

# CREBA and CREBB in two identified neurons gate long-term memory formation in *Drosophila*

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**Episodic events are frequently consolidated into labile memory but are not necessarily transferred to persistent long-term memory (LTM). Regulatory mechanisms leading to LTM formation are poorly understood, however, especially at the resolution of identified neurons. Here, we demonstrate enhanced LTM following aversive olfactory conditioning in *Drosophila* when the transcription factor cyclic AMP response element binding protein A (CREBA) is induced in just two dorsal-anterior-lateral (DAL) neurons. Our experiments show that this process is regulated by protein–gene interactions in DAL neurons: (1) *crebA* transcription is induced by training and repressed by *crebB* overexpression, (2) CREBA bidirectionally modulates LTM formation, (3) *crebA* overexpression enhances training-induced gene transcription, and (4) increasing membrane excitability enhances LTM formation and gene expression. These findings suggest that activity-dependent gene expression in DAL neurons during LTM formation is regulated by CREB proteins.**

memory consolidation | gene regulation | single neurons | CREBA | CREBB

**D**e novo protein synthesis promoted by recurrent learning of ten is a distinguishing feature of long-term memory (LTM) formation (1). A predominant hypothesis is that these new proteins “consolidate” synapse-specific changes in form and function, which represent elemental features of “engrams”—the systems-wide neuronal connections that facilitate network reactivation and memory retrieval (2). Learning-induced gene expression is typically controlled through the integrated action of many *cis*-regulatory elements and their associated transcription factors, of which cyclic AMP response element binding (CREB) proteins are well studied (3). LTM formation involves both activator and repressor transcription factors operating in linked positive and negative feedback loops (4). Whether these molecular events operate together within identified neurons or in separate foci of a memory circuit, however, has been more difficult to establish.

The *Drosophila* model system has been useful for revealing the molecules and circuits that underlie memory formation. Study of aversive olfactory learning continues to identify genes, proteins, and cell signaling cascades in an increasingly more complex memory circuit (5). Flies learn to associate odor (conditioned stimulus [CS]) with electric foot shock (unconditioned stimulus [US]) mediated by projection neurons (PNs) and dopaminergic neurons (DANs), respectively, which converge on the mushroom bodies (MBs), paired neuropils in the central brain. A single training session initiates sequences of neural activity with different durations in specific subpopulations of intrinsic MB neurons (5) that support genetically distinct phases of memory (short-term memory [STM]; middle-term memory [MTM]; anesthesia-resistant memory [ARM]), all of which eventually decay away (i.e., are decremental) (6). In addition to intrinsic MB neurons, these memory phases also depend on several types of extrinsic neurons (dorsal paired medial [DPM]; anterior paired lateral [APL]; protocerebral anterior medial [PAM]; protocerebral posterior lateral [PPL] and others) that form an extended neural network involved in olfactory memory formation (7).

Long-lasting memory of the aversive olfactory task is uniquely produced after repetitive spaced training. Genetic and pharmacological dissections have established that this LTM is dependent on CREB-activated gene expression and the synthesis of new proteins (6, 8). Neural output from the MB is required for LTM formation (and retrieval), and here too several classes of extrinsic (mushroom body output [MBON]) neurons with projections to various other neuropils have been described (7). Although these efferent neurons contribute to an extended network involved in LTM formation, *de novo* synthesis of proteins is required in only a few of them. More recently, an integrated molecular and circuit-based regulation of memory consolidation is being realized in the fly. Our group has identified several neurons in which protein synthesis is required for LTM consolidation. We found that MB output to two DAL neurons in the larger olfactory memory circuit induces protein synthesis- and CREB-dependent LTM. Subsequent feedback from DAL to MB neurons then appears necessary for LTM retrieval (8, 9).

In *Drosophila*, *crebA* and *crebB* genes encode CREB family proteins, CREBA and CREBB (the latter also known as dCREB2) (10, 11). *crebB* generates no fewer than nine distinct isoforms that function as transcriptional repressors and one reported activator

## Significance

**Most animals record only labile memories of single events, whereas the formation of persistent long-term memories (LTMs) usually requires recurrent experiences. Our study distinguishes these different memory types through a deconvolution of molecular/biochemical processes within specific neurons of an identified memory circuit. A training-responsive gene activator, CREBA, engages paired DAL neurons in this circuit by promoting protein synthesis-dependent LTMs, which can otherwise be antagonized by CREBB repressor proteins. Increased CREBA expression or elevated membrane excitability enhances LTMs even after only one training cycle. These findings exemplify a circuit gating mechanism via cellular changes in specific single neurons to distinguish one-time experiences from multiple sessions of learning for storage as persistent memory.**

