The Involvement of the Anterior Cingulate Cortex in Remote Contextual Fear Memory

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Although the molecular, cellular, and systems mechanisms required for initial memory processing have been intensively investigated, those underlying permanent memory storage remain elusive. We present neuroanatomical, pharmacological, and genetic results demonstrating that the anterior cingulate cortex plays a critical role in remote memory for contextual fear conditioning. Imaging of activity-dependent genes shows that the anterior cingulate is activated by remote memory and that this activation is impaired by a null α-CaMKII mutation that blocks remote memory. Accordingly, reversible inactivation of this structure in normal mice disrupts remote memory without affecting recent memory.

The formation of new memories involves protein synthesis–dependent changes in synaptic structure and plasticity in the hippocampus (*1–3*). However, these memories are not stored permanently in the hippocampus. In humans and animals, damage to the hippocampus preferentially disrupts recently acquired memories while sparing remotely acquired memories (*4–7*); these effects indicate that remote memories eventually become independent of the hippocampus. To study the role of extrahippocampal structures in remote memory, we used contextual fear conditioning. In contextual fear conditioning, mice form an association between a distinctive context and an aversive event that takes place in that context. When placed back into the context, mice exhibit a range of conditioned fear responses, including freezing (*5*). Contextual fear conditioning is ideally suited for the study of remote memory, because a single training session produces robust lifelong memory (*8*) that can be measured using automated procedures (*9*).

To identify extrahippocampal regions involved in processing remote contextual fear memories, we tracked the expression of genes (*zif268* and c-*fos*) modulated by neuronal activity (*10*). Separate groups of mice were trained with 0 or 5 footshocks and tested either 1 day later (recent memory test) or 36 days later (remote memory test) (*11*). Although context exposure that is not reinforced may itself produce lasting memories (*12*), we observed no time-dependent changes in cortical gene expression after recent or remote memory tests in the control mice that were not shocked (fig. S1). Therefore, we analyzed gene expression in the shocked mice normalized with respect to these stable levels in controls. This allowed us to isolate changes in gene expression associated with contextual fear memory, and control for gene expression associated with motor activity, general arousal, and other nonspecific aspects of the testing procedure (*13*, *14*). We focused our analyses on the anterior cingulate cortex (ACC), prelimbic (PL) and infralimbic (IL) regions of the medial prefrontal cortex, temporal cortex (TC), and visual cortex (VC), because of the proposed role for the cortex in remote memory (*15–20*). Zif268 expression was elevated in ACC after the remote (160.5 ± 4.6%), but not the recent (89.4 ± 7.4%), memory tests (Fig. 1A), which suggests that this region is preferentially involved in processing remote contextual fear memories. After the remote, but not recent, memory test, similar pronounced increases in gene expression were observed in IL, PL, and TC (Fig. 1A). The same temporally graded pattern was observed in ACC, IL, PL, and TC with Fos expression (Fig. 1B), which indicates that these results generalize to other activity-dependent genes, and are not specific to Zif268. Note that the distinct patterns of gene expression following recent and remote memory tests indicate that gene expression is not simply a correlate of freezing (or other fear-related) behaviors, because freezing levels were similar at both time points.

The results presented above suggest that processing of remote contextual fear memory involves coordinated activation of multiple cortical regions. We thus examined cortical activation in mutant mice (α-CaMKII$^{+/-}$), which have specific deficits in remote memory (*16*). Both the magnitude and the specificity of the contextual fear memory phenotype in the α-CaMKII$^{+/-}$ mice make them an ideal tool to examine neural systems for remote memory. Concurrent with their wild-type (WT) littermates (*11*), α-CaMKII$^{+/-}$ mice were contextually conditioned and tested either 1 or 36 days after training. Contextual fear was dramatically reduced in the α-CaMKII$^{+/-}$ mice at the longer retention delay when we used either freezing or activity suppression as a measure (*16*) (Fig. 2). The pronounced increase in Zif268 expression in ACC after the remote memory test was absent in the mutants (Fig. 1A). Rather, Zif268 expression was similar in mutants after the recent (82.1 ± 4.7%) and remote memory tests (93.2 ± 10.6%). The increased Zif268 expression associated with remote memory was also blocked in IL, PL, and TC (Fig. 1A), which indicated that cortical activation associated with remote memory may be widely blocked in the α-CaMKII$^{+/-}$ mice. Fos expression associated with remote memory was similarly blocked in ACC, IL, and PL in α-CaMKII$^{+/-}$ mice (Fig. 1B). It is thought that consolidation occurs in a reactivation-dependent manner, either during online (e.g., retrieval tests) or offline (e.g., sleep) situations (*19–22*). Reactivation may lead to the gradual refinement of the memory network and integration of that network with related, preexisting memories (*19*). Therefore, to assay synaptic remodeling underlying these processes during memory recall, we examined expression of growth-associated protein 43 (GAP-43), a marker of synaptogenesis (*23*), in ACC (*11*). GAP-43 expression was elevated after the remote memory test in WT (recent 11.5 ± 1.7, remote 19.2 ± 2.1; $P < 0.05$), but not α-CaMKII$^{+/-}$ (recent 10.2 ± 0.9, remote 11.2 ± 2.1; $P > 0.05$), mice. These results indicate that the α-CaMKII$^{+/-}$ mutation may impede cortical reorganization necessary for consolidation.

