of the embryo to partly develop (Fig. 5D), as compared with nonelectroporated embryos (*7*) or embryos electroporated with a mock plasmid (Fig. 5C). Similar results were obtained by electroporation of the general caspase inhibitor P35 (*26*) or treatment with the zVAD-fmk (fig. S6). Hence, the control by Shh of Ptc-mediated cell death represents a crucial event for neuroepithelial cell survival and neural tube development.

Thus, a signaling pathway is generated by Ptc that leads to apoptosis for the cell expressing Ptc in the absence of Shh. The trio of Shh, Ptc, and Smo then suggests a very subtle balance between the differentiating–life-sustaining signal mediated by Smo when Shh binds Ptc and the death-inducing signal derived from Ptc in the absence of Shh. The positive signal has a crucial impact in determining cell fate (*1*, *5*). In addition, the positive signal mediated either by Shh or other Hedgehog proteins may regulate the negative proapoptotic signal of Ptc. For example, Gli-3, involved in Shh-Ptc-Smo signaling, functions in apoptosis regulation (*29*) and interferes with the cell death induction observed in Shh mutant mice (*9*). However, the dogma proposes that cell death induction may only be the result of an absence of the proper signal for cell differentiation. This view would be difficult to reconcile with the observation that the developing neural tube of the $Ptc^{-/-}$ mouse embryo does not suffer cell deficits, but rather is overgrown, as expected for an absence of Ptc-induced cell death (*30*). Moreover, in chick embryos experimentally deprived of Shh-producing midline cells (No + FP), inhibition of cell death by transfection with the dominant-negative mutant for Ptc-induced cell death not only suppresses cell death but appears to partly allow spinal cord development (Fig. 5, F to H, and fig. S6). Thus, the control of cell death by Shh may also be an important part of the Shh role during central nervous system development. The Ptc-mediated death observed in the absence of Shh would then appear to be not just a consequence of a lack of cell differentiation but an active process contributing to spinal cord development.

References and Notes

1. T. M. Jessell, *Nature Rev. Genet.* **1**, 20 (2000).
2. N. M. Le Douarin, M. E. Halpern, *Curr. Opin. Neurobiol.* **10**, 23 (2000).
3. N. M. Le Douarin, M. A. Teillet, M. Catala, *Int. J. Dev. Biol.* **42**, 909 (1998).
4. M. Tessier-Lavigne, C. S. Goodman, *Science* **274**, 1123 (1996).
5. P. W. Ingham, A. P. McMahon, *Genes Dev.* **15**, 3059 (2001).
6. C. Chiang *et al.*, *Nature* **383**, 407 (1996).
7. J. B. Charrier, M. A. Teillet, F. Lapointe, N. M. Le Douarin, *Development* **126**, 4771 (1999).
8. J. B. Charrier, F. Lapointe, N. M. Le Douarin, M. A. Teillet, *Development* **128**, 4011 (2001).
9. Y. Litingtung, C. Chiang, *Nature Neurosci.* **3**, 979 (2000).
10. N. Miao *et al.*, *J. Neurosci.* **17**, 5891 (1997).
11. J. Britto, D. Tannahill, R. Keynes, *Nature Neurosci.* **5**, 103 (2002).
12. M. Murone, A. Rosenthal, F. J. de Sauvage, *Curr. Biol.* **9**, 76 (1999).
13. V. Marigo, R. A. Davey, Y. Zuo, J. M. Cunningham, C. J. Tabin, *Nature* **384**, 176 (1996).
14. D. M. Stone *et al.*, *Nature* **384**, 129 (1996).
15. J. Taipale, M. K. Cooper, T. Maiti, P. A. Beachy, *Nature* **418**, 892 (2002).
16. C. Wicking, E. McGlinn, *Cancer Lett.* **173**, 1 (2001).
17. P. Mehlen *et al.*, *Nature* **395**, 801 (1998).
18. F. Llambi, F. Causeret, E. Bloch-Gallego, P. Mehlen, *EMBO J.* **20**, 2715 (2001).
19. C. Tanikawa, K. Matsuda, S. Fukuda, Y. Nakamura, H. Arakawa, *Nature Cell Biol.* **3**, 216 (2003).
20. K. Thiebault *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 4173 (2003).
21. S. Rabizadeh *et al.*, *Science* **261**, 345 (1993).
22. D. E. Bredesen *et al.*, *Cell Death Differ.* **5**, 365 (1998).
23. C. Forcet *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 3416 (2001).
24. M. C. Bordeaux *et al.*, *EMBO J.* **19**, 4056 (2000).
25. N. A. Thornberry *et al.*, *J. Biol. Chem.* **272**, 17907 (1997).
26. C. Thibert *et al.*, data not shown.
27. C. Koike *et al.*, *Oncogene* **21**, 2670 (2002).
28. E. A. Barnes, M. Kong, V. Ollendorff, D. J. Donoghue, *EMBO J.* **20**, 2214 (2001).
29. K. Aoto, T. Nishimura, K. Eto, J. Motoyama, *Dev. Biol.* **251**, 320 (2002).
30. L. V. Goodrich, L. Milenkovic, K. M. Higgins, M. P. Scott, *Science* **277**, 1109 (1997).

