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Role of Secondary Sensory Cortices in Emotional Memory Storage and Retrieval in Rats

Tiziana Sacco[1], **Benedetto Sacchetti**[1,2,*]

[1]Department of Neuroscience, University of Turin, Corso Raffaello 30, I-10125 Turin, Italy.
[2]National Institute of Neuroscience, I-10125 Turin, Italy.

*To whom correspondence should be addressed. E-mail: benedetto.sacchetti@unito.it

Abstract

Visual, acoustic, and olfactory stimuli associated with a highly charged emotional situation take on the affective qualities of that situation. Where the emotional meaning of a given sensory experience is stored is a matter of debate. We found that excitotoxic lesions of auditory, visual, or olfactory secondary sensory cortices impaired remote, but not recent, fear memories in rats. Amnesia was modality-specific and not due to an interference with sensory or emotional processes. In these sites, memory persistence was dependent on ongoing protein kinase Mζ activity and was associated with an increased activity of layers II–IV, thus suggesting a synaptic strengthening of corticocortical connections. Lesions of the same areas left intact the memory of sensory stimuli not associated with any emotional charge. We propose that secondary sensory cortices support memory storage and retrieval of sensory stimuli that have acquired a behavioral salience with the experience.

During an emotional experience, sensory stimuli such as odors, sounds, and colors are associated with the affective qualities of that situation. Despite recent advances (1, 2), the question of how and where the brain stores permanent emotional memories remains elusive. Because memories involve the representation of past sensory and emotional events, they may be stored, in part, within the sensory cortex (3, 4). Nonetheless, lesions of sensory cortices do not prevent the formation of emotional memories (5–11). However, such lesions have been performed before (5, 6, 10, 11) or shortly after (7–9) learning, a time interval in which thalamus-amygdala circuits support the functional absence of sensory cortices (6, 12). No data are available on the involvement of the sensory cortex in long-term storage and retrieval of emotional memories. Therefore, we addressed two related questions: Are sensory cortices necessary for the storage and retrieval of remote fear memories? And if so, what is the role played by these sites?

Role of auditory cortices in fear memory. We first analyzed the involvement of the auditory neocortex in remote fear memories. Rats were trained to associate seven acoustic stimuli (i.e., conditioned stimuli, CSs) to an aversive unconditioned stimulus (US). The primary auditory cortex was lesioned 1 month later; this lesion was centered in area Te1 (13–15). Reconstructions of the smallest and largest extents of damage are shown in Fig. 1A [see also (15) and fig. S1]. Memory retention was assessed by measuring freezing behavior elicited by CSs previously paired with the US (Fig. 1B). During CS presentation, lesioned- and sham-operated animals showed equivalent freezing [mixed-design analysis of variance (ANOVA), $F_{1,19} = 0.42$, $P > 0.05$; group × trial interaction, $P > 0.05$].

