To Favor Survival Under Food Shortage, the Brain Disables Costly Memory

Pierre-Yves Plaçais and Thomas Preat*

The brain regulates energy homeostasis in the organism. Under resource shortage, the brain takes priority over peripheral organs for energy supply. But can the brain also down-regulate its own consumption to favor survival? We show that the brain of *Drosophila* specifically disables the costly formation of aversive long-term memory (LTM) upon starvation, a physiological state required for appetitive LTM formation. At the neural circuit level, the slow oscillations normally triggered in two pairs of dopaminergic neurons to enable aversive LTM formation were abolished in starved flies. Transient artificial activation of these neurons during training restored LTM formation in starved flies but at the price of a reduced survival. LTM formation is thus subject to adaptive plasticity that helps survival under food shortage.

Phenotypic plasticity in response to environmental changes can be adaptive or nonadaptive, depending on whether it provides the organism with higher fitness under the new conditions (*1*). Resource-mediated plasticity in general tends to be nonadaptive (*2*). For instance, because it is the central regulator of the organism's energy homeostasis, the brain enjoys primacy in the allocation of energy fluxes over supply to peripheral organs in case of resource shortage (*3*, *4*). This plasticity is nonadaptive because the brain is also the most energy-demanding of all the organs (*3*). It is much less known whether, in response to a decrease in food intake, the brain also adopts adaptive strategies and, for example, avoids excessive energy consumption by specifically inhibiting costly processes.

Because it relies on de novo protein synthesis (*5*, *6*), the formation of long-term memory (LTM) is a function that involves heavy metabolic machinery. In *Drosophila*, the substantial levy of LTM formation on the organism's metabolic resources has been directly observed (*7*). Both aversive and appetitive associative olfactory memories have been thoroughly studied over the past decades in *Drosophila*. Flies must be hungry to form (*8*–*10*) and retrieve (*11*) appetitive memory, which results from the paired delivery of sugar and odor. Typically, flies are starved for 20 to 24 hours before undergoing appetitive conditioning (*8*, *10*). These hungry flies form appetitive LTM after a single conditioning cycle (*8*, *10*). By contrast, aversive memory formation, which results from the simultaneous delivery of electric shocks and odor, does not require the flies to be hungry. Thus, studies on aversive memory have so far always been performed on fed flies. Two types of consolidated aversive memories are formed in *Drosophila*: LTM, which requires de novo protein synthesis, and anesthesia-resistant memory (ARM), which does not (*12*). Aversive LTM forms only after multiple cycles of associative training, spaced by rest intervals (spaced training) (*12*). ARM is formed after multiple massed training, without rest intervals, and after single-cycle training (*12*). LTM formation is gated by two pairs of dopaminergic neurons (DNs), named MV1 and MP1 (*13*), which project onto the mushroom body, a brain structure that plays an essential role in olfactory learning and memory (*14*). During the rest intervals of spaced training, synchronized oscillatory activity of these DNs drives the brain into the LTM consolidation pathway (*13*) by repressing the antagonist ARM pathway (*13*, *15*).

We investigated how the brain deals with the cost of aversive LTM formation in starved flies. We first studied the effect of two genetic mutations specifically affecting LTM: *crammer* (*cer*) (*16*) and *tequila* (*teq*) (*17*). As expected, when fed, these two mutants showed a strong memory deficit 24 hours after spaced training compared with wild-type *Canton-S* flies (Fig. 1, A and B). The same mutant lines showed normal performance after spaced training when deprived from food and provided with only water for 21 hours before conditioning and 24 hours after conditioning (Fig. 1, A and B). This finding suggested that after spaced training, starved flies did not form LTM but ARM, known to be insensitive to *cer* and *teq* mutations (*16*, *17*). Similar phenotypes were obtained with 18 and 24 hours of starvation before conditioning (fig. S1A), showing that turning off LTM occurred on an extended range of starvation length. To further investigate

Genes and Dynamics of Memory Systems, Neurobiology Unit, UMR 7637 École Supérieure de Physique et de Chimie Industrielles (ESPCI)/CNRS, 10 rue Vauquelin, 75005 Paris, France.