The CA1 region of the hippocampus is strongly activated during acquisition and recall of contextual fear conditioning (*13*), which suggests that this region has a critical role in processing contextual fear memories. In WT mice, gene expression was elevated in CA1 after the recent (Zif268: 126.1 ± 3.9%; Fos: 174.2 ± 6.3%), but not remote (Zif268: 79.9 ± 5.9%; Fos: 93.5 ± 11.4%), memory test (Fig. 1). These data suggest that consolidation involves the gradual disengagement of CA1 (*13*), coupled with progressive recruitment of cortical regions. Furthermore, because Zif268 expression was reduced below control levels after the remote memory test in WT mice, activity in CA1 may be inhibited

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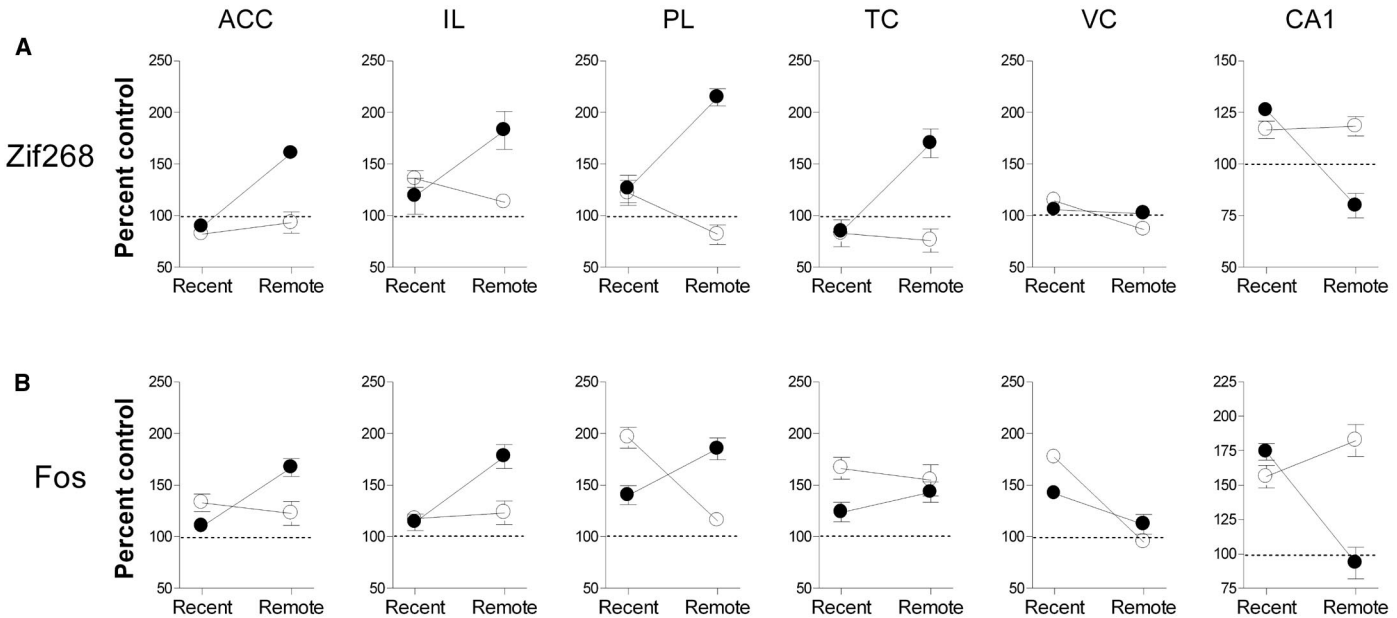