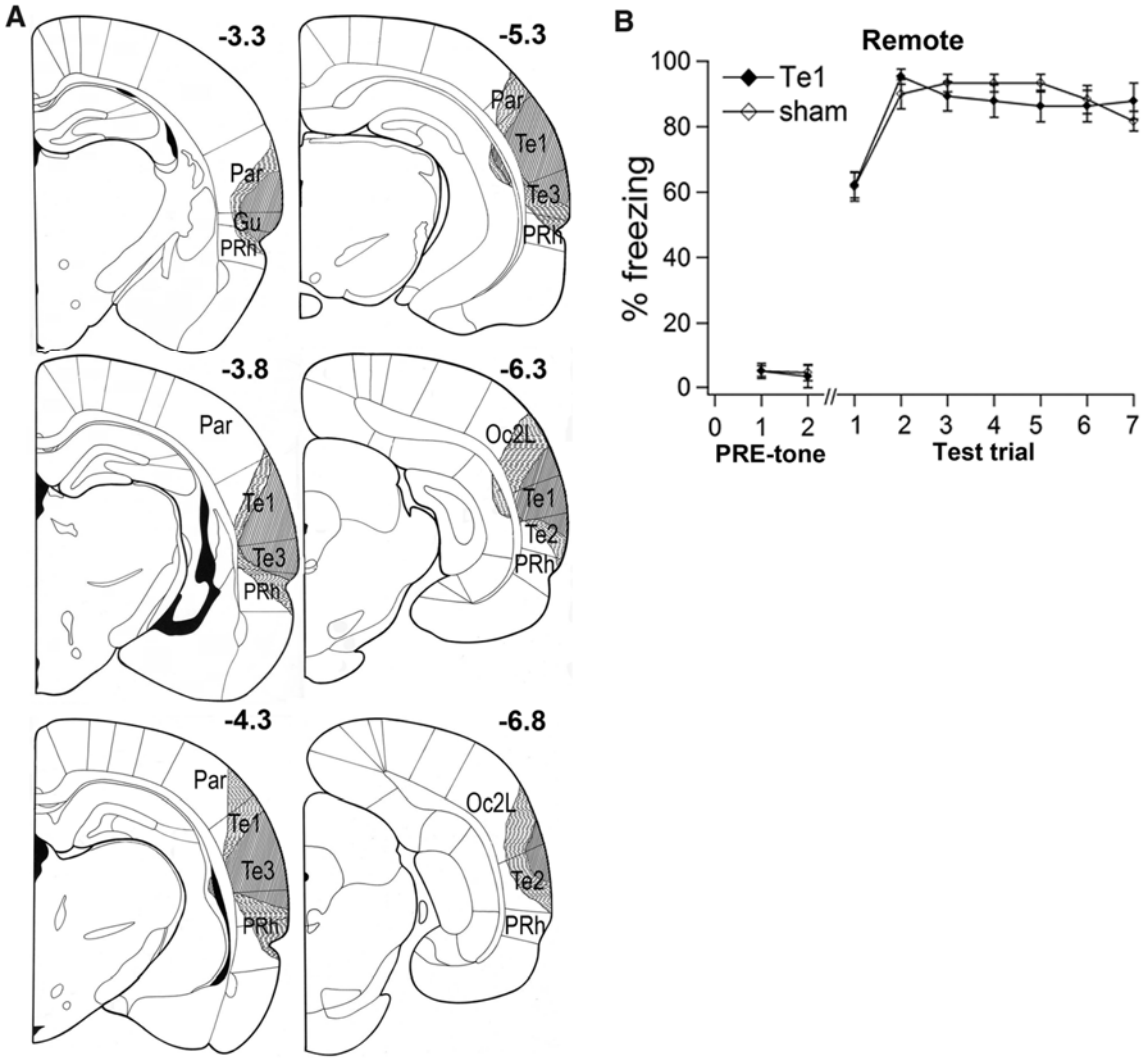

Fig. 1

Primary auditory cortex and fear memories. (A) Histological reconstruction of the smallest (gray) and largest (hatched) excitotoxic lesions aimed at the primary auditory cortex, area Te1. Negative numbers indicate posterior distance from bregma. Plates adapted from the atlas of Zilles (14). Ent, entorhinal cortex; Gu, gustatory cortex; Par, parietal cortex; PRh, perirhinal cortex; Te2 and Te3, secondary and tertiary auditory cortex. (B) Remote fear memories to acoustic CSs in Te1-lesioned (n = 11) and sham-operated (n = 10) animals. Fear response was measured as percentage of total immobility (freezing) both 2 min before (pre-tone) and during (test trial) presentation of seven CSs. All values are means ± SEM.

The primary auditory cortex is surrounded by a belt region that constitutes the secondary auditory area (13, 14). We therefore examined whether the secondary auditory cortex participates in remote fear memory storage and retrieval. The lesion was centered in the Te2 area (14) (Fig. 2, A and B, and fig. S2). Because Te2 is just above the posterior perirhinal cortex, and because a previous study reported that lesions of the entire perirhinal cortex abolished fear memories (9), in an additional group we disrupted the posterior perirhinal, but not Te2, cortex (fig. S1). In the latter case, sham-operated animals were those used in Te2 experiments. Figure 2C depicts freezing scores during a memory retention test in the lesioned and sham-operated rats. A mixed ANOVA revealed a significant effect for groups ($F_{2,36} = 68.66$, $P < 0.05$) and a significant group × trial interaction ($F_{12,216} = 1.83$, $P = 0.044$). A Newman-Keuls test showed that Te2-lesioned animals differed from the other two groups ($P < 0.05$). Collectively, the results indicate that lesions of the secondary auditory cortex, but not of the posterior perirhinal cortex or primary cortex, affect long-term fear memory.

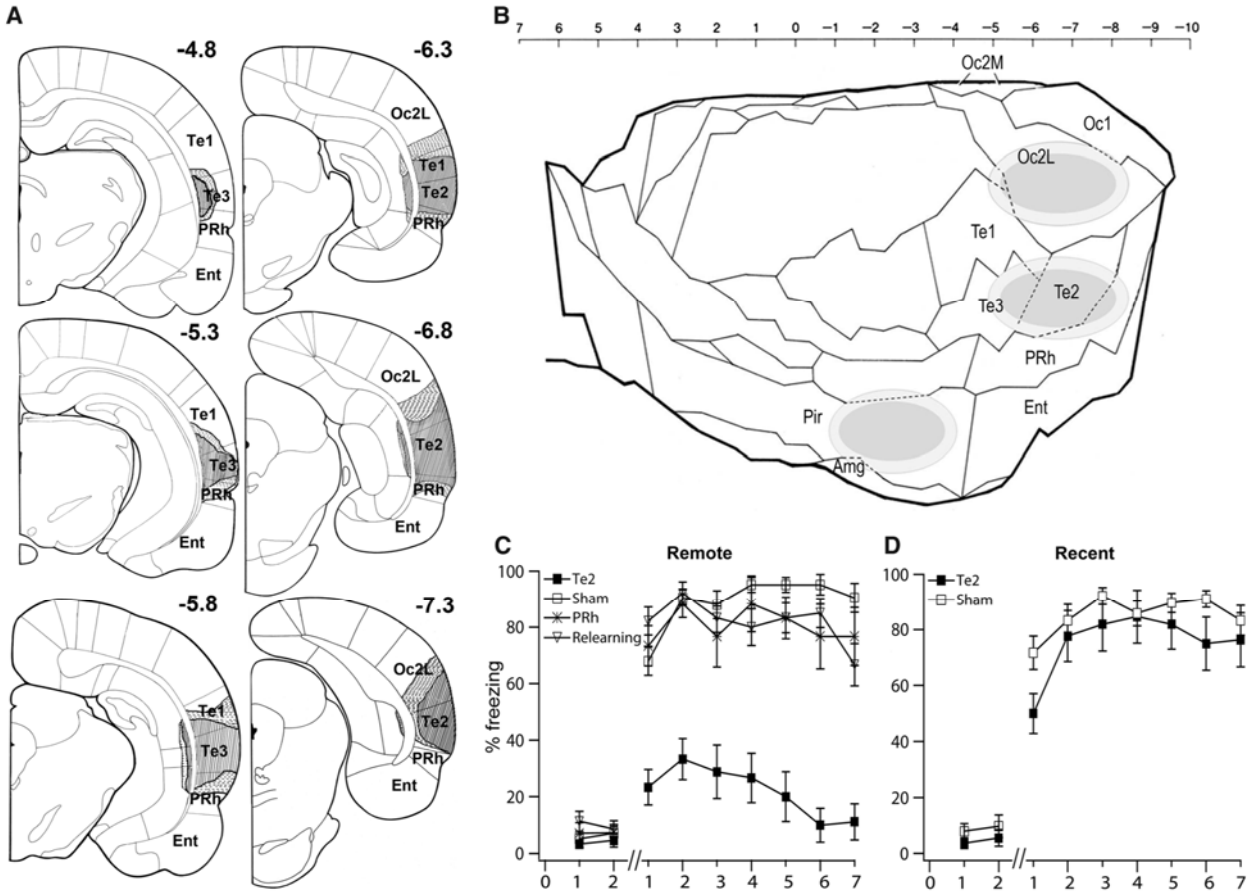

Fig. 2

Secondary auditory cortex and emotional memories. (A) Histological reconstruction of the excitotoxic lesions aimed at the Te2 area. Gray and hatched areas represent the smallest and the largest extent of the lesions, respectively. Negative numbers indicate posterior distance from bregma. Plates adapted from (14). (B) Schematic representation of the secondary sensory cortices included in the present study. The upper scale indicates positive and negative distances from bregma. Plate adapted from (14). (C) Remote fear memories in Te2-lesioned (n = 15), posterior perirhinal–lesioned (PRh, n = 10), and sham-operated (n = 14) animals. Te2-lesioned rats were able to reacquire CS-US association, as demonstrated by freezing levels comparable to those of control animals ($F_{1,27} = 2.083$, $P > 0.05$). (D) Recent fear memories in Te2-lesioned (n = 12) and sham-operated (n = 13) animals. Amg, amygdala; Ent, entorhinal cortex; Oc1, primary visual cortex; Oc2L and M, secondary occipital visual cortex; Pir, piriform cortex; Te3, tertiary auditory cortex.

