

---

*This copy is for your personal, non-commercial use only.*

---

**If you wish to distribute this article to others**, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

**Permission to republish or repurpose articles or portions of articles** can be obtained by following the guidelines [here](#).

**The following resources related to this article are available online at [www.sciencemag.org](http://www.sciencemag.org) (this information is current as of March 14, 2011):**

**Updated information and services**, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/331/6021/1207.full.html>

**Supporting Online Material** can be found at:

<http://www.sciencemag.org/content/suppl/2011/03/02/331.6021.1207.DC1.html>

This article **cites 22 articles**, 9 of which can be accessed free:

<http://www.sciencemag.org/content/331/6021/1207.full.html#ref-list-1>

This article appears in the following **subject collections**:

Neuroscience

<http://www.sciencemag.org/cgi/collection/neuroscience>

of plasmablasts by swIg<sup>+</sup> but not IgM<sup>+</sup> (Fig. 4G) memory cells after challenge. Thus, IgM<sup>+</sup> memory cells were not intrinsically hyporesponsive in immune hosts but were functionally inhibited by antigen-specific immunoglobulins produced either before challenge by plasma cells or after challenge by memory cell–derived plasmablasts. Inhibitory FcγRIIb (23) was probably not involved, because IgM<sup>+</sup> and swIg<sup>+</sup> memory cells expressed equal amounts of this receptor (fig. S2A).

PE- and allophycocyanin-specific naïve B cells accounted for about 1:5000 and 1:25,000 of all B cells in mice. These high frequencies are likely related to the presence of multiple epitopes on these large multimeric proteins (24). It will be of interest to use the enrichment approach to enumerate naïve B cells specific for monomeric antigens, although antigen multimerization may be required (25).

Naïve PE-specific B cells generated IgM<sup>+</sup> memory cells after immunization with PE and the adjuvants CFA (Fig. 2K), lipopolysaccharide, or alum (fig. S4), which suggested that this is a general feature of the primary immune response. These memory cells had few mutations in their IgM molecules, which indicated inefficient selection in germinal centers. It is possible that these poorly mutated IgM molecules had a high enough natural affinity for PE to trigger memory cell differentiation before extensive somatic mutation could occur.

The remarkable stability of the IgM<sup>+</sup> memory cells compared with swIg<sup>+</sup> memory cells was not related to selective enrichment of IgM<sup>+</sup> cells (fig. S5A), migration of swIg<sup>+</sup> cells to bone marrow (fig. S5B), or homeostatic proliferation (fig. S5C). The instability of swIg<sup>+</sup> memory cells may be

related to inhibitory signals through TACI (fig. S2A) (21) or deleterious off-target mutations induced by AID (26). Despite being shorter-lived and outnumbered by IgM<sup>+</sup> memory cells, swIg<sup>+</sup> memory cells dominated the secondary response because of a capacity to be activated in the presence of high-affinity neutralizing serum immunoglobulin. However, even swIg<sup>+</sup> memory cells could not produce germinal center cells, perhaps because their plasmablast progeny secreted enough immunoglobulin to clear the antigen very quickly. The failure to be activated efficiently in the face of immunoglobulin from swIg<sup>+</sup> memory cells or plasma cells suggests that IgM<sup>+</sup> memory cells do not contribute to the secondary response until these molecules decline. Serum immunoglobulin induced by certain subunit vaccines has been reported to decrease over time in humans (27), which suggests that IgM<sup>+</sup> cells could become the reservoirs of humoral immune memory for these vaccines. Because of their lower affinity and ability to produce germinal center cells, IgM<sup>+</sup> memory cells may also be useful for responding to antigenic variants produced by mutating pathogens.

