

Hunger and memory; CRTC coordinates long-term memory with the physiological state, hunger

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Animals form and store memory, which advantageously adjusts their behavior later on. Although the growing body of evidences suggests the basic mechanisms of memory, it is not clear whether and in which physiological state memory functions can be altered. Here we discuss our recent study that mild fasting facilitates long-term memory (LTM) formation in *Drosophila*.¹ Canonical LTM in flies is induced by multiple training with rest intervals, and is mediated by a transcription factor, CREB and its binding protein, CBP. However, fasting allows LTM formation (fLTM) only by single-cycle training, in a manner dependent on another CREB binding protein, CRTC. Although it has been controversial, we are convinced that gene expression in a specific neural structure, called mushroom body (MB), is required for LTMs. We also showed data suggesting that reduced insulin signaling during fasting activates CRTC, thereby inducing fLTM formation. These data provides the conceptual advance that flies adapt their mechanisms for LTM formation according to their internal condition, hunger state. Due to limited food resources in the wild, fLTM could be one of the major form of LTM in natural environment. Furthermore, our data also indicate a novel conception that improvement of memory deficit might be achieved by activation of CRTC.

and unavailability of food induces starvation response that affects their locomotor activity.³ Likewise, memory that allows animals to adjust their behavior based on past experiences, may also be affected by environmental conditions, although there were few evidences supporting this idea.

We sought to determine whether and in which physiological states, memory can be affected. We then encountered the evidence that, in *Drosophila*, formation of long-term memory (LTM), which requires de novo gene expression mediated by cAMP-responsive element binding protein (CREB)⁴⁻⁶ and lasts over 1 d, is distinct between aversive and appetitive conditioning paradigms. In aversive training, an odor is represented with electrical shocks, and flies learn to avoid the odor. Aversive LTM is formed by multiple trainings with rest intervals (spaced training), but not single-cycle training.⁷ Appetitive training consists of representation of an odor with sucrose reward, and thereafter flies approach to the odor. In contrast to aversive LTM, appetitive LTM is sufficiently formed by single-cycle training.⁵ The efficient formation of appetitive LTM by single-cycle training may be because neurons that convey the information of sucrose reward have a stronger reinforcement activity than that of electrical shocks. However, prior to appetitive training, flies are subjected to fasting that facilitates sucrose intake during the training. The hypothesis we raised here is that fasting prior to any training paradigm could facilitate LTM formation.

Keywords: long-term memory, *Drosophila*, CREB, CRTC, CBP, insulin signaling

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The brain functions are altered in response to environmental conditions; for example, light exposure affects sleep/wake activity,²

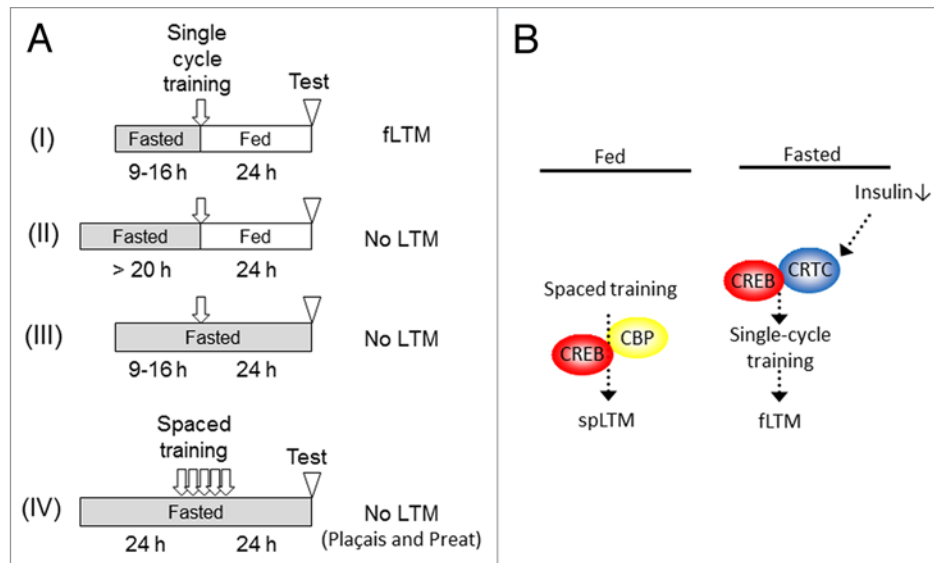


Figure 1. Mild fasting facilitates aversive LTM formation after single-cycle training. **(A)** Schematic diagrams of the experimental design and their effects on LTM. Fasting for 9–16 h prior to single-cycle training induces fLTM **(I)**. Fasting for 20–24 h prior to single-cycle training suppresses fLTM **(II)**. Fasting after single-cycle training suppresses fLTM **(III)**. Fasting before and after spaced training suppresses spLTM **(IV)**.¹² **(B)** In contrast to spLTM, which is dependent on CREB/CBP, fLTM is dependent on CREB/CRTC which is activated through reduced insulin signaling.