Because previous findings showed that pretraining lesions of the entire auditory area do not prevent CS-US association (5, 6), we then asked whether Te2-lesioned rats can form new fear memories. The animals that displayed amnesia underwent an additional CS-US training and 2 days later were tested for CS retention. All animals could reacquire CS-US association (Fig. 2C) (15). Finally, we tested whether a Te2 lesion hampers recent fear memories. Te2 was lesioned 1 day after CS-US pairing. During a memory retention test, there were no differences between groups ($F_{1,23} = 1.719$, $P > 0.05$) and no significant group × trial interaction ($P > 0.05$) (Fig. 2D); that is, post-acquisition lesion of the auditory cortex does not abolish recent fear memories (7).

Secondary sensory visual and olfactory cortices and emotional memories. To formulate a broader functional conceptualization of the involvement of secondary sensory cortices in emotional memories, we examined whether a lesion of secondary visual cortex affects fear memories related to visual CSs and whether a lesion of the posterior piriform cortex impairs olfactory fear memories. The secondary visual cortex lesion was centered in the lateral Oc2 (Oc2L) area (14) (Fig. 3A and fig. S3). During CS presentation, there were differences between groups ($F_{1,19} = 24.31$, $P < 0.05$) and a significant group × trial interaction ($F_{6,114} = 3.73$, $P < 0.05$) (Fig. 3B); hence, Oc2L lesions hamper remote memories. Lesioned animals could form new conditioned fear responses ($F_{1,19} = 0.627$, $P > 0.05$) (10, 11) (Fig. 3B). Finally, lesions of Oc2L performed 1 day after training did not impair recent fear memory ($F_{1,20} = 0.754$, $P > 0.05$; group × trial interaction, $P > 0.05$) (Fig. 3C).

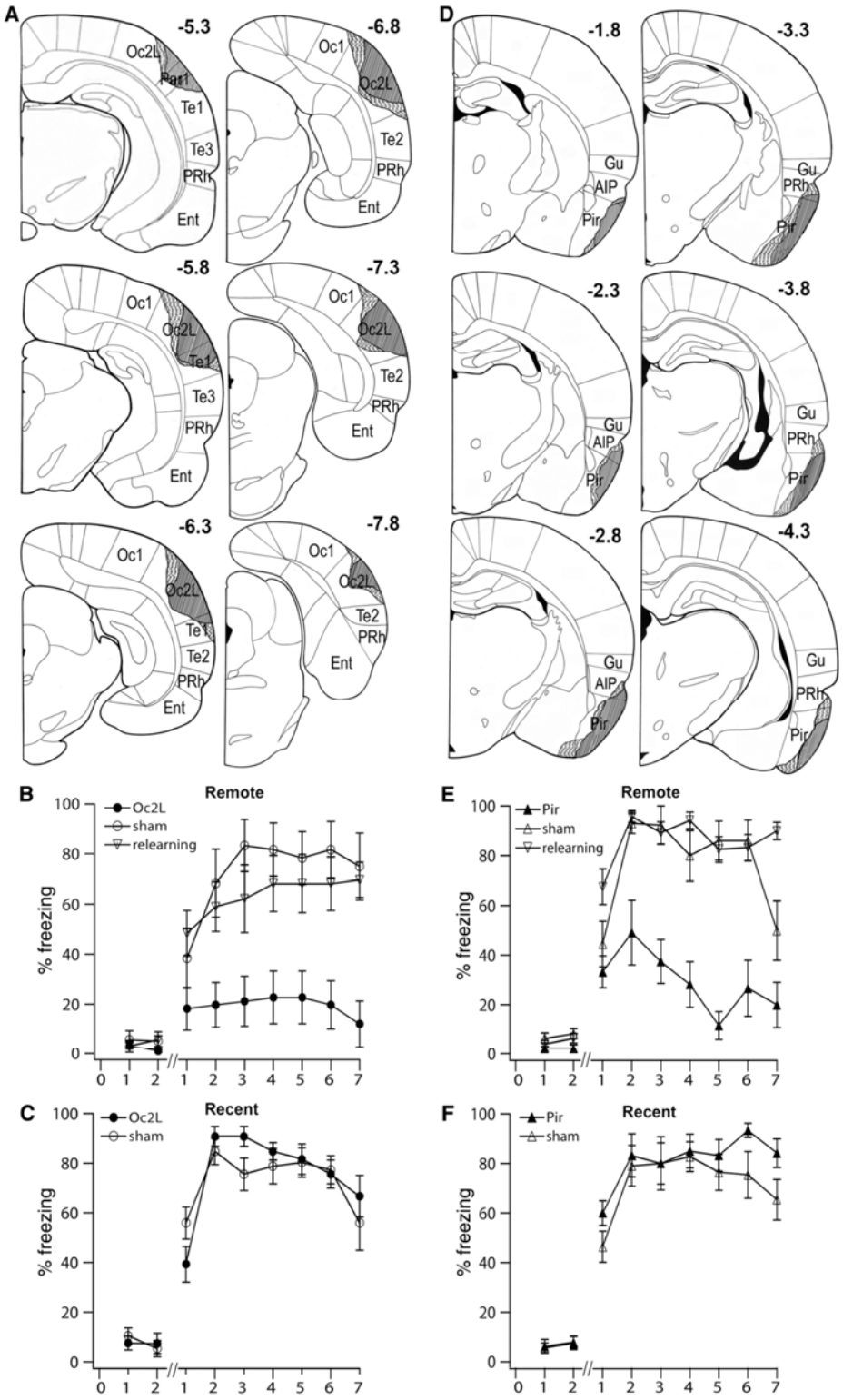

Fig. 3

Lesions of secondary visual cortex or of posterior piriform cortex impair remote fear memories. (A) Extent of Oc2L lesions. (B) Remote fear memories to visual CSs in lesioned (n =11) and sham-operated (n = 10) animals and in those retrained. (C) Recent emotional memories in Oc2L-lesioned (n = 11) and sham-operated (n = 11) rats. (D) Extent of posterior piriform cortical lesions. (E) Animals with posterior piriform cortex lesions (Pir, n = 12) are impaired in retention of remote memories relative to sham animals (n = 13). Lesioned animals were able to relearn CS-US association ($F_{1,23} = 2.225$, $P > 0.05$). (F) Posterior piriform cortex lesions do not affect recent fear memories ($F_{1,21} = 1.775$, $P > 0.05$; group × trial interaction, $P > 0.05$). Plates adapted from (14); perirhinal cortex (PRh) extent from (40). AIP, agranular posterior insular cortex; Ent, entorhinal cortex; Gu, gustatory cortex; Oc1, primary visual cortex; Te2 and Te3, secondary and tertiary auditory cortex.