#### References and Notes

1. L. J. McHeyzer-Williams, M. G. McHeyzer-Williams, *Annu. Rev. Immunol.* **23**, 487 (2005).
2. D. M. Tarlinton, *Immunol. Cell Biol.* **86**, 133 (2008).
3. R. W. Maul, P. J. Gearhart, *Adv. Immunol.* **105**, 159 (2010).
4. T. Yoshida *et al.*, *Immunol. Rev.* **237**, 117 (2010).
5. S. G. Tangye, K. L. Good, *J. Immunol.* **179**, 13 (2007).
6. M. Muramatsu *et al.*, *Cell* **102**, 553 (2000).
7. I. Dogan *et al.*, *Nat. Immunol.* **10**, 1292 (2009).
8. T. V. Le, T. H. Kim, D. D. Chaplin, *J. Immunol.* **181**, 6027 (2008).
9. M. M. Tomayko, N. C. Steinel, S. M. Anderson, M. J. Shlomchik, *J. Immunol.* **185**, 7146 (2010).

10. Materials and methods are available as supporting material on Science Online.
11. K. Hayakawa, R. Ishii, K. Yamasaki, T. Kishimoto, R. R. Hardy, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 1379 (1987).
12. B. Schitteck, K. Rajewsky, *Nature* **346**, 749 (1990).
13. F. Martin, A. M. Oliver, J. F. Kearney, *Immunity* **14**, 617 (2001).
14. C. C. Goodnow *et al.*, *Nature* **334**, 676 (1988).
15. J. Bell, D. Gray, *J. Exp. Med.* **197**, 1233 (2003).
16. A. Ridderstad, D. M. Tarlinton, *J. Immunol.* **160**, 4688 (1998).
17. D. J. Todd *et al.*, *J. Exp. Med.* **206**, 2151 (2009).
18. J. Freund, J. Casals, E. P. Hosmer, *Proc. Soc. Exp. Biol. Med.* **37**, 509 (1937).
19. D. Tarlinton, *Nat. Rev. Immunol.* **6**, 785 (2006).
20. F. Mackay, M. P. Cancro, *Semin. Immunol.* **18**, 261 (2006).
21. D. Seshasayee *et al.*, *Immunity* **18**, 279 (2003).
22. S. M. Anderson, M. M. Tomayko, A. Ahuja, A. M. Haberman, M. J. Shlomchik, *J. Exp. Med.* **204**, 2103 (2007).
23. J. V. Ravetch, *Curr. Opin. Immunol.* **9**, 121 (1997).
24. J. Y. Liu, T. Jiang, J. P. Zhang, D. C. Liang, *J. Biol. Chem.* **274**, 16945 (1999).
25. J. Newman, J. S. Rice, C. Wang, S. L. Harris, B. Diamond, *J. Immunol. Methods* **272**, 177 (2003).
26. M. G. Hasham *et al.*, *Nat. Immunol.* **11**, 820 (2010).
27. I. J. Amanna, N. E. Carlson, M. K. Slifka, *N. Engl. J. Med.* **357**, 1903 (2007).
28. We thank J. Walter and R. Speier for expert technical assistance and all members of the Jenkins lab for helpful discussions. This work was supported by grants from the NIH (R01 AI036914 and R37 AI027998 to M.K.J., F32 AI091033 and T32 CA009138 to J.T.), and the Intramural Research Program of the NIH, National Institute on Aging (R.W.M. and P.J.G.).

#### Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1201730/DC1  
Materials and Methods  
Figs. S1 to S5  
Tables S1 and S2  
References

15 December 2010; accepted 28 January 2011  
Published online 10 February 2011;  
10.1126/science.1201730

# Enhancement of Consolidated Long-Term Memory by Overexpression of Protein Kinase M $\zeta$ in the Neocortex

Reut Shema,<sup>1</sup> Sharon Haramati,<sup>1</sup> Shiri Ron,<sup>1</sup> Shoshi Hazvi,<sup>1</sup> Alon Chen,<sup>1</sup> Todd Charlton Sacktor,<sup>2</sup> Yadin Dudai<sup>1\*</sup>

Memories are more easily disrupted than improved. Many agents can impair memories during encoding and consolidation. In contrast, the armamentarium of potential memory enhancers is so far rather modest. Moreover, the effect of the latter appears to be limited to enhancing new memories during encoding and the initial period of cellular consolidation, which can last from a few minutes to hours after learning. Here, we report that overexpression in the rat neocortex of the protein kinase C isozyme protein kinase M $\zeta$  (PKM $\zeta$ ) enhances long-term memory, whereas a dominant negative PKM $\zeta$  disrupts memory, even long after memory has been formed.

