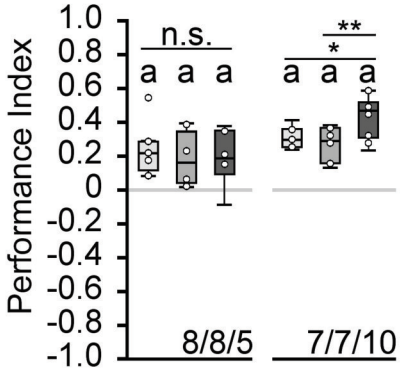


B



mef2-G4

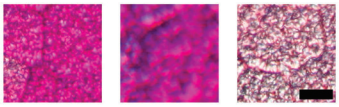
mef2-Gal4 *+GlyP-RNAi* *v+ GlyS-RNAi*



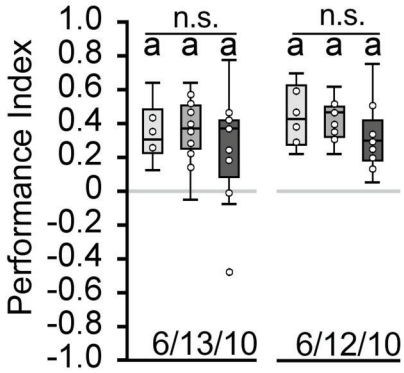
mef2-G4	+	-	+	+	-	+
GlyP-RNAi	-	+	+	-	-	-
GlyS-RNAi	-	-	-	-	+	+
	w1118			*w1118*		

C

FB-G4



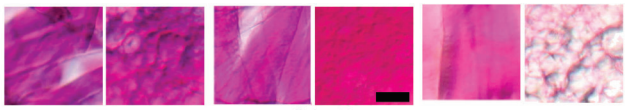
FB-Gal4 *+GlyP-RNAi* *v+ GlyS-RNAi*



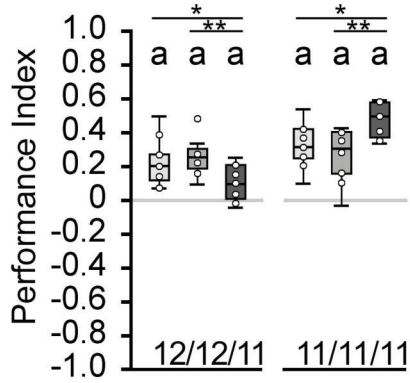
FB-G4	+	-	+	+	-	+
GlyP-RNAi	-	+	+	-	-	-
GlyS-RNAi	-	-	-	-	+	+
	w1118			*w1118*		