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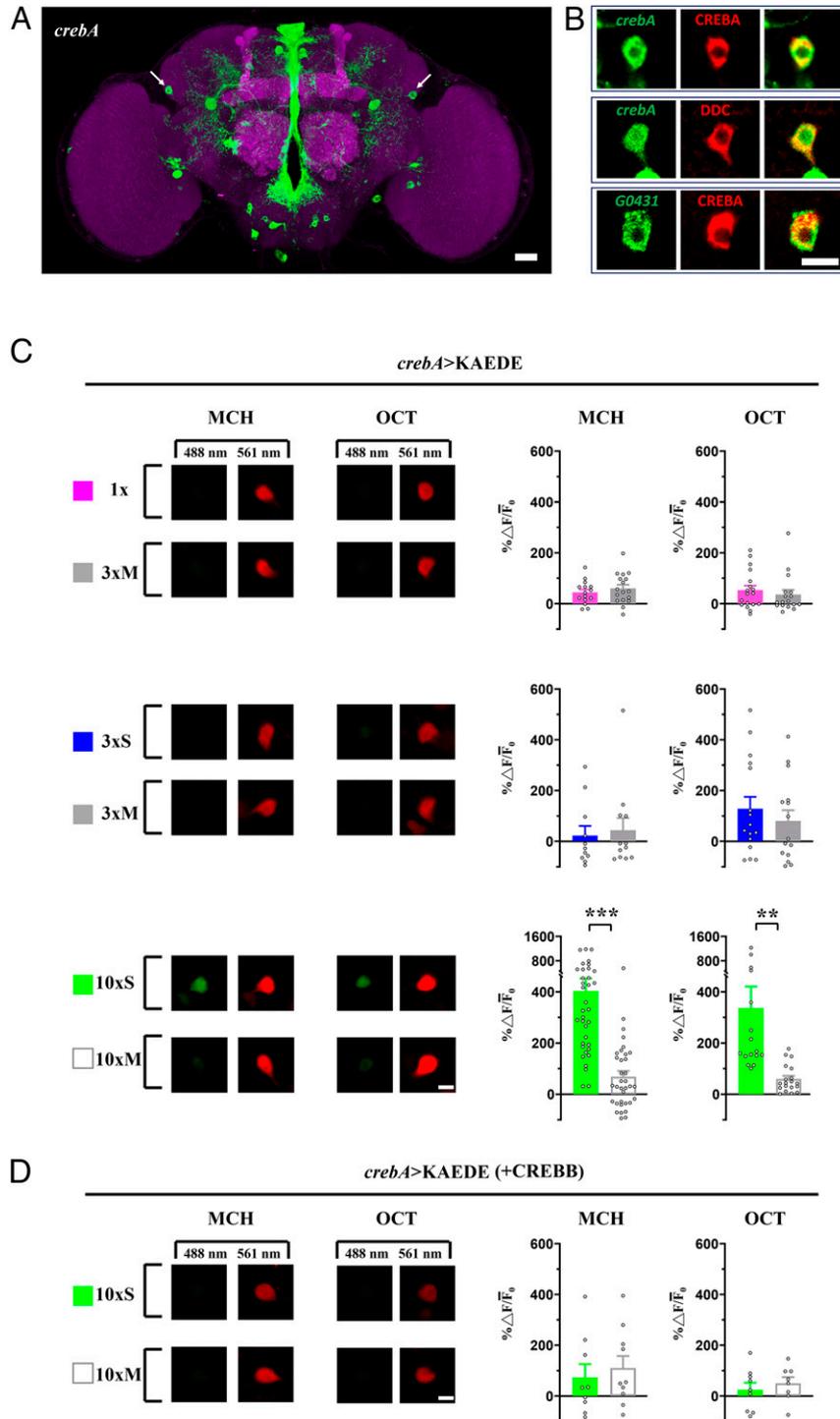
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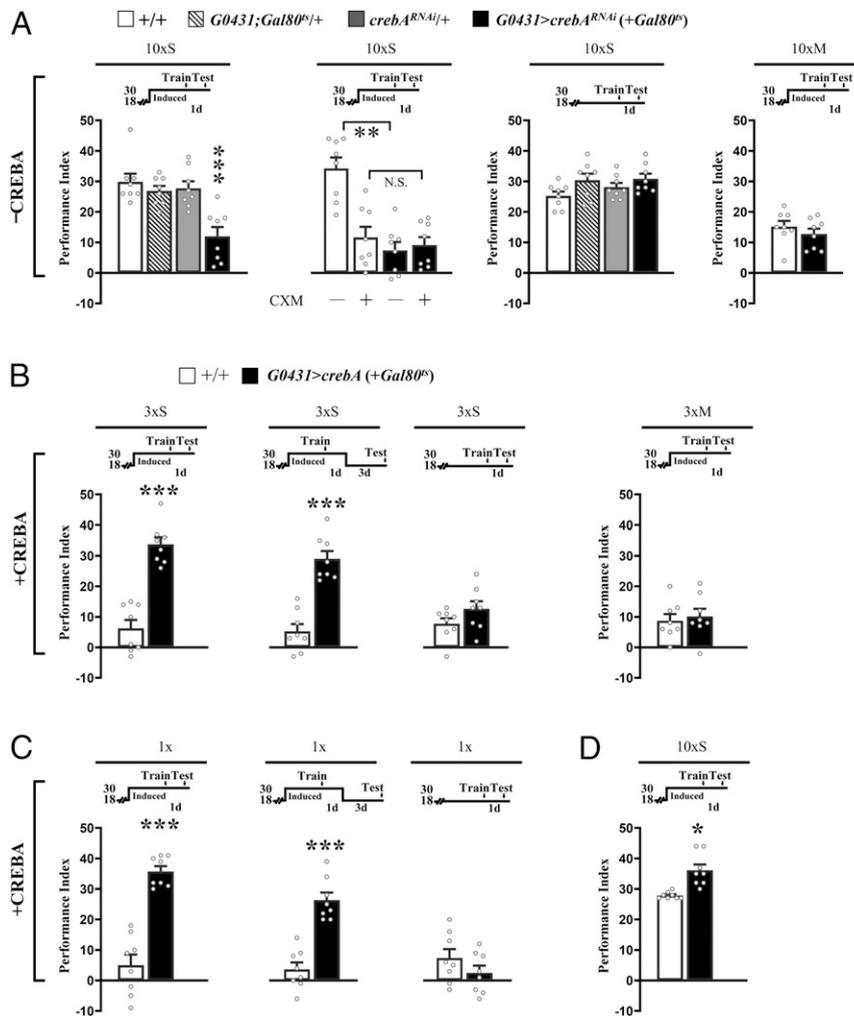
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(12). Activator and repressor *crebB* transgenes encoding these isoforms have been shown to enhance or impair LTM formation, respectively (13–16). In contrast, *crebA* encodes only one protein isoform, which is homologous to mammalian CREB3L1