**A**mnesic agents impair fresh memories during encoding and consolidation, and some can block reactivated long-term memories at reconsolidation (1). Furthermore, se-

lective brain lesions result in extensive loss of remote memories (2). Some agents have been proposed as potential memory enhancers, but their beneficial effect seems to be limited to the

encoding and immediate consolidation period (3–7). The importance of memory enhancement for treating cognitive decline calls for an intensive exploration of the effect of manipulating components of the memory storage machinery on memory performance.

Protein kinase M $\zeta$  (PKM $\zeta$ ) is a persistently active, atypical protein kinase C isoform that is critical for maintaining the storage of long-term memory long after its initial consolidation (8, 9). We have previously reported that inhibition of PKM $\zeta$  in the insular cortex (IC) of the behaving rat by the pseudosubstrate zeta inhibitory peptide (ZIP) leads to erasure of long-term memory of conditioned taste aversion (CTA), an associative type of memory, up to at least 3 months after encoding, without affecting the ability of the rat to relearn the same association and without

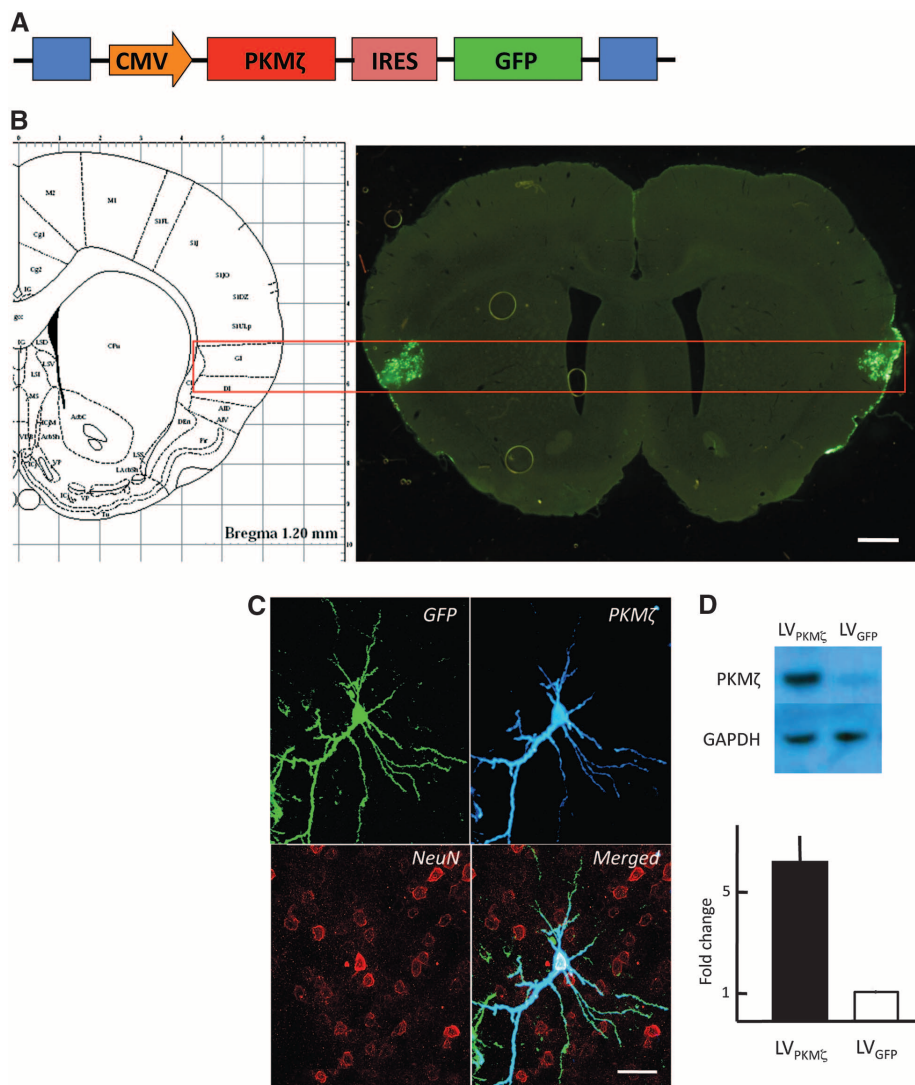
<sup>1</sup>Department of Neurobiology, Weizmann Institute of Science, Rehovot 76100, Israel. <sup>2</sup>Departments of Physiology, Pharmacology, and Neurology, The Robert F. Furchgott Center for Neural and Behavioral Science, SUNY Downstate Medical Center, Brooklyn, NY 11203, USA.