*To whom correspondence should be addressed. E-mail: thomas.preat@espci.fr

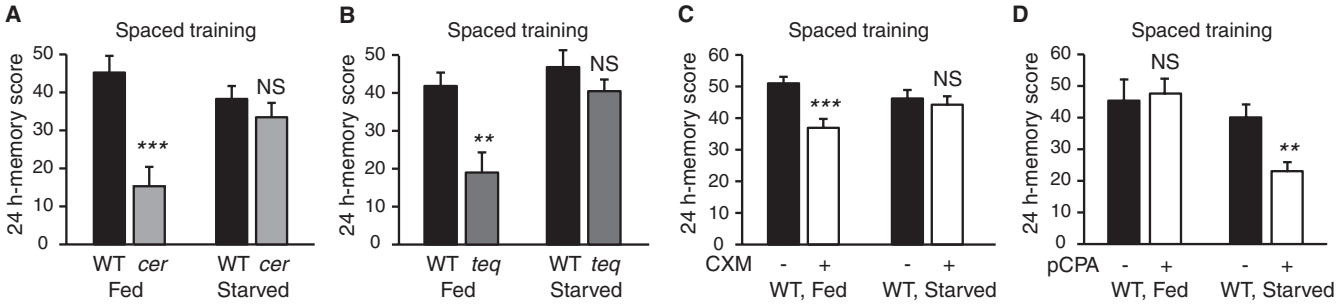

Fig. 1. Starvation prevents the formation of aversive LTM. (**A**) *cer* LTM mutant is defective for 24-hour memory after spaced training when fed (*t* test, t_{25} = 4.463, P = 0.00016, $n \geq 13$) but shows wild-type scores when starved for 21 hours before and 24 hours after conditioning (*t* test, t_{20} = 0.945, P = 0.36, n = 11). The interaction between genotype and starvation is statistically significant [two-way analysis of variance (ANOVA), $F_{(1,45)}$ = 8.230, P = 0.0063]. (**B**) *teq* LTM mutant is defective for 24-hour memory after spaced training when fed (*t* test, t_{20} = 3.573, P = 0.0019, n = 11) but performs normally when starved before and after conditioning (*t* test, t_{20} = 1.178, P = 0.25, n = 11). The interaction between genotype and starvation is statistically significant [two-way ANOVA, $F_{(1,40)}$ = 4.306, P = 0.044]. (**C**) Wild-type fed flies form CXM-sensitive LTM after spaced training (*t* test, t_{48} = 4.024, P = 0.00022, n = 25), but the memory of starved wild-type flies is unaffected by CXM treatment (*t* test, t_{48} = 0.5182, P = 0.61, n = 25). The interaction between CXM treatment and starvation is statistically significant [two-way ANOVA, $F_{(1,96)}$ = 5.47, P = 0.021]. (**D**) The LTM of wild-type fed flies is insensitive to pCPA (*t* test, t_{20} = 0.278, P = 0.78, n = 11), contrary to the memory formed by starved flies (*t* test, t_{24} = 3.404, P = 0.0023, n = 13). The interaction between pCPA treatment and starvation is statistically significant [two-way ANOVA, $F_{(1,44)}$ = 4.331, P = 0.043]. Asterisks illustrate results from two-tailed unpaired *t*-tests: **P < 0.01; ***P < 0.001; NS, not significant, P > 0.05. WT, wild-type *Canton-S* flies. Error bars indicate SEM.

whether starvation prevents LTM from being formed in wild-type flies, we assessed the sensitivity to the protein synthesis inhibitor cycloheximide (CXM) of the memory formed after spaced training. The memory formed by fed flies was altered by CXM absorption, revealing LTM formation, whereas CXM treatment had no effect on the memory score of starved flies (Fig. 1C). Conversely, the ingestion of DL-p-chlorophenylalanine (pCPA), an inhibitor of serotonin synthesis that specifically affects ARM (*13*, *18*), had no effect on the memory formed by fed flies but impaired that of starved flies (Fig. 1D). Starvation thus disables aversive LTM formation and directs the brain into the ARM pathway. Refeeding flies with sucrose just before training restored LTM formation (fig. S1, B to D). Aversive ARM does not last as long as LTM (*12*), and it is less costly than LTM (*7*), suggesting that in starved flies the aversive LTM pathway is shut down to save energy.