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Supporting Online Material

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Materials and Methods

Figs. S1 to S6

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Amygdalar and Hippocampal Theta Rhythm Synchronization During Fear Memory Retrieval

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The amygdalohippocampal circuit plays a pivotal role in Pavlovian fear memory. We simultaneously recorded electrical activity in the lateral amygdala (LA) and the CA1 area of the hippocampus in freely behaving fear-conditioned mice. Patterns of activity were related to fear behavior evoked by conditioned and indifferent sensory stimuli and contexts. Rhythmically synchronized activity at theta frequencies increased between the LA and the CA1 after fear conditioning and became significant during confrontation with conditioned fear stimuli and expression of freezing behavior. Synchronization of theta activities in the amygdalohippocampal network represents a neuronal correlate of conditioned fear, apt to improve neuronal communication during memory retrieval.

Considerable progress has been made in our understanding of the synaptic circuits and plasticity that underlie emotional learning, specifically during Pavlovian fear conditioning, and the involvement of the amygdala therein (*1*, *2*). Evidence suggests an alteration of neuronal responsiveness to fear-conditioned stimuli in the amygdala, sometimes paralleled by behavioral changes (*3*). During emotional arousal and various types of rhythmic activities during sleep, neurons in the amygdala produce theta activity (*4*, *5*). Although these activities facilitate synaptic plasticity and memory in extended neuronal networks, their relevance for the expression of fear or fear memory remains unclear. Of the extensive afferent and efferent connections of the amygdala, interactions with the hippocampus are particularly important for memory formation (*6*, *7*). Amygdala lesions attenuate hippocampal synaptic plasticity and block the memory-enhancing effects of direct hippocampal stimulation (*8*, *9*). Further, behavioral stress as well as stimulation of the amygdala interferes with synaptic plasticity in the hippocampal formation (*10*–*12*). This interaction appears to be bidirectional, given that tetanic stimulation of hippocampal efferent fibers can induce long-term potentiation in the LA (*13*). The present study was designed to characterize patterns of neural activity in amygdalohippocampal pathways related to the retrieval and expression of conditioned fear. The rationale was to use Pavlovian fear conditioning as a simple, well-established model of emotional learning (*1*) and to focus on

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interactions between the LA, which is the major input station of sensory signals to the amygdala, and the CA1 area of the hippocampus, with which the LA is mutually and prominently interconnected (*14*).

Mice were fear conditioned through the explicitly paired presentation of conditioned (CS^+) and unconditioned (US) stimuli, and their responses during fear-memory retrieval were compared with those of control animals undergoing explicitly unpaired training (*15*). Conditioned freezing behavior was monitored in the retrieval session to assess fear memory and the emotional relevance of the CS^+ and an indifferent control stimulus (CS^-). In addition, risk-assessment behavior (overt orienting and stretched attending) was examined as a control measure of species-specific defensive behavior with minimum locomotor activity. The results show a pronounced and selective fear response in conditioned mice, and a moderately aversive or ambiguous response of controls to the CS^+. The behavioral response to the CS^- was not different between groups (fig. S1).