\*To whom correspondence should be addressed. E-mail: yadin.dudai@weizmann.ac.il

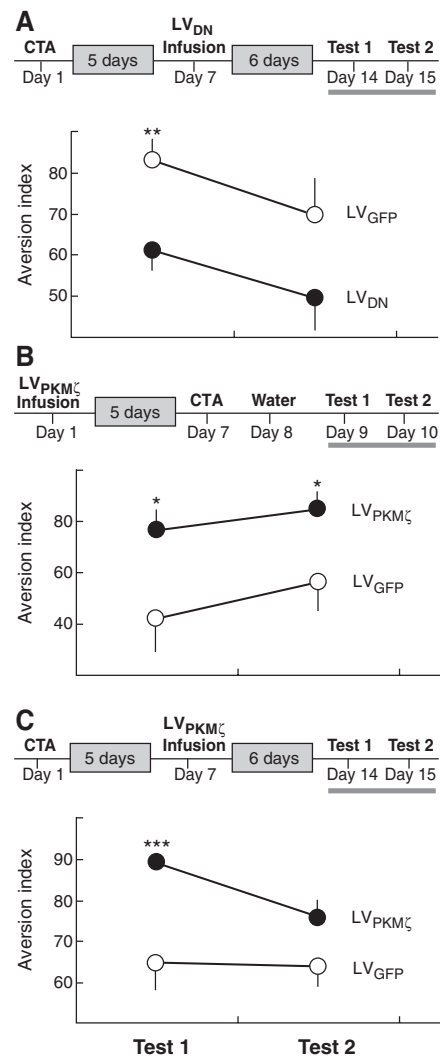
impairing recognition memory (10, 11). The training itself leads to a persistent increase in the level of the endogenous PKM $\zeta$  protein in the IC (fig. S1). We wanted to investigate the effect on CTA memory of targeted modulation of the level of PKM $\zeta$  in the IC. We therefore designed and generated lentiviruses expressing PKM $\zeta$  (LV<sub>PKM $\zeta$</sub> ) or a dominant negative (DN) version of PKM $\zeta$  mutated at the active domain (LV<sub>DN</sub>, PKM $\zeta$ -K281W) (Fig. 1A), and microinfused them, or control lentiviruses (LV<sub>GFP</sub>) (GFP, green fluorescent protein), bilaterally into the IC (Fig. 1, B

and C). Under the conditions used, 43  $\pm$  9% of the neurons (identified by NeuN staining,  $n = 6$  hemispheres) at the core of the injected volume expressed the GFP reporter. Overexpression of the PKM $\zeta$  protein was evident (Fig. 1D).

Rats infected in the IC with LV<sub>DN</sub> 6 days after single-trial CTA training and tested for CTA memory 7 days later displayed a marked reduction in memory (Fig. 2A). In parallel in vitro experiments, the DN inhibited PKM $\zeta$  activity tested on an exogenous kinase substrate (fig. S2). These data support our previous conclusion that