Fig. 1. Expression of activity-dependent genes after recent or remote memory tests. In order to isolate changes associated with memory, gene expression in the shocked groups is expressed as a percentage relative to controls that were not shocked. Changes in gene expression in different brain regions are shown for WT (black circles) and α-CaMKII$^{+/-}$ (open circles) mice after recent or remote memory tests. (**A**) Zif268 expression was elevated in WT, but not α-CaMKII$^{+/-}$ mice, after the remote memory test in ACC [Genotype × Delay interaction $F(1,28) = 16.82$, $P < 0.05$], IL [$F(1,28) = 9.82$, $P < 0.05$], PL [$F(1,28) = 33.73$, $P < 0.05$], and TC [$F(1,28) = 15.52$, $P < 0.05$], but not VC [$F(1,28) = 0.52$, $P > 0.05$]. In the CA1 region of the hippocampus, Zif268 expression was elevated after the recent, but not remote, memory tests in WT mice. In contrast, Zif268 expression was elevated at both time points in α-CaMKII$^{+/-}$ mice [$F(1,28) = 25.93$, $P < 0.05$]. (**B**) Changes in Fos expression were qualitatively similar to those in Zif268 for WT and α-CaMKII$^{+/-}$ mice. Fos expression was elevated in WT, but not α-CaMKII$^{+/-}$ mice, after the remote memory test in ACC [$F(1,28) = 14.03$, $P < 0.05$], IL [$F(1,28) = 9.69$, $P < 0.05$], PL [$F(1,28) = 46.37$, $P < 0.05$], but not TC [$F(1,28) = 1.83$, $P > 0.05$] nor VC [$F(1,28) = 1.39$, $P > 0.05$]. In CA1, Fos expression was elevated after the recent, but not remote, memory tests in WT mice. In contrast, Fos expression was elevated at both time points in α-CaMKII$^{+/-}$ mice [$F(1,28) = 30.78$, $P < 0.05$].

during processing of remote memories. In contrast, Zif268 levels remained elevated after the remote memory test in CaMKII$^{+/-}$ mice, which do not express behavioral memory at this time point. Therefore, the absence of this inhibitory feedback in the mutants may allow new encoding to occur.

Reduced cortical activation of Zif268 and Fos in the α-CaMKII$^{+/-}$ mice after remote memory tests most likely reflects differences in the organization of memory in these mutants. However, it is possible that the α-CaMKII$^{+/-}$ mutation disrupts regulation of these activity-dependent genes. We therefore conducted additional control experiments (*11*). First, we examined gene expression associated with training (Fig. 3A). Consistent with the observation that α-CaMKII$^{+/-}$ mice acquire contextual fear conditioning normally, we found that Zif268 and Fos expression was similar in WT and α-CaMKII$^{+/-}$ mice across different cortical regions. Furthermore, gene expression in the cortex was similar in WT and α-CaMKII$^{+/-}$ mice after removal from their home cage (Fig. 3B) and in controls that were not shocked (fig. S1). Therefore, the regulation of Zif268 and Fos appears to be normal in the α-CaMKII$^{+/-}$ mice under conditions associated with either low or high levels of neural activation, which suggests that changes in gene expression observed after remote memory tests are a consequence of changes in mnemonic processing in these mutants.

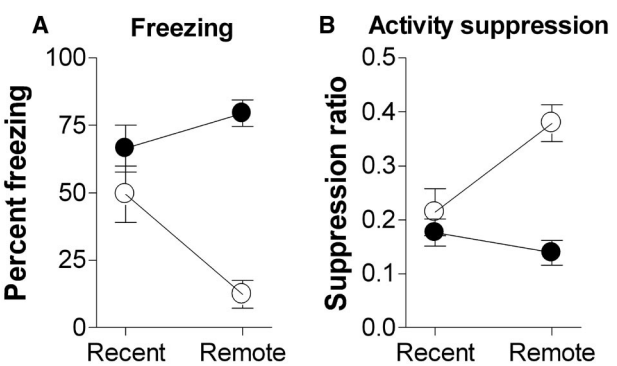

Fig. 2. Contextual fear memory in α-CaMKII$^{+/-}$ mice (open circles) versus WT mice (black circles). To assess memory in α-CaMKII$^{+/-}$ mice and WT mice, two behavioral indices of conditioned fear were measured in the same mice: (**A**) freezing; (**B**) activity suppression (*9*). Whereas WT mice exhibited robust levels of freezing and activity suppression in both the recent (1 day after training) and remote (36 days after training) retention tests, freezing [Genotype × Delay interaction, $F(1,28) = 10.62$, $P < 0.05$] and activity suppression [Genotype × Delay interaction $F(1,28) = 9.69$, $P < 0.05$] were markedly reduced at the longer retention delay in the α-CaMKII$^{+/-}$ mice.

The results presented above indicate that specific cortical sites are activated by remote memory processes and that this activation is absent in mice with remote memory deficits. To directly test whether these cortical sites are required for remote memory, we examined the effects of transient inactivation using lidocaine (*11*). Because remote memories are likely stored in distributed cortical networks (*19*, *24*), they may be resistant to focal disruption (*25*). However, executive structures like PL and ACC, which are both robustly activated by remote contextual memory, are thought to play an integrative role in memory (*26*–*28*) and may therefore be amenable for targeted disruption. Lidocaine infusions into ACC disrupted contextual fear memory at remote (18 and 36 days), but not recent (1 or 3 days) time points (Fig. 4A). Similar infusions into the neighboring PL had no effect on either 1-day-old or 36-day-old contextual fear memories (Fig. 4B). Although the imaging data show that a broad cortical network is activated by remote memory, these inactivation results