The piriform cortex represents the most extensive olfactory area. In it, information is represented in a topographic fashion, with more sensory representations maintained in the anterior part and more associative representations in the posterior part (16, 17). Therefore, we lesioned the posterior part of the piriform cortex 1 month after olfactory fear learning (Fig. 3D and fig. S4). These lesions disrupted remote fear memories ($F_{1,23} = 48.872$, $P < 0.05$; group × trial interaction ($F_{6,138} = 2.82$, $P < 0.05$) (Fig. 3E) but did not impair the capacity of relearning CS-US association (Fig. 3E) and did not affect recent fearful memories (Fig. 3F and fig. S5).

Collectively, the data indicate that lesions of the secondary cortices affect remote fear memories, whereas recent memories are left intact. These results suggest that amnesia, when present, is specifically related to memory impairment and is not secondary to sensory or motor disturbance. To more fully address this issue, we used the cell-permeable zeta inhibitory peptide (ZIP). ZIP inactivates protein kinase Mζ (PKMζ), an autonomously active protein kinase C isoform that is required for encoding long-term memory traces in several brain sites (18, 19). ZIP injection elicits amnesia without interfering with basal synaptic activity and without inducing large-scale neuronal damage (18, 19). Indeed, amnesia is still present when the peptide has been eliminated (18).

We injected ZIP into Te2, Oc2L, or piriform cortex 1 day or 1 month after fear learning. Memory was tested 2 days after injection. Controls received the scrambled inactive version of ZIP (Fig. 4, A and B). ZIP injection into Te2 impaired remote fear memories ($F_{1,19} = 22.98$, $P < 0.05$; group × trial interaction, $P > 0.05$), but not recent fear memories ($F_{1,14} = 0.10$, $P > 0.05$; group × trial interaction, $P > 0.05$) (Fig. 4, A and B, and fig. S6). PKMζ inactivation may disrupt remote memory storage, in which case the effect of ZIP would be persistent, or information retrieval, in which case the effect would be transient (18–20). We thus continued testing ZIP-treated animals 2 weeks after injection, to unveil memory spontaneous recovery. No evidence for spontaneous recovery was observed in ZIP-injected rats (Fig. 4C and fig. S6), thus suggesting that ZIP effects on long-term memories are durable. Immunocytochemistry after injections of biotin-labeled ZIP showed that the peptide diffused specifically within the Te2 without spreading to adjacent brain regions (fig. S7A). Histological analysis revealed no large-scale damage in ZIP-injected rats (fig. S7B). Similar results were obtained by injecting ZIP in Oc2L (Fig. 4, D to F, and figs. S6 and S7) and in the posterior piriform cortex (Fig. 4, G to I, and figs. S6 and S7).