and has been shown to function as a leucine-zipper transcription factor essential for embryonic development (10, 17). A role for CREBA in adult memory formation has not yet been shown.



**Fig. 1.** *crebA* expression in DAL neurons was enhanced after 10xS training and repressed by CREBB. (A) CREBA in dissected brains (green), counterstained with anti-DLG immunostaining (magenta). Arrows = DAL neurons. (Scale bar, 20  $\mu$ m.) (B) Single optical slices of DAL neuron. *crebA* expression (green) colocalized with CREBA antibody (red) (Top) and with DDC antibody (red) (Middle) and G0431-Gal4 expression (green) colocalized with CREBA antibody (red) (Bottom). (Scale bar, 10  $\mu$ m.) (C) *crebA* promoter activity after 1x, 3xM, and 3xS training with both MCH and OCT as reported by de novo KAEDE fluorescent protein synthesis (Left), estimated by the ratio of new (green, 488 nm) and preexisting (red, 561 nm) proteins (%  $\Delta F/F_0$ ) (Right). For each brain, single DAL neuron optical slices were imaged under identical conditions. Training effects of 1x compared with 3xM (Top) and effects of 3xS compared with 3xM (Middle) were not significant. The *crebA* promoter was activated after 10xS but not 10xM (Bottom). (Scale bar, 10  $\mu$ m.) (D) *crebA* activity was repressed by *crebB* overexpression (+CREBB) after 10xS as compared with 10xM training. (Scale bar, 10  $\mu$ m.) Bars represent mean  $\pm$  SE,  $n \geq 8$ /bar. \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .



**Fig. 2.** CREBA bidirectionally regulates LTM in DAL neurons. (A) Acute RNAi down-regulation of CREBA ( $-CREBA$ ) in DAL impaired 1-d memory after 10 $\times$ S (Left). LTM was similarly blocked by CXM feeding (Center Left). Memory was unaffected at 18  $^{\circ}$ C after 10 $\times$ S (Center Right) and at 30  $^{\circ}$ C after 10 $\times$ M (Right). (B) Overexpressing *crebA* (+CREBA) in DAL neurons at 30  $^{\circ}$ C enhanced 1-d memory after 3 $\times$ S (Left) and persisted at least 4 d (Center Left). Memory was unaffected at 18  $^{\circ}$ C after 3 $\times$ S (Center Right) and at 30  $^{\circ}$ C after 3 $\times$ M (Right). (C) Similarly, overexpressing *crebA* in DAL neurons enhanced 1-d memory after 1 $\times$  (Left), persisting at least 4 d (Center). Memory was unaffected at 18  $^{\circ}$ C after 1 $\times$  (Right). (D) Overexpressing *crebA* in DAL neurons enhanced 1-d memory after 10 $\times$ S. In all figures, temperature control schedules are indicated (Top). Flies were raised at 18  $^{\circ}$ C and then transferred to 30  $^{\circ}$ C to remove GAL4 inhibition by GAL80<sup>ts</sup> for 3 d before training. Bars represent mean  $\pm$  SE,  $n = 8$ /bar. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

Here, we demonstrate a biochemical gating mechanism at the single neuron level within a memory circuit in which antagonistic regulatory events and changes in neural excitability lead to LTM formation. Our results show that LTM formation depends on transcriptional activation by CREBA in DAL neurons. Endogenous “reporter” genes, *calcium/calmodulin-dependent protein kinase II* (*CaMKII*) and *period* (*per*), are up-regulated specifically during recurrent learning (spaced training), by increased neuronal excitability during LTM consolidation, or by ectopic overexpression of *crebA*. Moreover, we show that CREBB can antagonize transcriptional activation by CREBA in DAL neurons, suggesting that interactions between CREBA and CREBB in DAL neurons together modulate LTM formation in flies.