**Fig. 1.** Overexpression of PKM $\zeta$  in the IC. (A) Schematic map of the lentiviral construct designed to overexpress PKM $\zeta$  and GFP. The PKM $\zeta$  gene (or the DN mutation, PKM $\zeta$ -K281W) is under the control of a cytomegalovirus (CMV) promoter, followed by an internal ribosome entry site (IRES) and a GFP reporter. (B) A representative picture of a coronal section of the rat brain (bregma = 1.2 mm, 25  $\mu$ m width section), depicting GFP-infected cells in the bilateral IC. Scale bar, 100  $\mu$ m. (C) A neuron stained with GFP (green), PKM $\zeta$  (blue), and NeuN (red) and the merged picture of all the above. To allow clear single-neuron presentation, the picture was taken toward the periphery of the infected area. Scale bar, 20  $\mu$ m. (D) Upper panel: Western blot with an antibody specific for the catalytic domain of PKM $\zeta$ , depicting PKM $\zeta$  expression in the IC infused with the lentiviral vector containing the PKM $\zeta$  gene (LV<sub>PKM $\zeta$</sub> ), compared to that infused with GFP alone (LV<sub>GFP</sub>). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a loading control. Lower panel: Quantification of the increase in PKM $\zeta$  expression as a consequence of infection with LV<sub>PKM $\zeta$</sub> .



**Fig. 2.** Expression of a DN mutation of PKM $\zeta$  blocks, whereas overexpression of PKM $\zeta$  enhances, long-term memory in the IC of the behaving rat. (A) The IC of rats was infused bilaterally with LV<sub>DN</sub> (Fig. 1) 6 days after CTA training, followed by testing 7 days later (two subsequent tests, a day apart, were used to quantify experimental extinction). Memory was significantly disrupted by DN PKM $\zeta$  overexpression. Here and below, test days are underlined in the protocol flowchart. LV<sub>GFP</sub>,  $n = 9$ ; LV<sub>DN</sub>,  $n = 10$ . (B) LV<sub>PKM $\zeta$</sub>  was infused bilaterally into the IC 6 days before CTA training, using a weak US to circumvent the ceiling effect of the conventional CTA training and hence permit detection of potential memory enhancement (see methods in the SOM). Memory was tested starting 2 days after training. Memory was significantly enhanced, and no extinction was evident. LV<sub>GFP</sub>,  $n = 7$ ; LV<sub>PKM $\zeta$</sub> ,  $n = 9$ . (C) LV<sub>PKM $\zeta$</sub>  was infused bilaterally into the IC 6 days after CTA training, and memory was tested starting a week later. Memory was significantly enhanced by overexpression of PKM $\zeta$ , and extinction is evident. LV<sub>GFP</sub>,  $n = 23$ ; LV<sub>PKM $\zeta$</sub> ,  $n = 28$ . \* $P < 0.05$ , \*\*\* $P < 0.01$ , \*\*\*\* $P < 0.001$ . In (C), the statistics are for experimental versus control on test 1 and experimental on test 1 versus test 2.

the effect of ZIP on CTA memory in the IC is due to the inhibition of PKM $\zeta$  (10, 11).

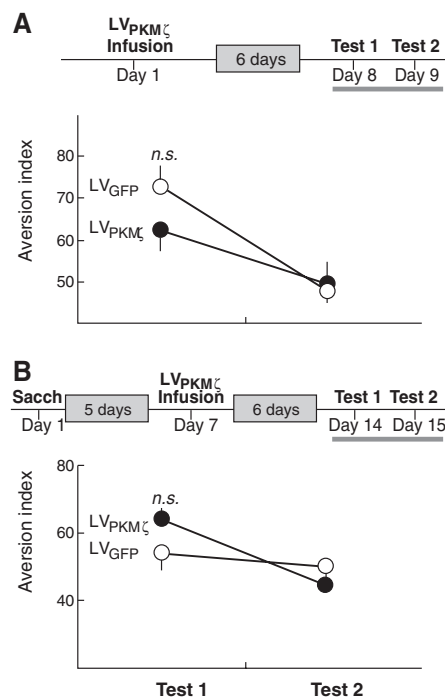
Whereas infection of the IC with LV<sub>DN</sub> disrupted long-term CTA memory, overexpression as a consequence of infection with LV<sub>PKM $\zeta$</sub>  significantly enhanced long-term memory. Memory enhancement was positively correlated with the extent of LV<sub>PKM $\zeta$</sub>  infection in the IC (fig. S3). It was evident both when the infection was performed before CTA training (Fig. 2B) and when performed at day 7 after CTA training (Fig. 2C), a time point when the long-term CTA trace in the IC is already consolidated (12). Because the strong unconditioned stimulus (US, LiCl) used in conventional CTA training results in a ceiling effect that could mask potential memory enhancement, in the LV<sub>PKM $\zeta$</sub>  experiments we used a diluted LiCl solution as the US (13). This US produced little CTA memory when tested at day 7 after training [aversion index (AI) = 64.1  $\pm$  4.7,  $n$  = 7 rats], which is significantly lower than that observed in the PKM $\zeta$ -overexpressing group tested a week later (Fig. 2C; AI = 89.1  $\pm$  2.5,