At the same time as the behavioral assessment, we determined electrophysiological activity by recording field potentials in both the LA and the CA1 of the dorsal hippocampus (*15*). In control animals, activity in the CA1 was distributed around the theta frequency, covering a relatively wide frequency range with only short periods of rhythmic patterns. No prominent pattern of activity was observed in the LA (Fig. 1, A to C), and cross-correlation analyses did not show any significant synchronization of activity between the two brain areas (Fig. 1, D and H). Activity in the CA1 or the LA did not differ during stimulus and prestimulus periods, nor could a difference be observed between CS^+ and CS^- periods. In fear-conditioned animals, theta activity prevailed in the CA1 under all stimulus conditions (Fig. 2). Activity in the LA before and during CS^- presentation resembled the activity in control animals, in that there was no indication of a predominant pattern or frequency (Fig. 2, A to C). Upon presentation of the CS^+, activity in the LA shifted into a highly rhythmic pattern centered at the theta frequency band (Fig. 2, F and G). Cross-correlation analyses revealed a progressive increase in synchronized activity at a frequency of 4 to 8 Hz during the CS^+ (Fig. 2H and fig. S2). By averaging the cross-correlograms from four consecutive CS^+ presentations and taking the second positive peak as a quantitative measure, a significant ($P < 0.004$, $t = 3.531$; Student's t test) increase in theta synchronization could be demonstrated in fear-conditioned animals (mean ± SEM: 0.125 ± 0.012; $n = 8$), as compared with control animals (0.048 ± 0.020; $n = 6$). A partial, insignificant increase was observed in fear-conditioned mice during the CS^- (0.085 ± 0.029 as compared with 0.044 ± 0.021 in controls) (*16*).

Atropine-sensitive type 2 theta activity (4 to 8 Hz) has been shown to occur in the hippocampal formation during periods of immobility, whereas atropine-resistant type 1 theta activity (8 to 14 Hz) is observed during exploration (*17*, *18*). Type 2 theta can be elicited by strongly arousing stimuli, such as confrontation with predators or noxious stimuli (*19*). We therefore deter-