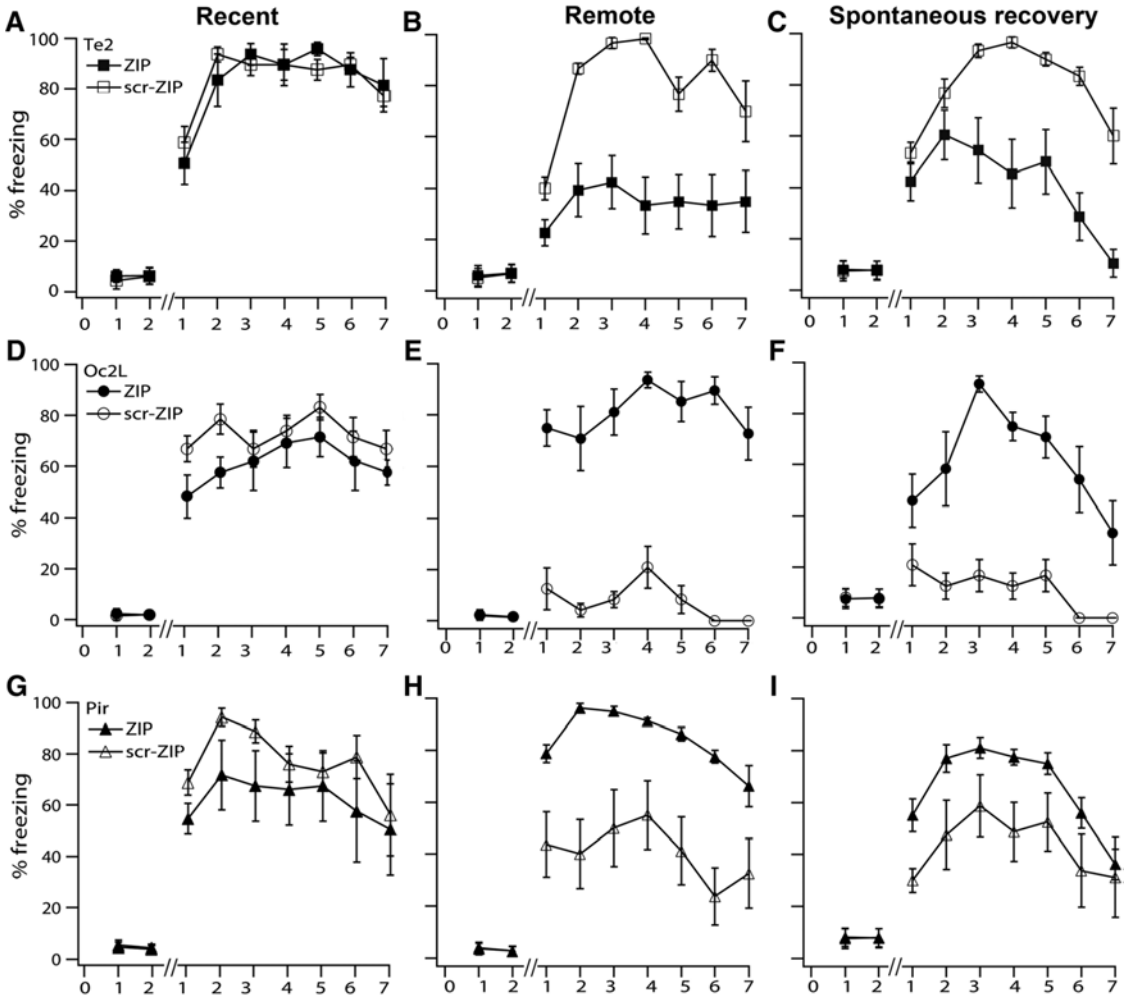

Fig. 4

Impairment of remote fear memories after injection of the PKMζ inhibitor ZIP. (A) ZIP (n = 8) or the scrambled version of ZIP (scr-ZIP, n = 8) was injected into Te2 1 day after training. (B) ZIP injection into Te2 (n = 11) 1 month after training hampered remote memory relative to control (n = 10) animals. (C) ZIP-treated rats did not show memory spontaneous recovery ($F_{1,19} = 17.26$, $P < 0.05$; group × trial interaction, $F_{6,114} = 3.88$, $P < 0.05$). (D) Recent memory to visual CSs was similar in rats infused with ZIP (n = 7) or scr-ZIP (n = 7) ($F_{1,12} = 2.91$, $P > 0.05$). (E and F) ZIP administration into Oc2L (n = 8) affected remote memories relative to control (n = 8) animals tested 2 days ($F_{1,14} = 95.26$, $P < 0.05$) (E) and 2 weeks ($F_{1,14} = 46.86$, $P < 0.05$) (F) after injection. (G) Recent olfactory fear memories were similar in ZIP-treated (n = 7) and control (n = 7) rats ($F_{1,12} = 1.29$, $P > 0.05$). (H and I) ZIP injection into posterior piriform cortex (Pir, n = 8) impaired remote memory both 2 days ($F_{1,14} = 23.41$, $P < 0.05$) (H) and 2 weeks ($F_{1,14} = 7.47$, $P < 0.05$) (I) after injection.

Modality-specific involvement of sensory cortices in fear memories. The involvement of secondary sensory cortices in memory storage raises the question of whether each cortex encodes memories specifically related to the sensory modality elaborated by the area, or whether these sites play a general role in memory processes irrespective of the diverse sensory modalities present during the emotional experience. We therefore investigated the effect of lesions of each sensory cortex on fear memories related to CSs of different sensory modalities. Figure 5A illustrates freezing elicited by acoustic CSs in rats with a lesion in Oc2L or in the posterior piriform cortex, as well as in unoperated animals. ANOVA indicated no differences among groups ($F_{2,25} = 0.171$, $P > 0.05$) and a nonsignificant group × trial interaction ($P > 0.05$) (see also fig. S6).

Figure 5B shows freezing to visual CSs in Te2-lesioned, posterior piriform–lesioned, and unoperated animals. Te2-lesioned animals froze less than did posterior piriform–lesioned and unoperated rats ($F_{2,24} = 18.168$, $P < 0.05$; group × trial interaction, $P > 0.05$). In rodents, the lateral posterior nucleus of the visual thalamus projects to Te2 (13, 21, 22), and this pathway may be involved in fear conditioning to visual stimuli (9, 22). Furthermore, Te2 neurons are also activated by visual stimuli (23) and objects (24) as well as by polymodal auditory-visual stimulation (23). On the other hand, the Te2 lesion also extends to part of Oc2L (Fig. 2A). Figure 5C depicts freezing to olfactory CSs in rats with lesions in Te2 or Oc2L and in the unoperated group. There were no differences among groups ($F_{2,26} = 0.396$, $P > 0.05$; group × trial interaction, $P > 0.05$).