## Results

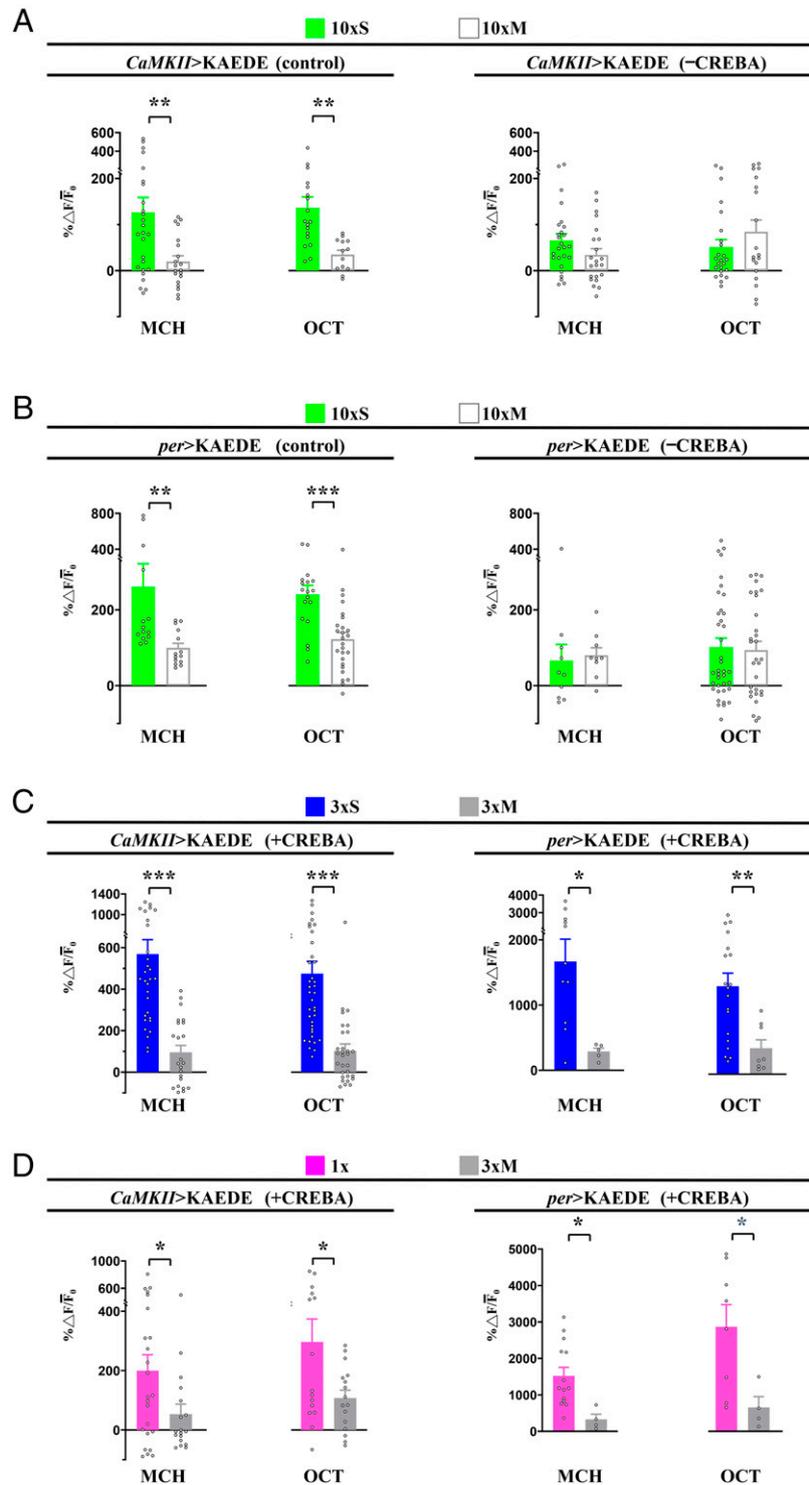
**CREBA in DAL Neurons Positively Regulates LTM Formation.** We first established a cellular function for CREBA. In a mouse F9 teratocarcinoma cell culture assay, significant PKA-dependent transcriptional activation of a *CRE-luciferase* transgene by CREBA was observed (SI Appendix, Fig. S1). Further, this up-regulation was antagonized by cotransfection of a CREBB repressor. To identify CREBA-positive neurons in the fly brain, we obtained and

confirmed a *Gal4* insertion in the *crebA* gene (BG00224; Berkeley *Drosophila* Genome Project [BDGP]) (SI Appendix, Table S1). *crebA-Gal4* driving a *UAS-mCD8::GFP* green fluorescent protein transgene revealed a sparse neuronal pattern that included the DAL neurons (Fig. 1A). These observations were verified by (1) immunostaining with CREBA antibody (Fig. 1B, Top and SI Appendix, Fig. S2A), (2) colocalized *crebA* expression with Dopa Decarboxylase (DDC) immunostaining known to label DAL neurons (Fig. 1B, Middle), and (3) CREBA immunostaining with the DAL neuron-specific driver *G0431-Gal4* (Fig. 1B, Bottom) (8). Strong and specific expression in DAL neurons further motivated our investigation of CREBA in the context of LTM formation.