In fed flies, MV1 and MP1 DNs (Fig. 2A) exhibit synchronized sustained activity, which on spaced training enables LTM formation (*13*).

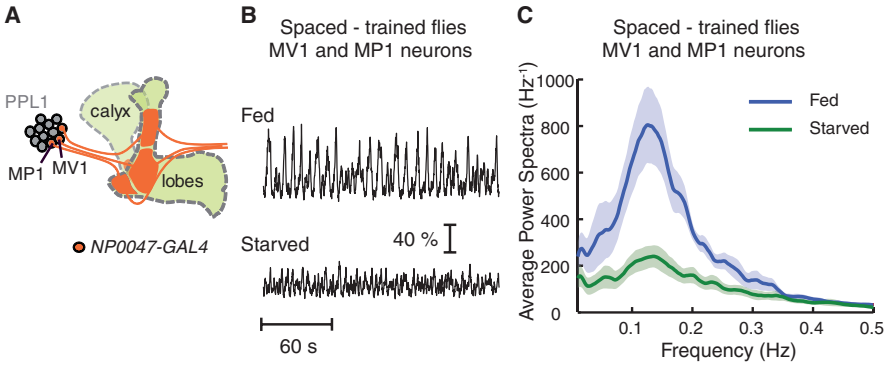

Fig. 2. Starvation silences the oscillations in MV1 and MP1 DNs that gate LTM. (**A**) Three bilateral pairs of DNs from the PPL1 cluster, among which the pairs of MV1 and MP1 neurons are included in the expression pattern of the *NP0047-GAL4* driver. These neurons symmetrically innervate the ipsi- and contralateral mushroom bodies, but each neuron type targets distinct parts of the mushroom body lobes. (**B**) Two illustrative examples of MV1 and MP1 neurons' activity after spaced training in a fed fly, featuring large, regular oscillations, and in a starved fly, where the signal amplitude is greatly reduced. (**C**) Average power spectra of MV1 and MP1 neurons' activity recorded after spaced training in fed ($n = 9$) and starved ($n = 10$) flies. Fed flies exhibit a characteristic peak, revealing an oscillatory behavior absent in starved flies.

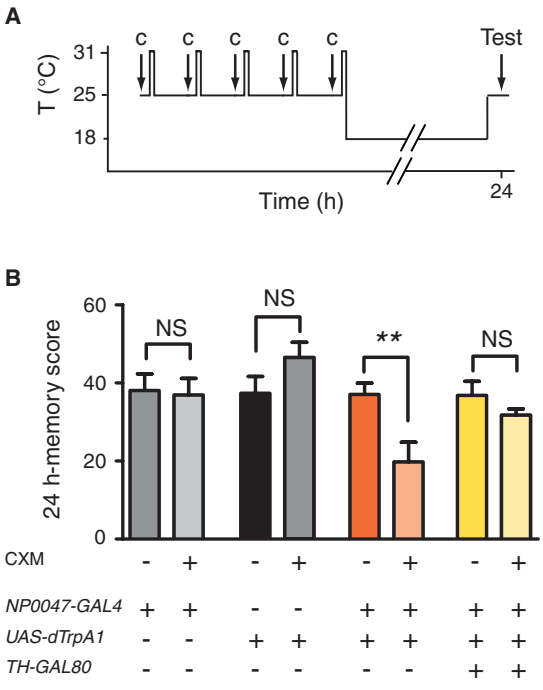

Fig. 3. Activation of oscillatory DNs during spaced training restores LTM formation in starved flies. (**A**) Experimental procedure coupling spaced training with a series of 1-min temperature shifts to 31°C immediately after each of the five cycles of conditioning (indicated by "c"). Neurons expressing *dTrpA1* are thus activated after each cycle. (**B**) Starved flies that do not express *dTrpA1* fail to form CXM-sensitive LTM. The activation of neurons labeled in *NP0047-GAL4* restores LTM formation (*t* test, $t_{25} = 3.099$, $P = 0.0048$), whereas the activation of the same set of neurons except the three pairs of DNs labeled in *NP0047-GAL4* does not (*t* test, $t_{23} = 1.265$, $P = 0.22$). The interaction between genotype and CXM treatment is statistically significant [two-way ANOVA, $F_{(3,96)} = 4.116$, $P = 0.0086$]. Asterisks report results from two-tailed unpaired *t* tests: $^{**}P < 0.01$; NS, not significant, $P > 0.05$. $n \geq 12$ for all genotypes. Error bars indicate SEM.