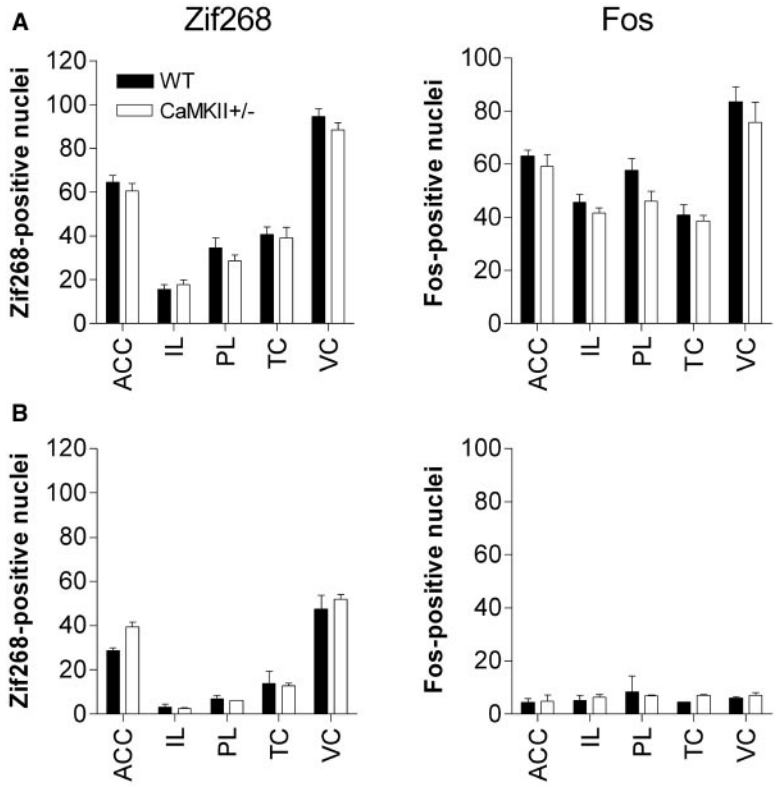

Fig. 3. Regulation of Zif268 and Fos in the cortex is normal in α-CaMKII$^{+/-}$ mice. The number of Zif268- and Fos-positive nuclei are shown for WT (black bars) and α-CaMKII$^{+/-}$ (white bars) mice. (**A**) Gene expression induced in the cortex after acquisition of contextual fear conditioning. Training-associated expression of Zif268 or Fos was similar in WT and α-CaMKII$^{+/-}$ mice in each of these regions (P values > 0.05). (**B**) Gene expression in the cortex in the home cage condition. There were no differences in Zif268 or Fos expression between WT and α-CaMKII$^{+/-}$ mice in each of these regions (P values > 0.05).

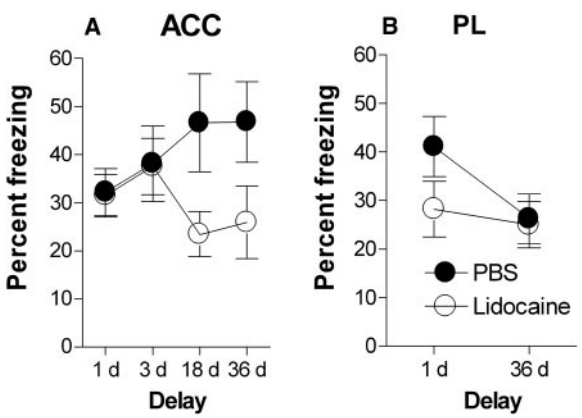

Fig. 4. Targeted pharmacological inactivation of ACC and PL. (**A**) Lidocaine-induced inactivation of ACC disrupts retrieval of remote, but not recent, contextual fear memories. Planned comparisons indicated that freezing levels were reduced in lidocaine-infused mice in the retention tests on the 18th or 36th day, but not the 1st or 3rd day after training (P values < 0.05). (**B**) Lidocaine-induced inactivation of PL did not disrupt retrieval of contextual fear memories. Planned comparisons indicated that freezing levels in phosphate-buffered saline (PBS)– and lidocaine-infused mice were not different (P values > 0.05).

identify ACC (but not PL) as an essential node within this network for processing remote memory.

Modeling, neuropsychological, and neurophysiological studies have suggested a central role for hippocampal-cortical networks in memory consolidation (*19*, *20*, *22*). Interactions between the hippocampus and cortex following initial learning lead to the gradual establishment of enduring memories in distributed cortical networks that are independent of the hippocampus. Here, we used brain imaging to identify cortical regions involved in processing fear memories. Our data show that processing fear memories involves the activation of multiple association cortical regions, consistent with the proposal that enduring memories are stored in distributed cortical networks. Cortical activation was greater after remote, rather than recent, memory tests, consistent with an increasingly important role for the cortex over time. In mice with specific deficits in remote memory, the pronounced cortical activation associated with remote memory was absent. These data suggest that CaMKII is necessary for the maturation and elaboration of cortical circuits underlying remote memory. In normal mice, imaging and inactivation experiments identified the ACC as a critical node in a broader cortical network processing remote memory. During memory encoding, ACC is thought to play an integrative role in cognitive control processes [e.g., attention, conflict monitoring (*26*, *27*, *29*)]. It remains to be determined whether the ACC is a site for memory storage per se or whether it mediates analogous processes for integrating multiple cortical representations underlying remote memories.