$P$  < 0.001). Hence overexpression of PKM $\zeta$  did not retard the normal fading of memory but rather enhanced the already faded memory. To exclude the possibility that the overexpression shifted the cortex into a persistently aversive state, in which continual expression of the aversion would mimic stronger CTA memory, we tested the effect of microinfusion of LV<sub>PKM $\zeta$</sub>  into the IC on aversion to a saccharin conditioned stimulus (CS) in the absence of CTA training. No significant effect on aversion was detected when infection was performed in the IC of naïve rats before they had ever encountered saccharin (Fig. 3A) or in the IC of rats that had previously been exposed to saccharin (Fig. 3B). We also did not detect differences in the volume of liquid consumed in the instrumental situation of the CTA conditioning protocol by the rats infected in the IC with LV<sub>PKM $\zeta$</sub>  (total liquid consumed on the first exposure to saccharin, LV<sub>PKM $\zeta$</sub>  = 8.29  $\pm$  0.58 ml, LV<sub>GFP</sub> = 8.36  $\pm$  0.69 ml,  $n$  = 18 and 15, respectively). We thus conclude that overexpression of PKM $\zeta$  in the IC has no significant effect on the sensorimotor or motivational faculties required to express CTA.

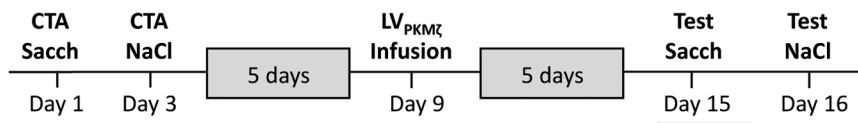
Does PKM $\zeta$  overexpression affect only the most recently acquired memory? The answer is no. When trained consecutively on two CSs with clearly distinguishable taste qualities, saccharin and NaCl (11), overexpression enhanced the long-term memory of both (Fig. 4).

Our data show that whereas inhibition of PKM $\zeta$  in the IC by competition with a DN mutation of the enzyme disrupts long-term CTA memory, overexpression of PKM $\zeta$  in the IC enhances memory, including memory formed long before the enzyme was overexpressed. Recently, a model was proposed to account for the erasure of memory by the inhibition of PKM $\zeta$ , according to which disruption of persistent activity of the enzyme disrupts trafficking of GluA2-containing AMPA receptors into the postsynaptic membrane and hence annuls the use-dependent increase in synaptic efficacy (9, 14). The inhibition of memory by the DN thus makes sense. But how does overexpression of PKM $\zeta$  increase memory already formed?