We wondered whether these two pairs of neurons were involved in starvation-induced LTM shutdown. We performed in vivo calcium-imaging experiments to record the activity of MV1 and MP1 neurons at the level of their mushroom body projections. Calcium levels were monitored by using the genetically encoded fluorescent reporter *GCaMP3* (*19*), the expression of which was targeted to MV1 and MP1 neurons by using the *NP0047-GAL4* driver (*13*). The amplitude of the sustained activity in MV1 and MP1 neurons was lowered in naïve starved flies compared with naïve fed ones (fig. S2). Even in the absence of conditioning, starvation thus tended to reduce MV1 and MP1 neurons' activity. This is consistent with a previous study showing that blocking MP1 neurons in fed flies could mimic the starvation required to retrieve appetitive memory (*11*). In fed flies, large-amplitude oscillations are characteristically observed in MV1 and MP1 neurons after spaced training (*13*) (Fig. 2B), resulting in a peaked power spectrum (Fig. 2C). Starved flies failed to develop such large oscillations (Fig. 2B), and so the characteristic peak was dampened in the power spectrum (Fig. 2C).

We wondered whether forcing MV1 and MP1 neurons' activity during spaced training would be sufficient to restore LTM formation in starved flies. We made use of the thermosensitive cation channel *dTrpA1*, which allows artificial activation of targeted neurons at temperatures above 28°C (*11*, *13*, *20*). We performed spaced training at permissive temperature (25°C), but after each cycle of conditioning the flies underwent a 31°C air flow for the first minute of the rest interval (Fig. 3A). In flies expressing *dTrpA1* under the control of *NP0047-GAL4* driver, which drives expression in three pairs of DNs including MV1 and MP1, such brief activation is sufficient to trigger oscillatory activity of MV1 and MP1 neurons for 15 to 30 min (*13*). The activation of *NP0047-GAL4* neurons was sufficient to restore protein synthesis-dependent LTM in starved flies, as reported by CXM sensitivity (Fig. 3B). The combination with *TH-GAL80*, which specifically inhibits *dTrpA1* expression in the three pairs of DNs (*13*, *21*), prevented LTM rescue (Fig. 3B). Last, we checked that the spaced training itself, without forcing DN activity, did not induce LTM (fig. S3).

Why does the brain shut down aversive LTM formation under nutritional shortage? Because of the metabolic cost of LTM formation, fed flies that form aversive LTM die prematurely when deprived of food and water after training compared with flies that form ARM (*7*). We therefore hypothesized that LTM-gating neurons were disabled on starvation to avoid a nonvital energy expense that could compromise survival. To test this hypothesis, we sought to determine whether forcing aversive LTM formation in starved flies would shorten their survival. We subjected starved flies to spaced training, combined with DN activation after each cycle as described above, and measured their survival to food and water

deprivation. Coupling activation of three pairs of DNs to spaced training shortened survival duration by about 30% (Fig. 4). Activation of the same neurons in the absence of spaced conditioning had no effect on survival duration (Fig. 4 and fig. S4, A and B). We also checked that the combination of DN activation with an unpaired protocol, where flies receive odorants and shocks separately and form no memory, did not significantly affect survival (fig. S4C). Hence, this marked reduction of survival was unequivocally attributable to the formation of aversive LTM in hungry flies.

Under food shortage, the brain will not just simply self-allocate available resources: It also trims its own metabolic expenses by turning off selected costly processes. In the present case, the shutdown of aversive LTM formation is achieved through the inhibition of a dopaminergic circuit that normally gates LTM formation (*13*), by switching memory formation from LTM to ARM. Starved flies accordingly showed enhanced ARM performance, either after a single cycle of training (fig. S5A) or after massed training (fig. S5B). This ARM increase occurred at all starvation lengths tested (fig. S5C). The mechanism of LTM gating in *Drosophila* had been previously described (*13*, *15*), but its ecological relevance remained unclear. The present work highlights a role, if not the prime one, of this LTM-gating mechanism, which is to prevent survival-threatening energy expense in a critical nutritional emergency situation. It was shown recently that the longevity of *Drosophila* males was decreased in selected lines with a higher LTM and lower ARM ability. Conversely, the longevity of males was increased in selected lines with lower LTM and improved ARM ability (*22*). This further illustrates the interplay between the cost of LTM formation and the organism's fitness. The LTM-gating mechanism we described might serve as a mechanistic basis for the modulation of LTM ability under selective pressure.