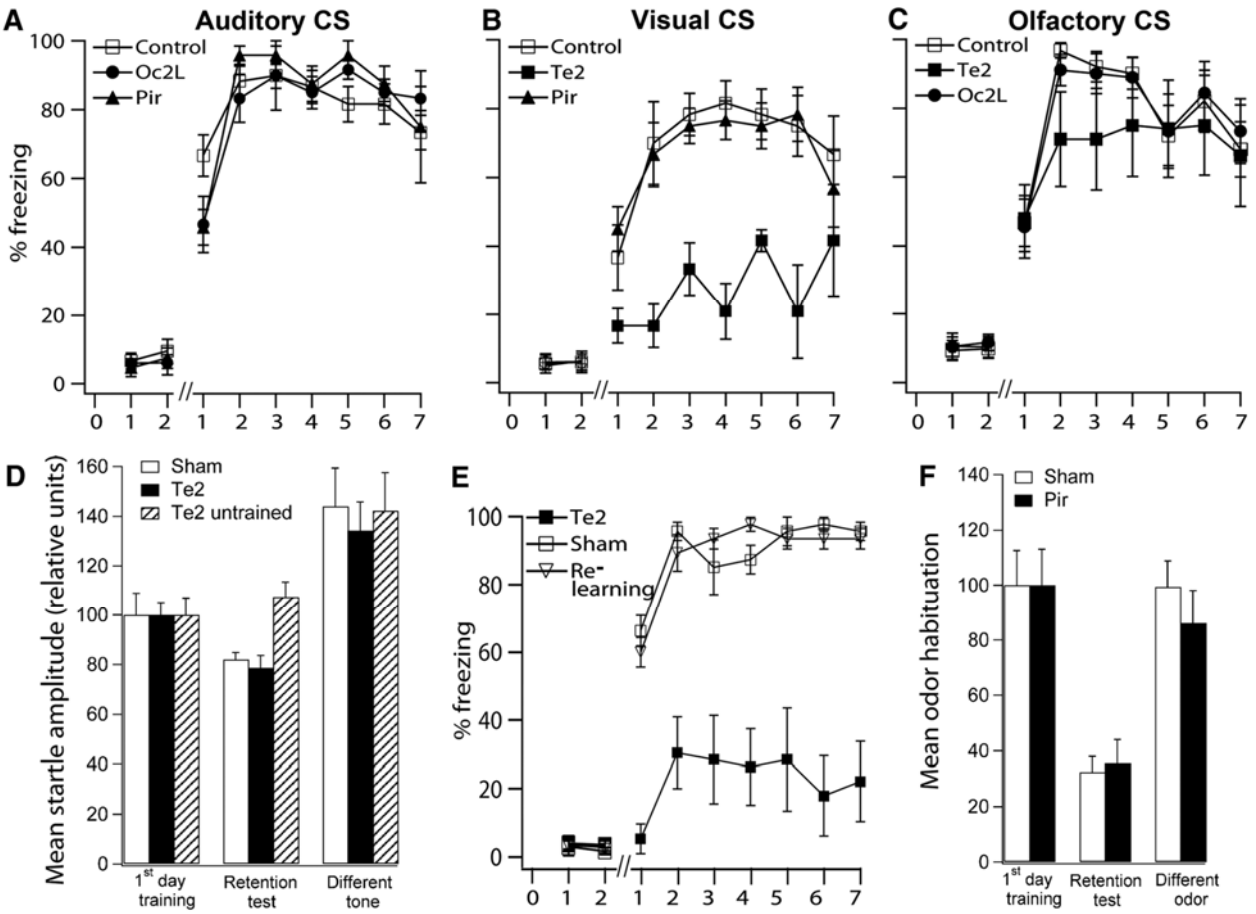

Fig. 5

Role of sensory cortices in emotional memory. (A) Acoustic fear memories in control (n = 10), Oc2L-lesioned (n = 10), and posterior piriform cortex–lesioned (Pir, n = 8) rats. (B) Visual fear memory in control (n = 9), Te2-lesioned (n = 8), or posterior piriform–lesioned (Pir, n = 10) animals. (C) Olfactory fear memory in control (n = 12), Te2-lesioned (n = 8), or Oc2L-lesioned (n = 9) rats. (D) Startle habituation during the first day of training, during the retention trial, and during the presentation of a new tone in Te2-lesioned (n = 12) and sham-operated (n = 12) rats and in those untrained (n = 10). (E) Remote fear memories to white-noise CSs were impaired in Te2-lesioned (n = 8) rats relative to sham-operated animals (n = 8). Te2-lesioned animals could form new fear memories ($F_{1,14} = 0.007$, $P > 0.05$). (F) Olfactory habituation in the posterior piriform–lesioned animals (Pir, n = 8) and in sham-operated animals (n = 10).

Collectively, these results indicate that each secondary sensory cortex is involved in emotional memories related to a specific sensory modality. The modality-specific involvement of sensory cortices suggests that lesions of these sites do not affect innate fear behavior. To address this further, we tested animals in two well-established models of anxiety—the open field and elevated plus maze tests—and also tested their unconditioned fear in the presence of a predator odor. No differences were detected among groups in all cases (15) (fig. S8).

Role of secondary cortices in fear memories. Secondary sensory cortices may encode the physical features of the sensory stimuli ("recognition memory"). Alternatively, these sites may store the emotional meaning acquired by sensory stimuli during an emotional experience. To discriminate between the two possibilities, we tested whether lesions of secondary cortices impair long-term recognition memory of stimuli not associated with any overt emotional consequences. If lesions of sensory cortices do not affect such memories, we can reliably exclude the proposition that these sites play a crucial role in encoding the physical features of sensory stimuli. Sudden auditory stimuli elicit a startle response; if the stimuli are presented repeatedly, startle response habituates across days and this habituation is retained for weeks. Long-term habituation memory relies on the memory of the sensory stimuli presented repeatedly. Therefore, we tested whether Te2 lesions affect such recognition memory.

Four weeks after training, animals were lesioned in Te2 or sham-operated. Figure 5D presents mean startle amplitude measured during memory retention trial. A mixed ANOVA revealed differences between the first day of training and the day of testing ($F_{1,22} = 12.32$, $P < 0.05$). Thus, recognition memory is still present 1 month after training. There were no differences between groups ($F_{1,22} = 1.36$, $P > 0.05$; group × trial interaction, $P > 0.05$). We also analyzed the startle reflex in Te2-lesioned animals not submitted to the habituation training. In these rats, startle response was measured 4 weeks before the lesion and shortly after it (Fig. 5D). The two measurements did not differ significantly ($F_{1,9} = 1.08$, $P > 0.05$), thus indicating that (i) Te2 lesions did not affect startle reaction, and (ii) the decrement in startle amplitude observed in the habituated animals is produced by the habituation protocol and is not due to the mere passage of time. During the retention trial, one-way ANOVA showed differences among groups ($F_{2,31} = 6.13$, $P < 0.05$). A Newman-Keuls test individuated differences between the untrained group and the other groups ($P < 0.05$), but not between the sham-habituated and Te2-habituated animals. Thus, Te2 lesion did not affect long-term habituation memory. After the memory test, we measured startle response to a new sound never before experienced in order to verify the capacity of the animals to discriminate between novel and familiar stimuli (Fig. 5D). One-way ANOVA showed no differences among habituated and untrained animals ($F_{1,9} = 0.4$, $P > 0.05$).

The acoustic stimuli used in the habituation paradigm differed from those presented in the fear conditioning experiments (a white noise versus a pure tone). Therefore, we verified the impact of Te2 lesion on remote fear memories produced by the association of white noises (CSs) with footshock (US) (Fig. 5E). ANOVA showed differences between lesioned and control animals ($F_{1,14} = 58.18$, $P < 0.05$; group × trial interaction, $P > 0.05$). Thus, a Te2 lesion hampered emotional memories irrespective of the type of acoustic stimuli used. Again, lesioned animals could relearn CS-US association (Fig. 5E).

We then examined whether a lesion of the posterior piriform cortex affects long-term habituation to an olfactory stimulus (Fig. 5F). A mixed ANOVA indicated differences

between the first day of training and the day of testing ($F_{1,16} = 56.98$, $P < 0.05$); that is, long-term habituation was present 1 month after training. The same statistical analysis showed no difference between lesioned and sham-operated rats ($F_{1,16} = 0.91$, $P > 0.05$; group × trial interaction, $P > 0.05$), thus suggesting that a posterior piriform lesion did not affect habituation memory. Student's t test confirmed the lack of difference between lesioned and control groups during the retention trial ($t_{16} = -0.81$, $P > 0.05$). Control and lesioned animals were also similar in their capacity to discriminate between familiar and novel odors ($t_{16} = -0.01$, $P > 0.05$) (Fig. 5F).