If this is the case, fasting should induce LTM formation after single-cycle aversive training as well. As we expected, 1 d memory was significantly enhanced by 9–16 h of fasting prior to single-cycle aversive training (Fig. 1A, I), although longer fasting for 20–24 h did not enhance memory (Fig. 1A, II). The 1 d memory enhancement was impaired by administration of the protein synthesis inhibitor, which blocks de novo gene expression, demonstrating that memory component enhanced by fasting is LTM. We thus referred to fasting-dependent LTM as fLTM, and to canonical LTM generated by spaced training as spLTM (Fig. 1B). We also showed that blocking CREB activity in the memory center, mushroom bodies (MBs) impairs fLTM as well as spLTM. CREB activity was blocked by expressing the dominant negative form of CREB (CREB2b) in MBs. However, in recent report, the same CREB2b induced in MBs using a different expression system did not show any effect on LTM, leading a different conclusion that CREB in MBs is dispensable for LTM formation.⁸ We demonstrated that ectopic expression of CREB2b by their expression system was less inducible than ours, suggesting that their CREB2b expression was not sufficient to block endogenous CREB activity. Thus, our results convinced that, as other

past studies suggested,^{4,5} CREB activity in MBs is required for LTM.

We next sought to determine the molecular pathway required for fLTM formation. CREB requires co-activators, CBP (CREB-binding protein) or CRTC (cAMP-regulated transcriptional co-activator) to activate transcription.⁹ By knockdown experiments targeted to MBs, we found that spLTM is dependent on CBP, and fLTM is dependent on CRTC. Therefore, CBP and CRTC are exclusively required for spLTM and fLTM, respectively (Fig. 1B). Furthermore, expression of constitutive active form of CRTC in MBs is sufficient to recapitulate fLTM formation. We have also demonstrated that appetitive LTM produced by single-cycle appetitive training is dependent on CRTC, but not CBP.

In mammalian metabolic tissues, CRTC is regulated by insulin signaling. During fasting, insulin signaling is suppressed, which in turn activates CRTC.^{9–11} Reduced insulin signaling might be essential for CRTC-dependent fLTM formation. Consistent to this idea, we have shown that, when insulin signaling is suppressed genetically, CRTC is activated in MBs and fLTM is recapitulated

without fasting, mediated by CRTC. Taken together, our results support the model in which fasting reduces insulin signaling and activates CRTC in MBs, thereby inducing fLTM formation (Fig. 1B).

LTM formation requires de novo gene expression, and therefore consumes energy. When food is available, fLTM is suppressed, which would reduce consumption of energy. Once food resources are run out, flies might have to begin a risky foraging, which could occur constantly in the natural environment with limited food resources. The biological meaning of aversive fLTM would be to allow flies to safely perform foraging. However, this seems paradoxical, because flies consume more energy to form fLTM in a fasting state, where they have to obtain energy source. It is known that flies increase the locomotor activity during fasting,³ which consumes energy but could lead to a success in foraging. Thus, consuming more energy during fasting would be one of survival strategies in flies. Under severer fasting conditions, aversive fLTM is not beneficial because flies should care less about aversive stimuli to pursue available food before starving to death. Consistent to this idea, we found that fLTM formation was suppressed by fasting for 20–24 h prior to the training (Fig. 1A, II), and by fasting before and

after the training (Fig. 1A, III). Plaçais et al., reported that continuous fasting after spaced training suppresses dopaminergic neurons, thereby blocking spLTM formation (Fig. 1A, IV).¹² Suppression of dopaminergic neurons may also be important to impair fLTM formation by prolonged fasting. Together, aversive LTMs in flies are finely optimized due to fasting states for their survival. Furthermore, considering that, in the wild, spaced training-like stimuli would not frequently occur, and food resources are always limited, we speculate that flies could preferentially utilize fLTM over spLTM.

Our results suggest that fasting or activation of CRTC might be able to improve memory deficits, by inducing fLTM formation. However, because fasting can either facilitate or suppress LTM formation depending on its duration, fasting itself is not an ideal mean to treat memory deficits. Therefore, pharmacological activation of CRTC can be a reliable method to improve memory deficits. CRTC also plays an important role in metabolism in metabolic tissues.⁹ Thus, future study will be focused on the mechanism of CRTC regulation in the brain, and then we might be able to establish a novel method of memory improvement by manipulating CRTC activity specifically in the brain.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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