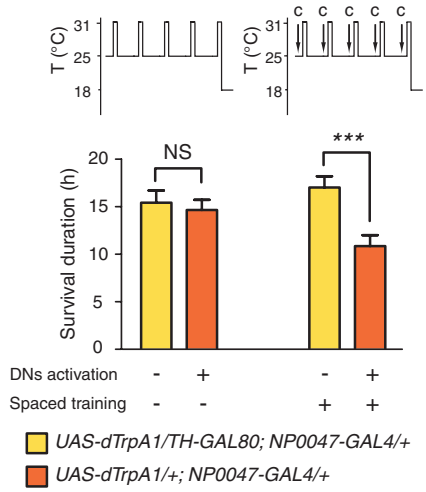

Fig. 4. Forcing aversive LTM formation in starved flies causes their premature death. Survival duration under water and food deprivation was measured for individual flies expressing *dTrpA1* under the control of *NP0047-GAL4*, either without (orange) or with (yellow) *GAL4* inhibition by *TH-GAL80*. The two conditions thus differ only by the activation of the three pairs of DNs in *NP0047-GAL4*. The various treatments are illustrated pictographically above the bar graph. A series of thermal activation alone yielded no difference between the two conditions (*t* test, $t_{52} = 0.4659$, $P = 0.64$, $n = 26$ to 28 flies for each condition). When spaced training was coupled to thermal shifts, DN activation, which forces LTM formation (Fig. 3), resulted in a ~30% decrease in survival duration (*t* test, $t_{70} = 3.696$, $P = 0.0004$, $n = 36$ flies for each condition). The interaction between DN activation and spaced training is statistically significant [two-way ANOVA, $F_{(1,122)} = 5.045$, $P = 0.0265$]. Asterisks report results from two-tailed unpaired *t* tests: ***$P < 0.001$; NS, not significant, $P > 0.05$. Error bars indicate SEM. Survival curves are shown in fig. S4.

Flies make appetitive LTM in a single conditioning cycle when they are starved. Why is the gating mechanism, and hence the starvation-induced shutdown, specific to aversive memory? Under natural conditions, reward learning occurs when a starved fly finds a food source. One can thus assume that the resulting refuelling largely exceeds the energy expense for LTM formation. In support of this argument, it has been shown that appetitive LTM forms only when flies ingest nutritious reward, whereas palatable rewards without caloric content produce only short-term memory (*23*).

How general is the mechanism evidenced here in *Drosophila*? In all species, stress may exert either positive or negative effect on memory formation depending on how stress is timed with conditioning (*24*). In particular, malnutrition (*25*) or immune response (*26*, *27*) can impair memory formation, but these impairments are consequences of stress and they do not help the animal adapting to it. On the contrary, we describe here a case of adaptive plasticity. Many of the features at play in the fly are also involved in the regulation of mammalian LTM. First, there is a growing body of evidence supporting the role of a loop between DNs of the ventrotegmental area (VTA) and the hippocampus in the control of information entry into LTM (*28*, *29*). Second, VTA DNs can exhibit a slow oscillatory firing pattern (*30*, *31*). Third, nutritional state interfere with LTM formation through cortisol receptors in the hippocampus, which among other functions, are involved in the regulation of both energy homeostasis (*3*) and long-term potentiation (*32*, *33*), a cellular basis of long-term memorization. Hence the scheme of LTM shutdown by starvation presented here may correspond, in a simplified version, to a conserved mechanism of interaction between the set point of energy homeostasis and the ability of LTM formation.

Note added in proof: In this issue of *Science*, Hirano *et al*. report that refeeding starved flies after conditioning facilitates aversive LTM formation (*34*).

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Supplementary Materials

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Materials and Methods
Figs. S1 to S5
References (*35*–*37*)

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