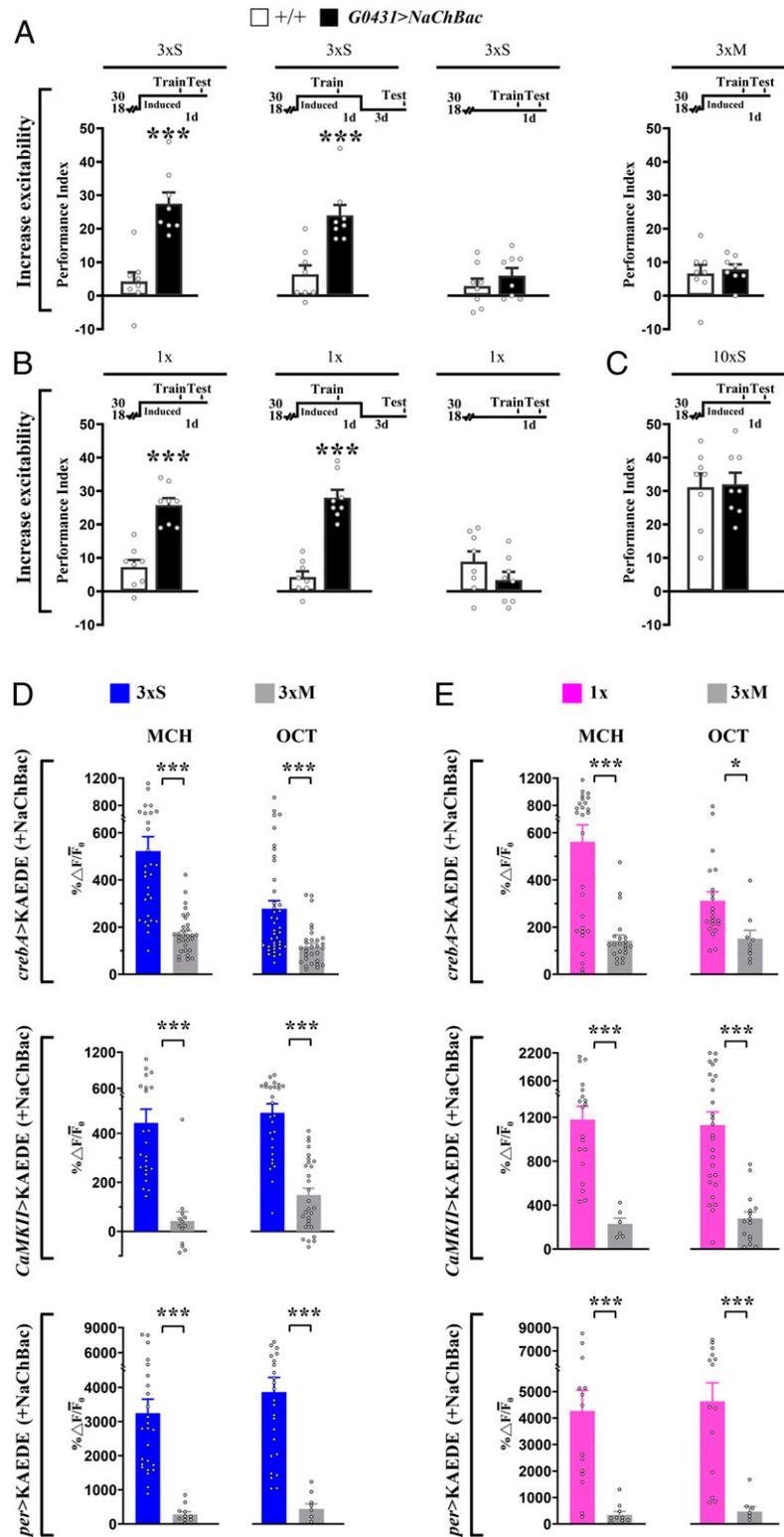
We used a photoconvertible fluorescent KAEDE reporter transgene in DAL neurons to monitor the effect of training on *crebA* expression. After converting preexisting green KAEDE into red via ultraviolet (UV) irradiation prior to training, we measured the accumulation of newly synthesized green KAEDE driven by *crebA-Gal4* after training (8). New expression of *crebA* was not detected after one training session (1 $\times$ ) or three spaced training sessions (3 $\times$ S) (Fig. 1C, Top two rows) but was elevated after 10 spaced training sessions (10 $\times$ S) (Fig. 1C, Bottom row). In a manner analogous to the in vitro

results above, we overexpressed *crebB* in DAL neurons and observed repression of 10xS training-induced *crebA* expression (Fig. 1D). Thus, CREBB appears to antagonize the transcriptional activity of *crebA* in vivo in DAL neurons.

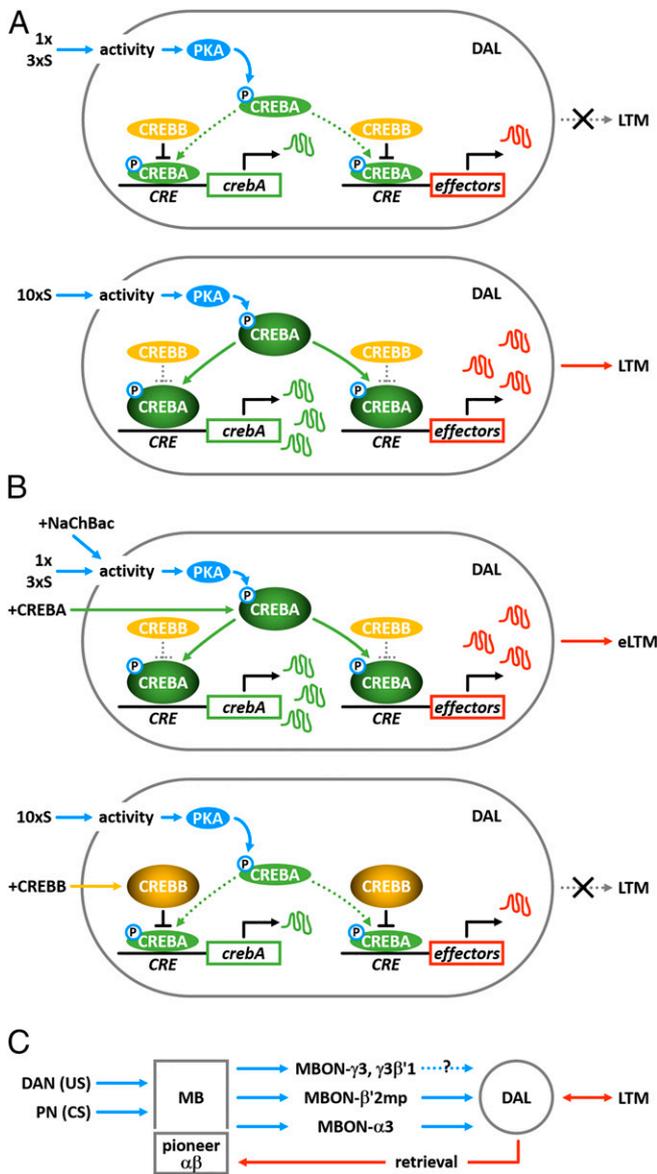
Using adult stage-specific transgenic RNA interference (RNAi) targeted by the *Gal4/UAS* system to knock down CREBA in DAL neurons under the temporal control of a temperature-sensitive *tub-Gal80<sup>ts</sup>* encoded protein (the inhibitory effect of GAL80<sup>ts</sup>