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Materials and Methods
Fig. S1
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NEUROSCIENCE

Zif and the Survival of Memory

Iván Izquierdo and Martín Cammarota

Neurobiologists have a firm understanding of how new memories are formed, but the mechanisms that render new memories permanent are still poorly understood. It is becoming clear that new memories first require a time-dependent consolidation process to ensure their permanence, and that recalled remote memories are labile, sometimes necessitating a reconsolidation process to reestablish their permanence. Two reports in this issue provide fresh insights into the mechanisms of memory consolidation and reconsolidation in the brains of rodents subjected to contextual fear conditioning (*1*, *2*). In this process, rodents form an association between a particular environment and an aversive event (usually a footshock) and when placed back in that context show a range of conditioned fear responses including freezing of movement. The two new studies show that the early activity-dependent gene *zif268* is crucial for the consolidation, reconsolidation, and survival of memories associated with contextual fear conditioning. Consolidation of memories associated with the fear response requires distinct biochemical processes in the hippocampus (*3*), and retrieval of such memories involves the anterior cingulate cortex (*4*), among other brain areas. On page 839, Lee *et al*. (*1*) suggest that Zif268 expressed in hippocampal neurons selectively promotes the consolidation and reconsolidation of recently learned fear memory in rats. Meanwhile, on page 881, Frankland *et al*. (*2*) propose that Zif268 in the anterior cingulate cortex favors retrieval of remote fear memory in mice.

There is considerable experimental support for the notion of memory consolidation, but strong evidence for reconsolidation of memories is still lacking. Reconsolidation is thought to strengthen the "neuronal traces" of memories reactivated by exposure to the original context in which they were acquired, ensuring that the memories remain permanent (*5*, *6*). Without memory reconsolidation, it is thought that reactivated memories, which become labile, may be lost (extinction). The evidence for memory reconsolidation to date rests mainly on the fact that infusion of the protein synthesis inhibitor anisomycin into the basolateral amygdala (*5*) or the hippocampus (*6*) at the time of testing for memory retrieval hinders retrieval in a subsequent test session. This does not always happen, however (*7*), and even when it does, further testing reveals an opposite phenomenon: the inhibition of the extinction of memory retrieval (*7*, *8*). Memory extinction is by far the most common outcome following reexposure to an unassociated learning cue (*7*–*9*) and consists of the inhibition of memory retrieval by pairing the cue (in the case of contextual fear conditioning, a footshock) with the lack of reinforcement (*7*, *9*). Extinction was first described by Pavlov nearly a century ago (*7*–*9*) and is a very adaptive form of learning, as it shoves undesirable memories backstage (*8*, *9*) and thus makes space for further learning or retrieval of other more important memories. The biochemical mechanisms involved in learning and retrieval can be saturated (*10*). Indeed, persistence of responses to a fear that is no longer present is at the root of phobias, panic disorder, and posttraumatic stress disorder (*1*, *7*). These illnesses are regularly treated precisely by the induction of memory extinction (*1*, *7*, *9*).

The need for the extinction of unnecessary memories in our daily cognitive life is best illustrated by the famous short story by Borges, "Funes the Memorious" (*11*). Funes could remember everything—for example, an entire day of his life. But to do this he needed another entire day, which is an impossibility. Further, because he couldn't extinguish his memories, he was unable to make generalizations about life and therefore was unable to think logically.

Despite the obvious physiological role for memory extinction and the shortage of evidence in favor of reconsolidation, the very idea of reinforcing certain important memories while forgetting trivial memories is somehow appealing. We all know that a single word can make us retrieve a full song, and we all feel that after this we have somehow improved its retention. Nonetheless—perhaps sadly, but in accord with the need to adapt to an ever-changing world—memory extinction always prevails in the end (*7*), as Pavlov and Borges predicted. Like it or not, repetition of the unassociated cue leads to extinction (*8*, *9*).

Several experiments are needed in order to accept reconsolidation as a real, indisputable phenomenon. First, reconsolidation must be shown to occur across a variety of behaviors. So far, it has been described only in aversive tasks that require and promote the inhibition of movement (freeze response) during contextual fear conditioning (*1*, *7*, *8*). Second, retrieval induced by the contextual cue alone should improve memory retrieval over long time periods, which has never been described. Third, it must be shown that pre- or posttesting treatments that hinder further test performance can do so long-term, and not just during one session. [There has

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been passing mention of this only in one experiment so far (*6*).] Fourth, at least one biochemical substrate must be found that affects reconsolidation selectively while not affecting consolidation of the original memory. This is essential, otherwise reconsolidation could be confused with new additional learning (*5*).