D

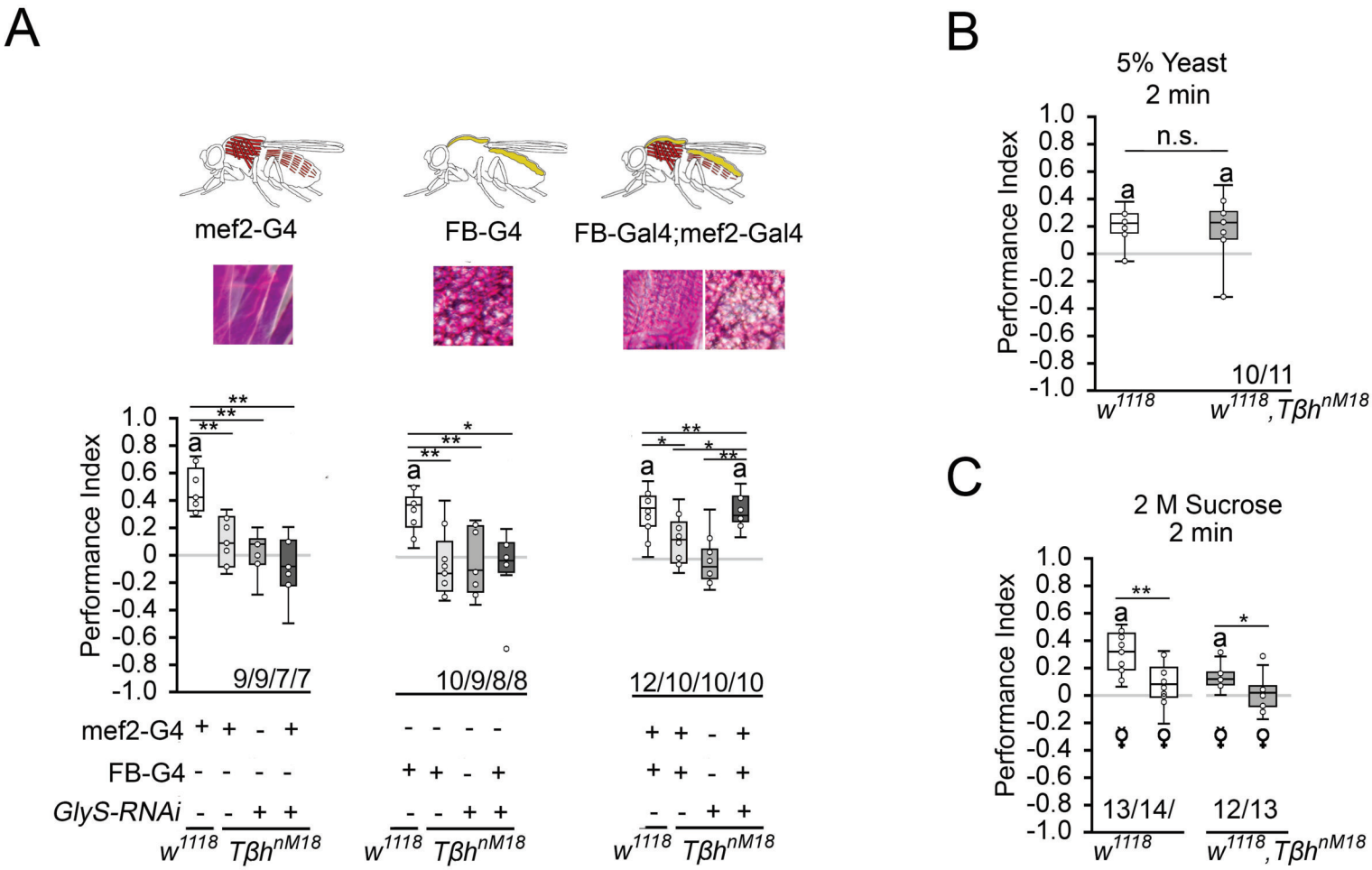
FB-Gal4;mef2-Gal4

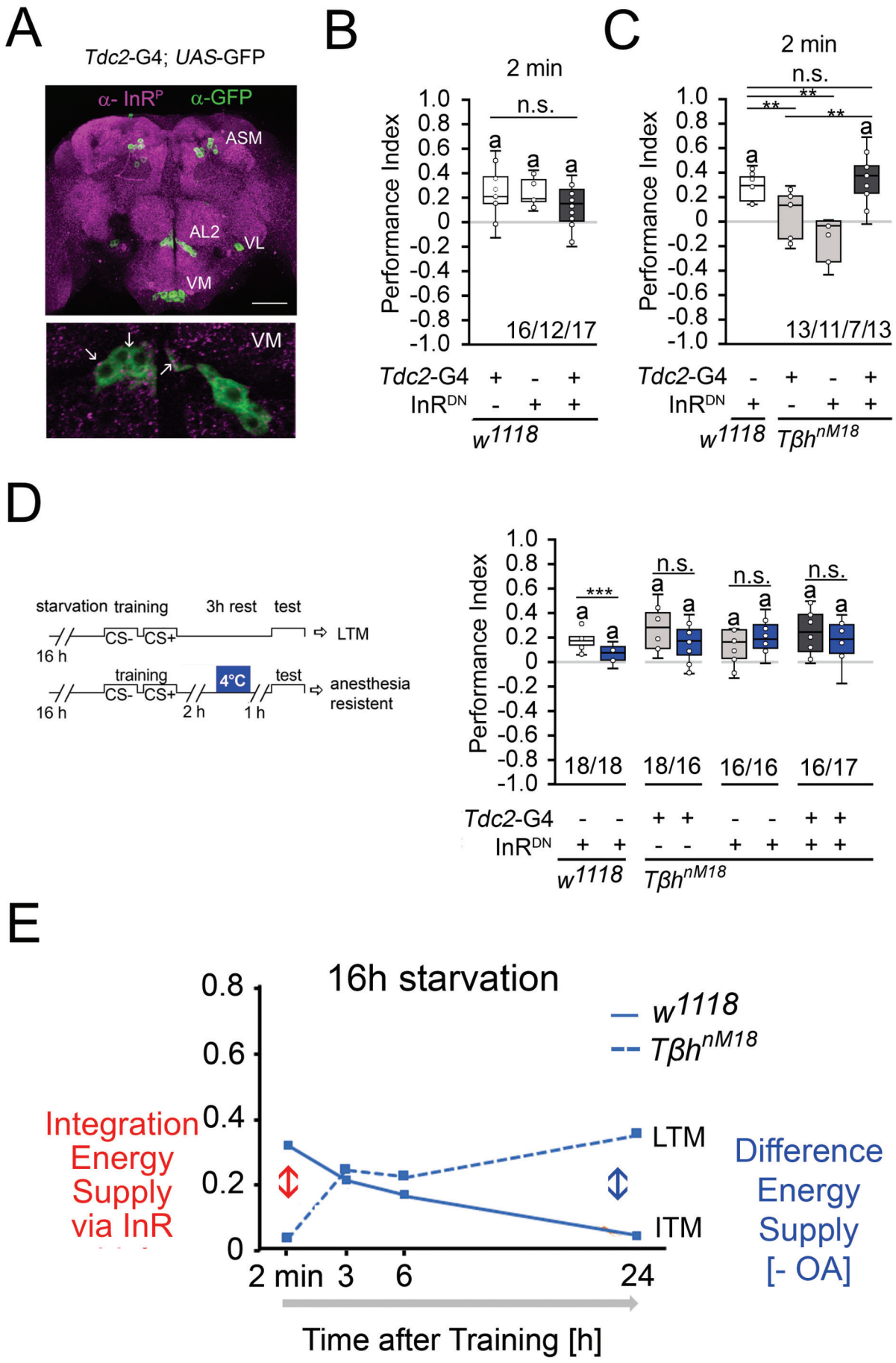


FB-Gal4; mef2-Gal4 *+GlyP-RNAi* *v+ GlyS-RNAi*

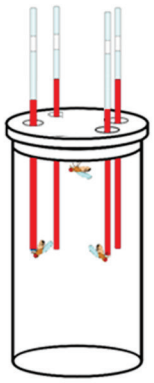


FB-G4;mef2-G4	+	-	+	+	-	+
GlyP-RNAi	-	+	+	-	-	-
GlyS-RNAi	-	-	-	-	+	+
	w1118			*w1118*		

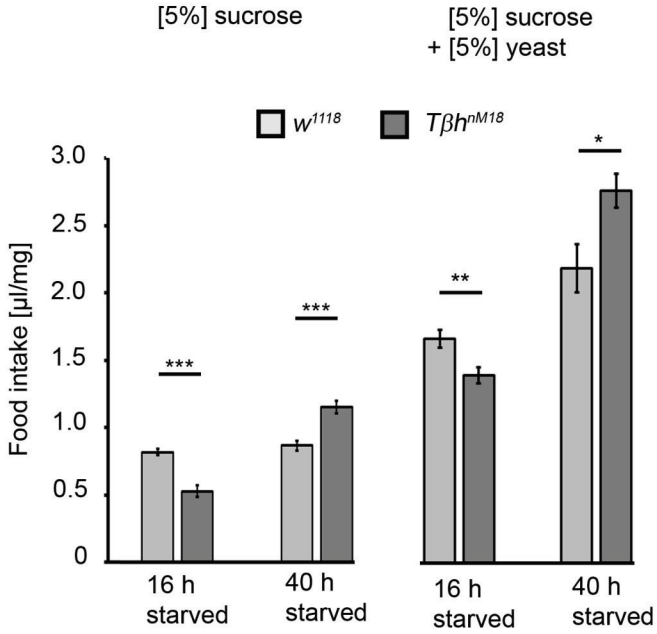

A
mef2-G4
FB-G4
FB-Gal4;mef2-Gal4
Performance Index
9/9/7/7
10/9/8/8
12/10/10/10
mef2-G4
FB-G4
GlyS-RNAi
w1118
TβhnM18
B
5% Yeast
2 min
n.s.
10/11
w1118
w1118,TβhnM18
C
2 M Sucrose
2 min
13/14/
12/13
w1118
w1118,TβhnM18


A
Tdc2-G4; UAS-GFP
α- InR^P
α-GFP
ASM
AL2
VL
VM
VM
B
2 min
n.s.
Performance Index