Considering the functional systems level; that is, what it is that the brain now does differently, three types of possibilities come to mind. One is that overexpression of PKM $\zeta$  enhances global, item-invariant operations, such as attention, incentive valence, or sensory or motor operations, all of which are required for memory expression but are not memory per se. Nevertheless, as noted above, there was no significant effect of infection with LV<sub>PKM $\zeta$</sub>  in the IC on sensorimotor and motivational attributes of either naïve or CS-exposed rats, as manifested in their normal approach to and consumption of saccharin and in their total liquid consumption. Rats whose IC was microinfused with LV<sub>PKM $\zeta$</sub>  6 days before training, as in Fig. 2B, displayed a normal immediate reaction to LiCl, augmenting the conclusion that the treatment and overexpression of PKM $\zeta$  did not significantly alter sensorimotor, including visceral, responses relevant to CTA [supporting online material (SOM)]. Furthermore, in rats whose IC was infected with LV<sub>PKM $\zeta$</sub>  on day 7 after conditioning, the enhanced long-term memory was still capable of experimental extinction, similar to a normal CTA trace obtained after the conventional, one-trial training (15) (Fig. 2C), implying no persistent shift in the reaction to the CS. All in all, the aforementioned data favor an effect on mnemonic functions. We cannot, however, completely exclude the possibility that overexpression of PKM $\zeta$  culminates in alterations of fine-tuning or implicit properties of the system that might amplify memory performance, yet are undetected in the nonmnemonic tests. A second possibility is that overexpression of PKM $\zeta$  results in the enhancement of global, item-invariant memory operations, of the type postulated to take part in memory retrieval, such as retrieval and search mode (16, 17). Finally, a third possibility is that overexpression of PKM $\zeta$  results in strengthening of item-variant memory operations (17), such as specific associations of specific items. The encoding of CTA in the IC was reported to be distributed, and specific associations are estimated to engage plastic changes in about 25% of the neurons (18), suggesting that the level



**Fig. 3.** Overexpression of PKM $\zeta$  in the IC does not alter innate preferences for saccharin. **(A)** The IC of rats was microinfused bilaterally with LV<sub>PKM $\zeta$</sub>  or LV<sub>GFP</sub>, followed by a multiple choice test (see methods in the SOM) for the preference for saccharin versus water on days 8 and 9. There was no significant difference between the groups in their preference for saccharin (see methods in the SOM). LV<sub>GFP</sub>,  $n$  = 16; LV<sub>PKM $\zeta$</sub> ,  $n$  = 17. **(B)** Rats were first exposed to saccharin, followed 6 days later by infusions into the IC of either LV<sub>PKM $\zeta$</sub>  or LV<sub>GFP</sub>. Preference for saccharin was tested 7 and 8 days later. Again, there was no significant difference between the groups in their preference for saccharin. LV<sub>GFP</sub>,  $n$  = 25; LV<sub>PKM $\zeta$</sub> ,  $n$  = 26.



**Fig. 4.** Overexpression of PKM $\zeta$  enhances long-term memory of multiple taste associations. Rats were trained on CTA using saccharin as the CS, and 2 days later using NaCl as the CS. Six days later, either LV<sub>PKM $\zeta$</sub>  (dark gray bars in bar graph) or LV<sub>GFP</sub> (light gray bars in bar graph) was microinfused bilaterally into the IC. The CTA memory tests were administered on days 15 and 16. The LV<sub>PKM $\zeta$</sub>  group showed marked memory enhancement for both associations;  $n$  = 8 in each group. \* $P$  < 0.005.

of overexpression that we obtained in the IC in the present study could affect multiple behaviorally relevant representations. The possibility that we indeed achieved strengthening of item-variant memory operations is perhaps the most exciting, but at this point in time we cannot dissociate item-invariant from item-variant effects, particularly given that overexpression appears to enhance more than one stored association.

As to the hardware implementation level of consolidated memory enhancement by PKM $\zeta$ , it is implausible that this is due to flooding the cortex indiscriminately with the overexpressed enzyme, because this could lead to nonselective change in synaptic weights or even to saturation of use-dependent plasticity. Indeed, saturating amounts of exogenously applied PKM $\zeta$ , when perfused directly into neurons, appear to produce potentiation of all of the cell's synapses (19). However, PKM $\zeta$  synthesized in neurons is captured at recently active synapses, by a process of synaptic tagging, enabling these specific synapses to maintain a persistent state of enhanced efficacy (20). Thus, it is plausible to assume that the overexpressed kinase may augment the endogenous increase (fig. S1). We did observe differential accumulation of overexpressed PKM $\zeta$  in dendritic spines in the IC in vivo (fig. S4), which is consistent with but does not prove the hypothesis. Selectivity resulting in strengthening memory might hence be achieved if the new enzyme molecules synthesized by the overexpressed gene are preferentially attracted to tagged synapses.