Lee *et al*. (*1*) have now carried out the last two sets of experiments successfully. They found that intrahippocampal administration of a Zif268 antisense oligodeoxynucleotide (ODN), when given at the time of the first of three memory retention tests, hindered retrieval for the two remaining sessions. Furthermore, they observed that this treatment did not affect consolidation of the original memory. In contrast, an antisense ODN for brain-derived neurotrophic factor (BDNF) selectively blocked consolidation but not reconsolidation. The investigators conclude that there is a double dissociation between consolidation and reconsolidation, and between BDNF and Zif268. Memory consolidation requires BDNF but not Zif268, whereas reconsolidation recruits Zif268 but not BDNF.

There are two other major forms of memory loss besides Pavlovian extinction: Freudian repression and amnesia. The mechanisms of Pavlovian extinction involve the activation of enzymatic pathways, gene expression, and protein synthesis in the hippocampus and amygdala (*7*, *8*). The mechanism of memory repression consists of the inhibition of hippocampal activity through the influence of prefrontal neurons at the time of memory retrieval (*12*). Amnesia is widely believed to result from real neuronal or synaptic loss that can be either morphological or functional. The passage of time is often accompanied by some degree of forgetting, even in the absence of brain disease, so doubtless research into the retrieval of remote memories will continue to thrive.

The hippocampus and several other cortical areas, including the anterior cingulate cortex, are necessary for the retrieval of memories associated with contextual fear conditioning (*4*). In their study, Frankland and co-workers (*2*) show that the anterior cingulate cortex is particularly crucial for retrieval of remote fear memory. They demonstrate that retrieval of remote fear memory in mice is accompanied by an increase in Zif268 and the product of another activity-dependent gene (c-*fos*) in the anterior cingulate cortex and other brain areas. This increase is not seen in animals with transient blockade of the nucleus accumbens or in mutant α-CaMKII mice in which the remote memory of contextual fear is impaired.

It would be useful to investigate whether the phenomena described by Lee *et al*. (*1*) and Frankland *et al*. (*2*) also are observed in tasks other than those involving contextual fear conditioning in a restricted environment. Such findings would reconsolidate the results discussed here and would ensure their future as a remote memory that can be readily retrieved.

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PHYSICS

Colloids as Big Atoms

Wilson Poon

Colloid science is important for applications ranging from drugs to dairy products. Less well known is that it can also illuminate basic physics questions, because in certain crucial respects, colloids behave as "big atoms." The report on page 847 of this issue by Aarts *et al.* (*1*) beautifully illustrates this approach, which was pioneered by Einstein. In particular, the results show that phenomena at the interface between a liquid and a vapor can be studied with a colloidal model.

Beginning with his doctoral thesis, Einstein showed that the incessant, random jiggling of colloidal particles known as Brownian movement was the visible manifestation of the "graininess"—the molecular nature—of the surrounding liquid. One consequence is that the density of particles as a function of height in a dilute suspension in sedimentation equilibrium is given by an equation that depends on the particle's buoyant mass, the gravitational acceleration, Boltzmann's constant, and the absolute temperature. It turns out that this equation also expresses the distribution of gas molecules in a constant-temperature atmosphere in gravity, where it is known as the barometric distribution. In other words, colloids made up of relatively large particles can behave in the same way as much smaller counterparts in the molecular world; for some purposes, colloids behave as "big atoms." Jean-Baptiste Perrin's experimental verification of the "colloidal barometric distribution" contributed toward his 1926 physics Nobel Prize and the widespread acceptance of the reality of molecules.

Today, the study of colloids is throwing new light on fundamental problems of condensed matter physics, from the kinetics of crystallization (*2*) to the nature of glassy states [(*3*, *4*); see (*5*) for a review]. In their work, Aarts *et al*. (*1*) use colloids to study the vapor-liquid interface. At conditions far from the critical point (the temperature and pressure beyond which vapor and liquid do not exist separately), such interfaces are macroscopically flat. Microscopically, however, thermal energy excites ripples in the interface. These capillary waves (which, like capillary rise, are governed by the surface tension) are important in diverse fields such as oceanography (where wind-excited capillary ripples amplify to giant waves) and the rupture of polymer films (which is bad for coatings). After a century of study, these ripples still hold surprises. Thus, recent x-ray scattering from capillary waves in organic liquids (*6*) shows that as we move down to molecular length scales, the surface tension first decreases and then increases again; the decrease is probably caused by the long-range nature of the dispersion (that is, van der Waals) forces between the molecules.

Vapor-liquid coexistence reflects a tug of war between intermolecular attraction and repulsion. Aarts *et al.* used inert polymers to induce attraction between hard-sphere colloids of diameter $d \approx 140$ nm. Polymer coils are excluded from the region between the surfaces of two nearby particles, creating an unbalanced osmotic pressure pushing them together—the particles effectively attract each other (see the figure, A). The range and strength of this so-called depletion attraction is directly proportional to the size and the concentration of the inert polymers, respectively (see the figure, B).