**Fig. 3.** CREBA bidirectionally regulates *CaMKII* and *per* transcription in DAL neurons. (A) The *CaMKII* promoter was activated in DAL neurons 24 h after 10xS but not after 10xM, as reported by de novo KAEDE fluorescent protein synthesis (Left) (refer to Fig. 1 for details). Elevated de novo KAEDE synthesis was suppressed following RNAi down-regulation of CREBA (-CREBA, Right). (B) The *per* promoter was similarly activated in DAL neurons 24 h after 10xS but not 10xM (Left). This activity was also abolished by RNAi down-regulation of CREBA (Right). (C and D) By contrast, levels of *CaMKII* (Left) and *per* (Right) gene activation were elevated by *crebA* overexpression (+CREBA) after 3xS and 1x but not after 3xM. Flies were raised at 18 °C and then transferred to 30 °C to remove GAL4 inhibition by GAL80<sup>ts</sup> for 3 d before training. Refer to *SI Appendix, Figs. S3 and S4* for representative images. Bars represent mean ± SE,  $n \geq 4$ /bar. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .



**Fig. 4.** Increased DAL neuron membrane excitability activates genes and enhances LTM. (A) *NaChBac* overexpression (+NaChBac) increased DAL neurons excitability and elevated 1-d memory after 3xS (Left), persisting for at least 4 d (Center Left). Memory was unaffected at 18 °C after 3xS (Center Right). Memory was similarly unaffected after 3xM (Right). (B) *NaChBac* overexpression also elevated 1-d memory after 1x (Left), persisting for at least 4 d (Center). Memory was unaffected at 18 °C after 1x (Right). (C) *NaChBac* overexpression in DAL neurons had no effect on 1-d memory after 10xS. (D and E) *crebA* (Top), *CaMKII* (Middle), and *per* (Bottom) activity in DAL neurons as reflected by de novo KAEDE synthesis (see Fig. 1) was elevated by *NaChBac* overexpression after 3xS and 1x. Refer to *SI Appendix, Fig. S5* for representative images. Bars represent mean  $\pm$  SE,  $n \geq 6$ /bar. \* $P < 0.05$ ; \*\*\* $P < 0.001$ .



**Fig. 5.** CREBA and CREBB interactions regulate transcription in DAL neurons and gate LTM formation. (A) In normal flies, 1x or 3xS training (Top) induces neural activity in the memory circuit, which includes DAL neurons. Increased neural activity elevates cAMP signaling and activates PKA, which then directly or indirectly leads to phosphorylation and activation of CREBA. However, basal levels of CREBB repressor inhibit activated CREBA from inducing effector gene expression, thereby blocking LTM formation. With 10xS training (Bottom) activated CREBA accumulates to levels sufficient to overcome basal CREBB repression and promotes transcription of downstream effector “memory genes.” One downstream gene is *crebA* itself, establishing a putative positive feedback loop and contributing to further CREBA accumulation. CREBA then induces (further) transcription of downstream effector genes, which yields long-lasting changes in intrinsic neuronal excitability and/or synaptic plasticity during memory consolidation. (B) Transgenic overexpression of *crebA* (+CREBA) or sodium channels (+NaChBac) (Top) provides sufficient CREBA or activity (respectively) to overcome basal CREBB antagonism, establish the CREBA-positive feedback loop, and induce effector gene expression, even with subthreshold 1x or 3xS training, hence the appearance of enhanced LTM (eLTM). Conversely, transgenic overexpression of *crebB* (+CREBB) (Bottom) yields enough CREBB to block activation of the CREBA feedback loop and transcription of downstream effector genes even after 10xS training, thereby preventing LTM formation. (C) DAL neurons contribute to a circuit-level feedback loop among neurons intrinsic and extrinsic to MBs during olfactory memory consolidation and retrieval. The association of odor (CS) via PNs and foot shock (US) via DANs first is registered as an increase in neural activity within MBs, which then promotes a persistent neural trace

protein on GAL4 expression at 18 °C is inactivated at 30 °C), we found an impairment in LTM formation after 10xS training (Fig. 2 A, Left). We observed no further reduction in 1-d memory after spaced training when flies were fed the protein synthesis inhibitor cycloheximide (CXM) (Fig. 2 A, Middle Left), indicating that the CREBA knockdown specifically blocked LTM (16). Control flies kept at 18 °C after 10xS training or at 30 °C after 10xM training were unaffected (Fig. 2 A, Middle Right and Right).