Cortical activity related to recent or remote aversive memories. An alternative method to investigate the involvement of neural sites in memory processes is based on the analysis of expression of early genes such as cfos and zif268. Such genes are required for synaptic plasticity and are used as an index of neuronal activation (25, 26). We tracked the level of the proteins encoded by zif268 in Te2 to investigate (i) whether this site is recruited by recent and/or remote fear memories, (ii) whether it is engaged by emotional and/or sensory memory, and (iii) the cortical layer(s) activated. We first analyzed zif268 expression in animals retrieving remote fear memory. zif268 was measured after the presentation of a tone in three different groups. In the first group, the tone was paired with the US 1 month before its presentation ("fear conditioned memory"); in the second group, 1 month before its presentation, the tone was presented unaccompanied by any emotional stimuli ("recognition memory"). The third group consisted of animals that had never perceived the tone previously. zif268 expression was analyzed separately in the different cortical layers without experimenter knowledge of the experimental condition (Fig. 6A). One-way ANOVA showed a significant difference among groups in layers II–III ($F_{2,41} = 11.929$, $P < 0.05$) and IV ($F_{2,41} = 7.466$, $P < 0.05$) but not in layers V ($F_{2,41} = 2.621$, $P > 0.05$) or VI ($F_{2,41} = 0.827$, $P > 0.05$). In all cases, a Newman-Keuls test showed that zif268 labeling increased in the conditioned groups ($P < 0.05$) (Fig. 6C). No differences were found between animals naïve to the sound and those that had perceived it previously, thus suggesting that novel and familiar acoustic stimuli activated Te2 in a similar manner. Laminae II–IV are intensely connected with the other cortices and with the thalamus (13, 25, 26). An increased activity in these layers has previously been reported in parietal layers after remote spatial memory retrieval. In that case, the authors suggested that such a laminar activity reflects the formation of corticocortical neural assemblies (26). Our data extend these findings to secondary sensory cortices and to fear memory, perhaps suggesting that memory storage and retrieval in the cortex engages superficial layers as a general rule.

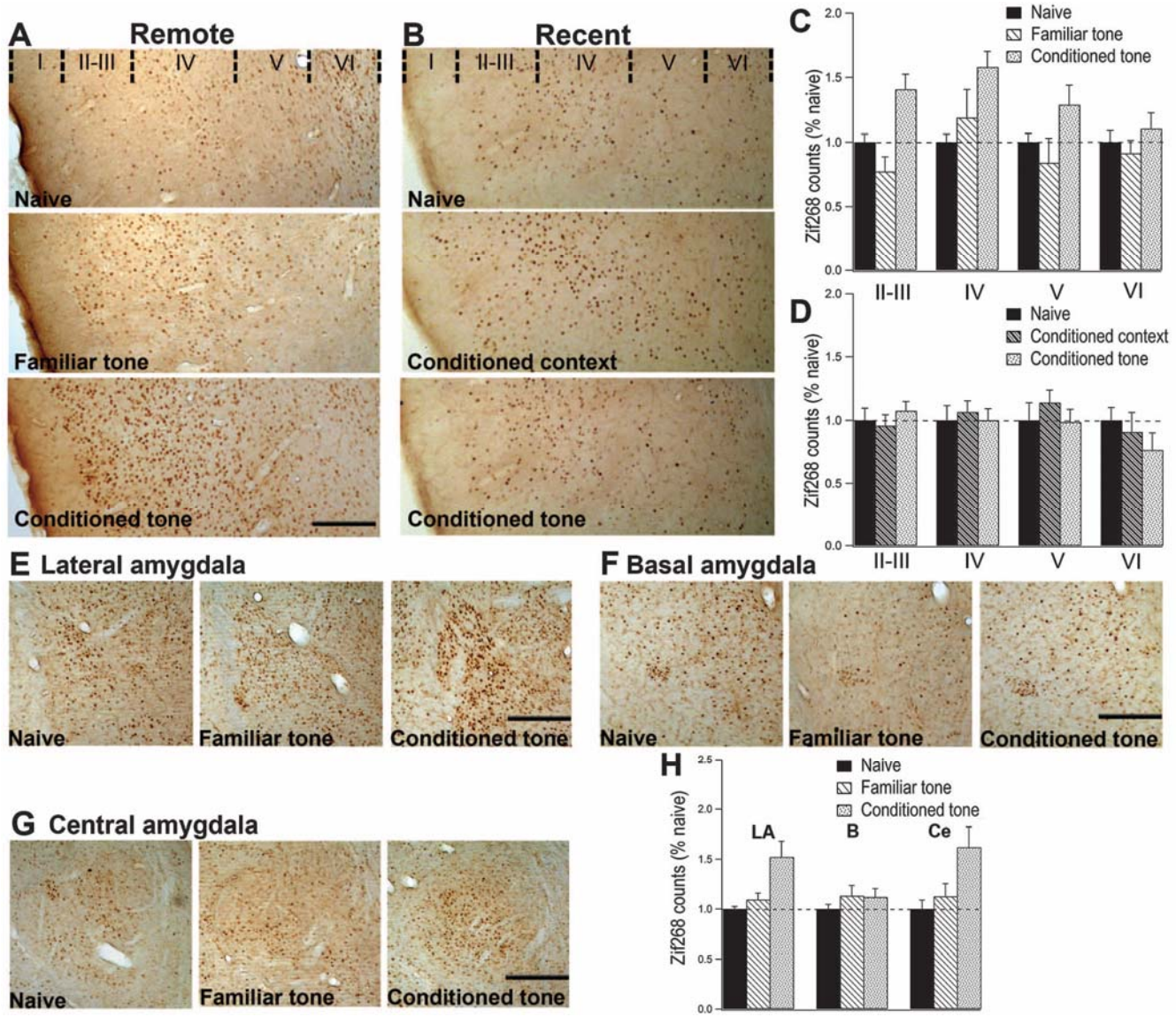

Fig. 6

zif268 protein expression in sensory neocortex and in the amygdala. (A and B) Photomicrographs of zif268 staining within Te2 cortical layers I–VI after testing for remote (A) or recent (B) fearful memory. Scale bar, 150 µm. (C) After remote memory test, zif268 counts relative to naïve animals significantly increased in conditioned rats in layers II–III and IV. (D) After recent fear memory test, zif268 analysis revealed no difference among groups in Te2 cortical layers II–III ($F_{2,22} = 0.58$, $P > 0.05$), IV ($F_{2,22} = 0.13$, $P > 0.05$), V ($F_{2,22} = 0.57$, $P > 0.05$), and VI ($F_{2,22} = 0.89$, $P > 0.05$). (E to G) After remote memory test, zif268 was also analyzed in the lateral, basal, and central nuclei of the amygdala. Scale bars, 230 µm. (H) Lateral (LA) and central (Ce), but not basal (B), amygdala activity was significantly enhanced after remote memory retrieval mainly in the tone-conditioned group. All data are means ± SEM.