The study of such colloid-polymer mixtures has yielded many new insights [see (*7*) for a review]. In particular, it has been

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The Involvement of the Anterior Cingulate Cortex in Remote Contextual Fear Memory

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Materials and methods

Mice

The α-CaMKII$^{+/-}$ mutation (*S1*) was maintained by crossing mutants that were 50% C57Bl/6NTacfBr and 50% 129Sv/J (F1 hybrid) with mice that were F1 hybrids of the same genetic background. For the inactivation studies, male C57Bl/6NTacfBr mice were used. All mice were maintained on a 12 h light/dark cycle. Behavioral experiments were conducted during the light phase of the cycle, and mice were at least 12 weeks old at the time of training.

Immunocytochemistry

Prior to contextual fear conditioning, α-CaMKII$^{+/-}$ mutant and WT littermate control mice were handled for 6 consecutive days. On the first 3 days, mice were removed from their home-cage and individually handled for 2 minutes in the vivarium. On days 4-6 mice were transported from the vivarium to a holding room. In order to habituate the mice to the general procedures used during training and testing, mice were then placed in a transport cage and taken to the room housing the contextual fear conditioning apparatus, where they were handled. One day following the completion of handling, mice were trained. The apparatus and general procedures (*S2, S3*) for contextual fear conditioning have previously been described. Briefly, during training mice were placed in the conditioning chamber for 7 minutes. After 2 minutes they were presented with 5 unsignaled footshocks (2 s duration, 0.75 mA, 1 minute apart). Control groups of mice were treated identically, except that they did not receive any shocks during training. Separate groups of trained and control mice were then tested 1- (recent group) or 36-days (remote group) later: WT recent/train (n=8); α-CaMKII$^{+/-}$ recent/train (n=8); WT remote/train (n=8); α-CaMKII$^{+/-}$ remote/train (n=8); WT recent/control (n=8); α-CaMKII$^{+/-}$ recent/control (n=3); WT remote/control (n=9); α-CaMKII$^{+/-}$ remote/control (n=3). During testing, mice were placed back in the conditioning chamber for 2 minutes, and freezing and activity were recorded using automated procedures described previously (*S2*). Activity suppression ratios were calculated by comparing activity levels during training (prior to shock delivery) to testing according to the following formula: ($activity_{test}$/($activity_{train}$+ $activity_{test}$)). Ninety minutes following the completion of testing, mice were perfused transcardially. Brains were prepared for immunocytochemistry using anti Fos (1:20000) and anti Zif268 (1:7500) primary rabbit polyclonal antibodies. A biotinylated goat anti-rabbit antibody (1:2000) was used as a secondary. Staining was revealed using the avidin-biotin peroxidase method (ABC kit)

coupled to diaminobenzidine as chromogen. GAP-43 immunofluorescence was processed using a primary rabbit polyclonal antibody (1:1000) and a fluorescein isothiocyanate (FITC)–conjugated goat anti-rabbit secondary antibody (1:600). For co-visualisation of nuclei, 1.5µg/ml of 4,6-diamidino-2-phenylindole (DAPI) was included in the mounting medium. Quantitative analysis of Fos and Zif268-positive nuclei was performed using a VisioLab 2000 image processing system. Structures were anatomically defined according to the Paxinos and Franklin atlas (*S4*). Immunoreactive neurons were counted bilaterally with a fixed sample window across at least three sections by an experimenter blind to the treatment condition. Counts in individual trained mice were normalized with respect to the group mean of their corresponding control groups (e.g., WT recent/train vs. WT recent/control, α-CaMKII$^{+/-}$ remote/train vs. α-CaMKII$^{+/-}$ remote/control etc). These normalized scores were expressed as a percentage, and these percentages were averaged across mice to yield group means.

Additionally, gene expression was examined following a) removal from home cage (WT, n=2; α-CaMKII$^{+/-}$, n=2) and b) contextual fear conditioning training (WT, n=6; α-CaMKII$^{+/-}$, n=6). Procedures for the trained mice were identical to those described above. Ninety minutes following training, mouse brains were extracted and processed for immunocytochemistry.