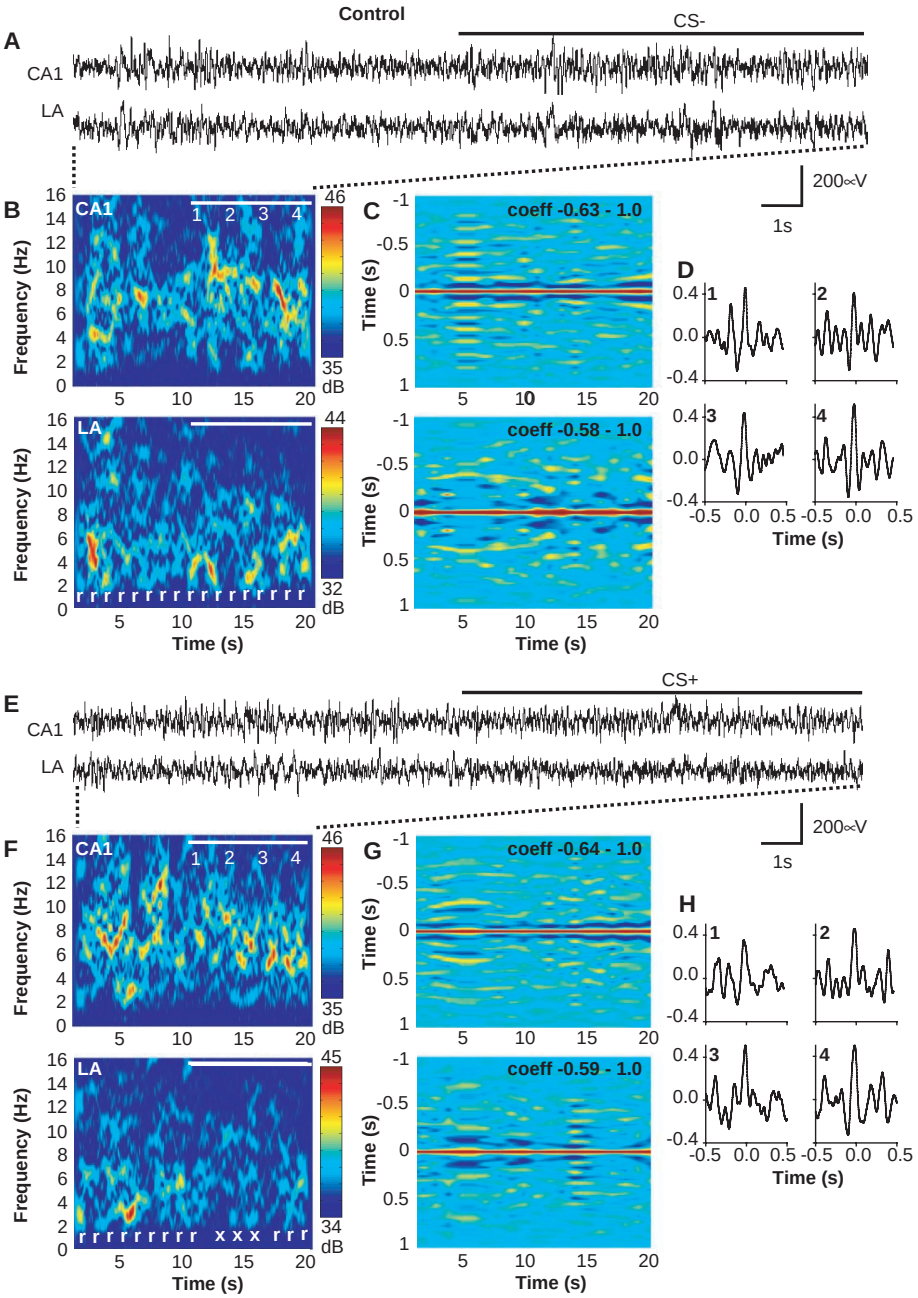


Fig. 1. Neural activity in the CA1 and the LA of a control animal during the presentation of CS^- [(A) to (D)] and CS^+ [(E) to (H)]. (**A** and **E**) Original traces of field-potential recordings in the CA1 (upper traces) and the LA (bottom traces) before and during CS^- or CS^+ presentation (bars above the traces). (**B** and **F**) Color-coded power spectra of the traces in (A) and (E) demonstrate CA1 theta activity in a frequency band of 4 to 12 Hz during the entire stimulus (white bar) and prestimulus phase. LA activity lacks such a prominent pattern [the time scales in (B) and (F) differ from those in (A) and (E)]. Behavior (r, risk assessment; x, exploration) is indicated near the bottom of the diagrams. (**C** and **G**) Autocorrelation analyses indicate only short periods of rhythmic activities in the CA1 and the lack of rhythms in the LA. The correlation coefficient is indicated in the upper right corner. (**D** and **H**) Cross-correlation analyses of activities in the LA and the CA1 during stimulus presentation reveal a low level of synchronization. Four successive 3-s intervals are shown [as indicated by numbers 1 to 4 in (B) and (F)], starting 1 s before presentation of the stimuli.

mined electrophysiological activity during periods of defined defensive behavior, such as freezing and risk assessment (*15*). Indeed, freezing of fear-conditioned mice during the CS^+ was associated with a significantly stronger theta synchronization (0.112 ± 0.019 at 5.0 ± 1.5 Hz, $P < 0.05$; $n = 8$) than the risk-assessment response of controls (0.050 ± 0.030 at 4.4 ± 0.6 Hz; $n = 6$) (Fig. 3A). An association of theta synchronization and freezing behavior was also evident in three control animals, which displayed freezing intervals of sufficient length for a cross-correlation analysis (0.141 ± 0.034). Both training groups showed low levels of synchronized theta activity when displaying risk-assessment behavior during the CS^- (0.068 ± 0.016 in fear-conditioned animals; 0.055 ± 0.009 in controls) and strong hippocampal theta activity without synchronization to the LA during exploration (Fig. 3B).

Together, our observations indicate that elicitation of conditioned freezing behavior is associated with type 2 theta activity and theta synchronization in amygdalohippocampal pathways (*20*). Theta synchronization may thus be functionally related to the retrieval and/or expression of conditioned fear.