The enhancement of memory long after encoding also raises the possibility that synaptic tagging and capture (21, 22) can mold memory over much longer periods of time than previously supposed, a mechanism that might be useful in situations such as the integration of new episodic items into long-term memory schemata (23) or, more generally, memory summation over prolonged periods of time (24).

The observation that overexpression of PKM $\zeta$  enhances memories long after they had been formed renders it plausible to consider PKM $\zeta$  a potential target not only for memory blockers (which might be useful, for example, in treating post-traumatic stress) but also for novel types of memory enhancers in the treatment of amnesia and cognitive decline.

#### References and Notes

1. Y. Dudai, *Annu. Rev. Psychol.* **55**, 51 (2004).
2. P. J. Bayley, J. J. Gold, R. O. Hopkins, L. R. Squire, *Neuron* **46**, 799 (2005).
3. C. Giurgea, *Cond. Reflex* **8**, 108 (1973).
4. E. A. Drier *et al.*, *Nat. Neurosci.* **5**, 316 (2002).
5. U. Staubli, G. Rogers, G. Lynch, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 777 (1994).
6. D. B. Sternberg, K. R. Isaacs, P. E. Gold, J. L. McGaugh, *Behav. Neural Biol.* **44**, 447 (1985).
7. Y.-P. Tang *et al.*, *Nature* **401**, 63 (1999).
8. E. Pastalkova *et al.*, *Science* **313**, 1141 (2006).
9. T. C. Sacktor, *Nat. Rev. Neurosci.* **12**, 9 (2011).
10. R. Shema, T. C. Sacktor, Y. Dudai, *Science* **317**, 951 (2007).
11. R. Shema, S. Hazvi, T. C. Sacktor, Y. Dudai, *Learn. Mem.* **16**, 122 (2009).
12. K. Rosenblum, N. Meiri, Y. Dudai, *Behav. Neural Biol.* **59**, 49 (1993).
13. D. E. Berman, S. Hazvi, J. Stehberg, A. Bahar, Y. Dudai, *Learn. Mem.* **10**, 16 (2003).
14. P. V. Miguez *et al.*, *Nat. Neurosci.* **13**, 630 (2010).
15. M. Eisenberg, T. Kobilo, D. E. Berman, Y. Dudai, *Science* **301**, 1102 (2003).
16. E. Tulving, *Elements of Episodic Memory* (Oxford Univ. Press, Oxford, 1983).
17. Y. Dudai, *Memory from A to Z. Keywords, Concepts and Beyond* (Oxford Univ. Press, Oxford, 2002).
18. S. E. Grossman, A. Fontanini, J. S. Wieskopf, D. B. Katz, *J. Neurosci.* **28**, 2864 (2008).
19. D. S. F. Ling *et al.*, *Nat. Neurosci.* **5**, 295 (2002).
20. S. Sajikumar, S. Navakkode, T. C. Sacktor, J. U. Frey, *J. Neurosci.* **25**, 5750 (2005).
21. U. Frey, R. G. M. Morris, *Nature* **385**, 533 (1997).
22. K. C. Martin *et al.*, *Cell* **91**, 927 (1997).
23. D. Tse *et al.*, *Science* **316**, 76 (2007).
24. A. Aydin, J. M. Pearce, *Anim. Learn. Behav.* **25**, 108 (1997).
25. The support of the Israel-U.S. Binational Science Foundation (Y.D and T.C.S), the Nella and Leon Benoziyo Center for Neurological Diseases (Y.D.), and of NIH R01 MH53576 and MH57068 (T.C.S.) is gratefully acknowledged. R.S. is supported by the Adams Fellowship program of the Israeli Academy of Sciences and Humanities. We thank R. Eilam for providing invaluable assistance in histochemistry.

#### Supporting Online Material

www.sciencemag.org/cgi/content/full/331/6021/1207/DC1  
Materials and Methods  
SOM Text  
Figs. S1 to S4  
References

9 November 2010; accepted 31 January 2011  
10.1126/science.1200215