16/12/17
Tdc2-G4
InR^DN
w1118
C
2 min
n.s.
**
**
**
Performance Index
13/11/7/13
Tdc2-G4
InR^DN
w1118
Tβh^nM18
D
starvation training
3h rest
test
LTM
16 h
CS-
CS+
training
4°C
test
anesthesia resistent
16 h
2 h
1 h
***
n.s.
n.s.
n.s.
Performance Index
18/18
18/16
16/16
16/17
Tdc2-G4
InR^DN
w1118
Tβh^nM18
E
16h starvation
w1118
Tβh^nM18
Integration Energy Supply via InR
LTM
ITM
Difference Energy Supply [- OA]
2 min
3
6
24
Time after Training [h]

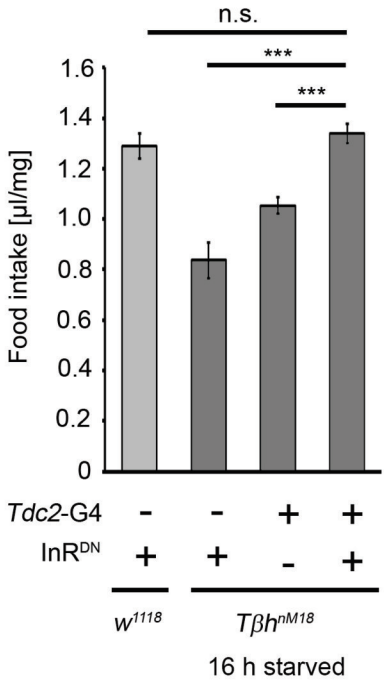
A



B


[5%] sucrose
[5%] sucrose
+ [5%] yeast
w1118
TβhnM18
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
TβhnM18
16 h starved