Both the hippocampus and the amygdala are known to participate in the formation of fear memories (*1*, *6*), and their relative contribution appears to depend on both the conditioning paradigm and training intensity used (*21*). Conditional freezing involves both the LA and the dorsal hippocampus, and although the latter may be more concerned with configural processing and contextual tasks, a lesion of the area still reduces cued conditioned freezing performance (*22*). We thus extended our analysis to amygdalohippocampal synchronization during contextually induced freezing behavior (Fig. 4). Again, we observed significant freezing behavior ($47.0 \pm 7.3\%$ of recording time; $P < 0.0001$, $t = 14.24$, Student's t test, $n = 5$; compared to the neutral context) that was associated with pronounced theta activity in the LA. As during cued retrieval, these theta oscillations in the LA were significantly synchronized with hippocampal rhythms (0.207 ± 0.051 at 4.3 ± 0.8 Hz; $P < 0.05$, $t = 2.627$, Student's t test, $n = 5$). No comparable rhythmicity and synchronicity were observed during risk assessment in the shock context or the neutral context. Although it has been suggested that foreground context (fig. S3) and background context conditioning are differently dependent on the dorsal hippocampus (*23*), the two conditioning methods were equally efficient in evoking synchronized rhythmic activity. Hence, communication along amygdalohippocampal pathways may be involved in the development and expression of fear-related emotions under different training conditions. Indeed, the temporally structured relay of signals between the amygdala and hippocampus during theta synchronization may allow a parallel processing of unitary and configural stimulus information related to cued and contextual fear memories.

The LA/CA1 network system seems to be well suited to rhythmically oscillate at theta