These results suggested that CREBA is a positive regulator of LTM formation. We tested this notion directly by overexpressing a *crebA* transgene and then subjecting flies to suboptimal training (3xS) (13). Along with *crebA*, we also tested two other putative activator transgenes and a repressor transgene encoded by *crebB* (13–16). Of these four candidate transgenes, adult stage-specific overexpression targeted to DAL neurons enhanced LTM only for *crebA* (Fig. 2 B, Left and SI Appendix, Fig. S2B). This effect of *crebA* persisted for at least 4 d (Fig. 2 B, Middle Left), while control flies kept at 18 °C after spaced training or at 30 °C after massed training were unaffected (Fig. 2 B, Middle Right and Right). *crebA*-enhanced LTM was also observed after 1x training and somewhat after 10xS training (Fig. 2 C and D).

Induced transgenic expression of *crebA* yielded immunopositive signals concentrated in the nuclei of DAL neurons, compared with sparse cytosolic signals in control flies (SI Appendix, Fig. S2C). Induced transgenic expression of *crebA<sup>RNAi</sup>*, on the other hand, decreased immunopositive signals in DAL neurons (but not in another central brain structure, the ellipsoid body [EB]) (SI Appendix, Fig. S2D). Together, these data suggest that LTM formation in DAL neurons is positively regulated by CREBA.

**CREBA Modulates *CaMKII* and *per* Transcription.** *CaMKII* and *per* are two genes, disruption of which are known to impair LTM and which appear to be transcriptionally regulated by CREBB (8). We were interested to know whether they were regulated by CREBA in DAL neurons during LTM formation. Consistent with their established roles in LTM formation, training-induced transcription of *CaMKII-KAEDE* or *per-KAEDE* in control flies was apparent after 10xS training. When transgenic *crebA<sup>RNAi</sup>* flies were subjected to 10xS training, however, transcription of these downstream genes was not observed (Fig. 3 A and B and SI Appendix, Fig. S3A). When transgenic *crebA* was overexpressed, however, *CaMKII* or *per* expression was seen after 3xS or even 1x training (Fig. 3 C and D and SI Appendix, Fig. S3B), but not without *crebA* overexpression after subthreshold 3xS or 1x training (SI Appendix, Fig. S4). Not surprisingly, overexpression of *CaMKII* or *per* transgenes themselves was not sufficient to enhance LTM after 3xS training, while overexpression of *crebA* alone was (SI Appendix, Fig. S2B). Presumably, CREBA regulates an ensemble of effector genes, the modulation of any one of which is not sufficient to enhance LTM. Nonetheless, these data show that CREBA positively regulates CREB-responsive downstream genes after space training.

#### Activity-Dependent Gene Expression in DAL Neurons Gates LTM Formation.

In our experiments above, training appeared to induce the transcription of *crebA* and its downstream effector genes, *CaMKII* and *per*. Did training, then, produce these cellular responses via an increase in neuronal activity, as is the case in other model systems (18, 19)? We explored this notion by increasing membrane excitability (and thereby the propensity of neural activity) in DAL neurons via ectopic expression of a sodium channel transgene, *UAS-NaChBac*, under temporal control of *tub-Gal80<sup>ts</sup>*. When the

among intrinsic and extrinsic MB neurons. Persistent output from MBs then drives neural activity directly or indirectly between MB and DAL neurons via several MBONs. After CREBA-induced changes in gene expression within DAL neurons is complete, output from DAL neurons projecting back to the dendrites of pioneer  $\alpha\beta$  neurons of the MB is required for memory retrieval.

inhibitory effect of GAL80<sup>ts</sup> was inactivated at 30 °C before training, transgenic expression of *NaChBac* was sufficient to enhance 1-d memory after 3×S or 1× training (Fig. 4 *A* and *B*, *Left*). This manipulation had no effect on 1-d memory after 3×M training (Fig. 4 *A*, *Right*), after 10×S training (Fig. 4C) or after 3×S or 1× training in transgenic flies kept at 18 °C when active GAL80<sup>ts</sup> blocked expression of the *UAS-NaChBac* transgene (Fig. 4 *A*, *Middle Right* and Fig. 4 *B*, *Right*). Finally, enhanced memory after 3×S or 1× training persisted for at least 4 d, which is 3 d after flies were shifted back to 18 °C when GAL80<sup>ts</sup> again blocked further expression of *UAS-NaChBac* (Fig. 4 *A*, *Middle Left* and Fig. 4 *B*, *Middle*). These latter results imply that training-induced neuronal activity is required to enhance LTM during acquisition and/or memory consolidation but not during memory retrieval. Finally, we extended these observations to the cellular level by showing that *crebA-KAEDDE*, *CaMKII-KAEDDE*, or *per-KAEDDE* each was induced after 3×S or 1× training when *NaChBac* was overexpressed (Fig. 4 *D* and *E* and *SI Appendix*, Fig. S5 *A* and *B*). Thus, these transcriptional responses appear to be activity-dependent during LTM formation.