Te2 is reciprocally connected with the amygdala (6, 9, 13), a crucial site for fear-related processes (1, 2). Therefore, we investigated whether the lateral, basal, and central regions of the amygdala are recruited by remote fearful memories. Analysis of zif268 expression revealed that lateral ($F_{2,31} = 9.94$, $P < 0.05$) and central ($F_{2,31} = 5.30$, $P < 0.05$) regions, but not the basal nucleus ($F_{2,31} = 0.03$, $P > 0.05$), were significantly activated by remote fear memory test (Fig. 6, E to H).

The increase of zif268 activity may be reflective of a mnemonic code but may also be a bias imparted by the expression of fear behavior displayed mainly by conditioned animals. To discriminate between the two possibilities, we examined zif268 proteins in the posterior piriform cortex, a region not engaged by acoustic fear learning. One-way ANOVA showed no differences between groups in all layers ($P > 0.05$) (fig. S9). Thus, zif268 activity was increased specifically in the brain regions engaged in acoustic fear memory.

We next proceeded to track zif268 protein expression in animals retrieving recent fear memories. To isolate auditory memory processes from nonspecific effects that may arise from a training procedure performed 24 hours before memory reactivation, we also examined zif268 activity in animals conditioned to the experimental context but not to the tone ("conditioned context"). In Te2 cortex, one-way ANOVA showed no differences among naïve, "conditioned context," and "conditioned tone" groups in all layers ($P > 0.05$) (Fig. 6, B and D). The results indicate that the secondary sensory cortices are preferentially involved in processing remote, rather than recent, fear memories. The distinct patterns of zif268 expression after recent and remote memory tests confirm that the activity of early genes in Te2 is not simply a correlate of fear-related behaviors, because freezing levels were similar at both time points (fig. S9). zif268 counts revealed a marked increase in the activity of lateral ($F_{2,19} = 5.69$, $P < 0.05$), basal ($F_{2,19} = 5.79$, $P < 0.05$), and central ($F_{2,19} = 3.92$, $P < 0.05$) amygdala in the "conditioned tone" group (fig. S10), thus suggesting that the amygdala is also involved in the early stage of memory formation (1, 2, 5–7).

Several observations indicate that the amnesia we observed is related specifically to interference with memory processes. As previously discussed, impairment in sensory perception or in the innate fear behavior can be reliably ruled out. Another effect that could have reduced learned fear might have resulted from deafferentiation or cell death in the amygdala or thalamic nuclei produced by cortical lesions of neurons that directly innervate these sites. However, amygdala or thalamic dysfunctions prevent CS-US association and impair recent fear memories (6, 7, 27–29), in marked contrast to our data. In addition, amygdala lesions hampered emotional memories irrespective of the sensory modality used as CS, in contrast to the modality-specific memory deficit that we observed. The modality-specific amnesia also allows us to rule out any "mass action effect," in which amnesia would be produced by a large cortical disruption independent of the area lesioned. The latter possibility is also ruled out by the fact that primary cortex lesions that were larger than the secondary cortex disruption did not abolish fear memories. Finally, amnesia may be due to the involvement of sensory cortices in the transmission of sensory information to the amygdala. However, in this case, memory impairment should have been present both immediately and 1 month after conditioning. Indeed, ZIP peptide is thought to interfere with synaptic plasticity and not with basal synaptic transmission (18).

Sensory cortical lesions do not impair recent memories or learning of a new memory trace (5–11), thus suggesting that other regions are important in these early stages. Previous (1, 2, 5, 6) and present data indicate that the lateral and central regions of the amygdala support the formation of recent fear memories. The lateral region is the recipient of afferents from thalamus and sensory cortices (1). Plastic changes related to memory formation occur in both thalamic (3) and lateral amygdala (1) neurons. The central nucleus, which receives extensive connections from the lateral amygdala and

projects to several brainstem regions, may be involved in the organization of specific autonomic and behavioral responses. At this early stage, the cerebellum is also recruited to set the more appropriate responses to new stimuli and/or situations (30, 31). As fear memories mature, they become dependent on sensory cortices. Because previous (28, 29) and present results also support a role for the amygdala in the retrieval of remote memories, it may be that permanent memories are widely distributed across sensory cortices and amygdala neurons (as well as other sites). However, because of its anatomical and functional connections, the amygdala may provide the necessary link between neural sites that encode memory and autonomic or motor effectors. Our data do not allow us to discriminate between these possibilities; they indicate only that the amygdala alone is not sufficient to support permanent fear memories.

The anterior cingulate cortex also participates in the storage of permanent fear memories (25). This site plays an integrative role in emotional and cognitive control processes (e.g., attention, error detection and correction). In addition, it may also encode information about the aversive components of an emotional experience (32). Indeed, it may interact with sensory cortices to provide the integration among the multiple representations occurring in sensory cortices during memory storage.

Both previous and present data support the view that secondary cortices encode the emotional valence acquired by sensory stimuli with the experience. Previous findings have shown that plasticity related to long-term acoustic habituation takes place in the lower auditory system (i.e., brainstem and auditory nuclei) but not in the auditory cortex (33, 34), and that olfactory long-term habituation is related to olfactory bulb activity (35). Novel and familiar sounds determine a similar Te2 neuronal activation [(36) and present results], whereas Te2 activity increases significantly if the sounds have acquired a behavioral value (34). Finally, Te2 neurons show conditioning-induced changes in firing probability in response to CSs (37) and predict behavioral responses in a conditioned task (38). Also in the posterior piriform cortex, neurons exhibit activity according to the valence acquired with the experience by an odor cue (17, 39).

Collectively, our data provide the basis for a new conceptual framework for the storage of emotional memories. Visual, acoustic, and olfactory stimuli associated with a highly charged emotional situation take on the affective qualities of that situation. Secondary cortices that perform high-level sensory analysis combine sensory processing and memory plasticity to encode the behavioral salience of perceiving stimuli. Such information becomes widely distributed throughout the cortex, each secondary sensory cortex coding the valence of stimuli of a specific modality. Such a memory storage mechanism results in a synaptic strengthening of corticocortical connections that may provide the integrated view of the whole emotional experience during memory recall.

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