Pharmacological inactivation

Under chloral hydrate anesthesia and using standard stereotaxic procedures, stainless-steel guide cannulae (22 gauge) were implanted into the anterior cingulate cortex (ACC) or prelimbic cortex (PL). At least one week later, mice were handled for 3 consecutive days in the vivarium. At the completion of handling, mice were trained with 3 unsignaled footshocks (2 s duration, 0.75 mA, 1 minute apart). Separate groups of mice were tested 1-36 days later. Ten minutes prior to testing mice were briefly anesthetized with halothane, to aid insertion of the injection cannula (28 gauge). To inactivate ACC and PL we used lidocaine, a sodium channel blocker that transiently suppresses neuronal firing and thus avoids possible compensatory changes that can occur with permanent lesions. Mice received 0.5 µl infusions of lidocaine (4%) or PBS at a rate of 0.2 µl /min. The injection cannula was left in place for 2 minutes following the infusion and the mice were returned to their home cages prior to testing. Testing was conducted as described above. Anatomical specificity is a critical issue when using targeted pharmacological approaches to inactivate brain regions (*S5*). Therefore, we evaluated the extent of inactivation following lidocaine infusions by examining Zif268 expression after a lidocaine injection into ACC. These results indicated inactivation following lidocaine infusions was confined to ACC, approximately 0.7 mm in diameter.

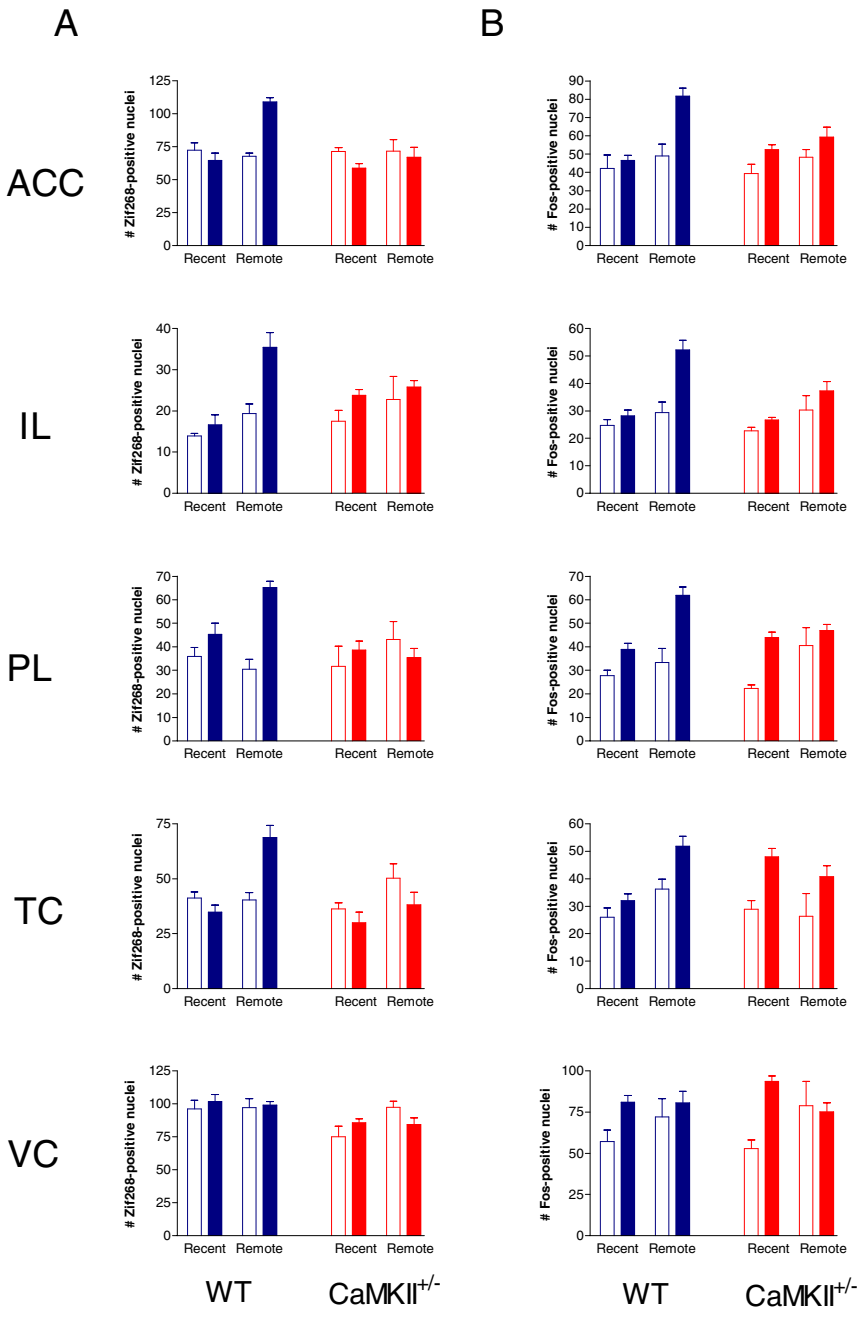

Fig. S1. Raw number of (A) Zif268- and (B) Fos-positive nuclei in anterior cingulate cortex (ACC), infralimbic (IL) and prelimbic (PL) cortices, temporal cortex (TC) and visual cortex (VC) following memory tests in WT (blue) and CaMKII$^{+/-}$ (red) mice. Mice were tested either 1-day (recent) or 36-days (remote) following training. During training mice received either 0 (open bars) or 5 (closed bars) footshocks.

References and Notes

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