## Discussion

CREBA and CREBB both are expressed in DAL neurons (20), and transgenic manipulations of CREBB have shown an impairment of 1-d memory after 10×S training (8). These studies, however, did not investigate a functional role for CREBA in DAL neurons and, in particular, did not query whether LTM might be enhanced. Here, we have focused on a role for CREBA in LTM formation. We first established in vitro that CREBA induced expression of a *CRE-luciferase* reporter gene in a PKA-dependent manner and was blocked by CREBB (*SI Appendix*, Fig. S1). Then, we used CREBA antibody, a DAL specific *Gal4* driver and a *crebA*-driven *KAEDDE* reporter to confirm that CREBA not only was expressed in DAL neurons but also responded transcriptionally to 10×S (but not 3×S or 1×) training (Fig. 1 *A–C*). In this in vivo context, we also showed that 10×S training-induced expression of *crebA* in DAL neurons was antagonized by overexpression of a *crebB* (repressor) transgene (Fig. 1D).

These observations suggested that CREBA in DAL neurons might serve as a positive regulator of protein synthesis-dependent LTM. Indeed, inducible transgenic manipulations of *crebA* only in DAL neurons were sufficient to impair 1-d memory after 10×S training (similar to inhibition of protein synthesis) using *crebA<sup>RNAi</sup>*, or to enhance 1-d memory after 1× or 3×S training by overexpressing wild-type *crebA*. Importantly, LTM remained enhanced 4 d after 1× or 3×S training even when induction of transgenic *crebA* ceased 3 d earlier (Fig. 2). Together, these results suggest that CREBA in DAL neurons is involved in learning and/or memory consolidation but not necessarily in memory retrieval.

*CaMKII* and *per* are two “downstream” genes that are CREB responsive, are expressed in DAL neurons and impair LTM when disrupted (8). Using *CaMKII* and *per*-driven *KAEDDE* reporter transgenes, we have shown that expression of both genes is induced normally after 10×S training, is blocked after such training by induced expression of a *crebA<sup>RNAi</sup>* and is enhanced after 1× or 3×S training when a *crebA* transgene is inducibly expressed (Fig. 3). Here too, these transgenic manipulations are not required for *CaMKII* or *per* expression and LTM to persist for 4 d after training and 3 d after transgenic manipulations are blocked.

This role for CREBA in DAL neurons during learning and memory consolidation suggested that the transcriptional response might be activity dependent. We explored this possibility by expressing a *NaChBac* transgene in DAL neurons, which served to increase

membrane excitability and presumably neural activity in response to training. We found that induced expression of *NaChBac* in DAL neurons was sufficient to enhance 1-d memory and to enhance expression of *crebA*, *CaMKII*, and *per* after 1× or 3×S training (Fig. 4). Together, these observations have suggested a model, which we describe in Fig. 5. The model illustrates how CREBA and CREBB interact to regulate transcription in DAL neurons and the activity-dependent transcriptional response to gate LTM formation.

CREB-dependent long-term memory formation first was shown in *Drosophila* using inducible transgenes, which were expressed throughout the fly (16). Acute expression of a transgenic *crebB* repressor blocked LTM after 10×S training, whereas similar manipulations of a synthetic *crebB* activator transgene enhanced LTM (13–16). An early attempt to identify specific neurons underlying LTM implicated MBs, wherein MB-specific transgenic expression of a *crebB* repressor was reported to impair LTM after 10×S training (21). A subsequent study revealed, however, that this behavioral impairment derived from developmental defects in MB structure due to chronic expression of the *crebB* transgene. In contrast, induced expression of a *crebB* transgene only in adult-stage MBs did not impair LTM and did not produce any developmental defects (8). In neither study was a positive (CREB) regulator identified nor was enhanced LTM evaluated.

One-trial learning is usually insufficient to produce protein synthesis-dependent LTM, except for those experiences important for survival (22, 23). Here, we have demonstrated that LTM can form after a single training session when “memory genes” in DAL neurons are genetically manipulated. Learning-related and CREB-dependent changes in membrane excitability are well known and explain aspects of neuronal plasticity underlying memory consolidation (19). Regulation of ion channel genes by CREBA and CREBB transcription factors, for example, modulate plasticity in alcohol tolerance in *Drosophila* (24). CREB-dependent regulation of gene expression in DAL neurons appears sufficient to promote systems memory consolidation by modulating neural excitability. Further studies may elucidate whether neural circuits involved in motivation and attention also modulate DAL neurons during LTM formation (25, 26) and whether such prolonged neural activity also produces synaptic plasticity in DAL neurons (19, 27).

## Materials and Methods

In this report we used an automated olfactory aversive learning task (6) and assessed LTM after elevating or blocking CREBA-dependent gene activation in temporally and spatially targeted domains. Similarly, we bidirectionally modulated the expression of CREBA in DAL neurons to examine the effects on training-responsive gene transcription using a *Gal4*-targeted UV-sensitive *KAEDDE* reporter system (8). Spatial and temporal regulation of Na<sup>+</sup> channel activity with transgene overexpression in DAL neurons was used to evaluate impacts of elevated membrane excitability on LTM and on training-responsive genes using the *KAEDDE* reporter system (8). See *SI Appendix*, *Materials and Methods* for details of fly strains, reagents, and all procedures.

**Data Availability.** All study data are included in the article and/or *SI Appendix*.

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