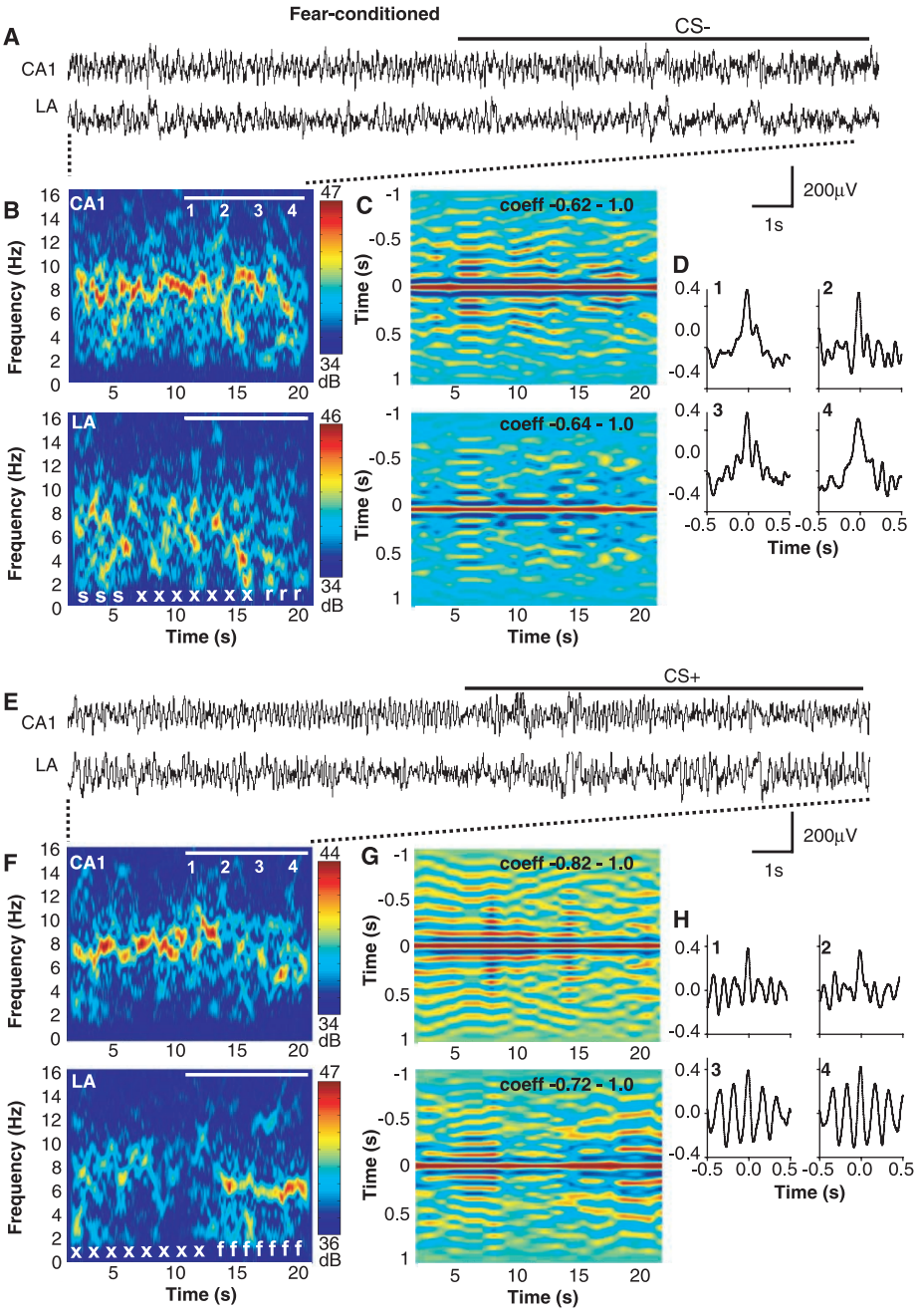
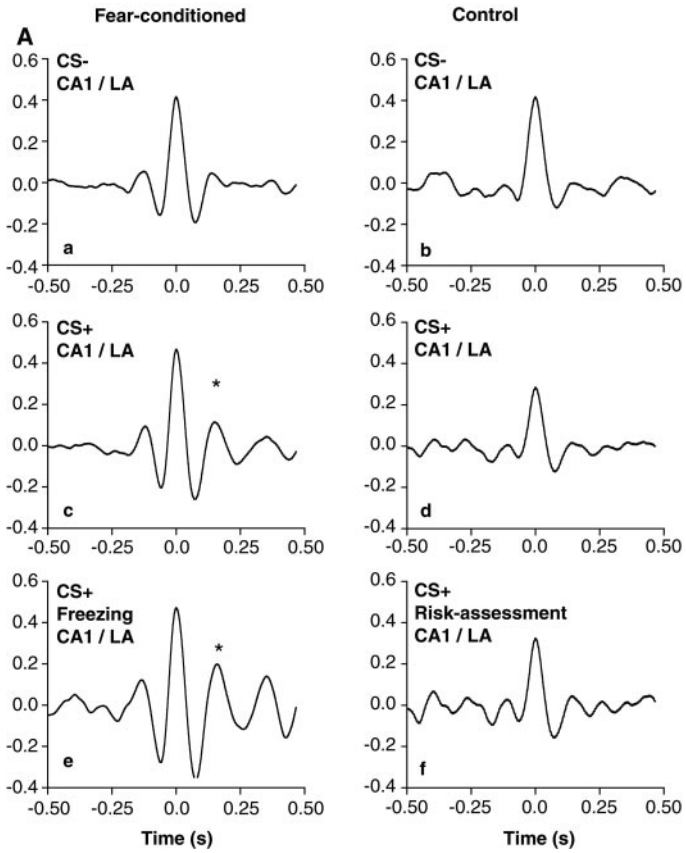


Fig. 2. Neural activity in the CA1 and LA of a fear-conditioned animal during presentation of CS^- [(A) to (D)] and CS^+ [(E) to (H)]. (**A** and **E**) Original traces of field-potential recordings in the CA1 (upper traces) and the LA (bottom traces) before and during CS^- or CS^+ presentation (bars above the traces). (**B** and **F**) Color-coded power spectra of the traces in (A) and (E) [which have time scales that differ from those in (B) and (F)]. Similar to control animals, broad-range theta activity is seen in the CA1 but not in the LA during the CS^-. However, CS^+ presentation is associated with highly rhythmic theta activity at around 5 Hz in both brain areas and the expression of freezing behavior (f) (s, stereotypic behavior; x, exploration; r, risk assessment). (**C** and **G**) Autocorrelation analyses indicate short epochs of rhythmic activities in the CA1 alone during exploratory behavior and an increased rhythmic activity in the LA during freezing. (**D** and **H**) Cross-correlation analyses reveal a progressive increase of correlated theta activity in the two brain areas during presentation of the CS^+ but not the CS^-. Four successive 3-s intervals are shown [(as indicated by numbers 1 to 4 in (B) and (F)], starting 1 s before presentation of the stimuli.



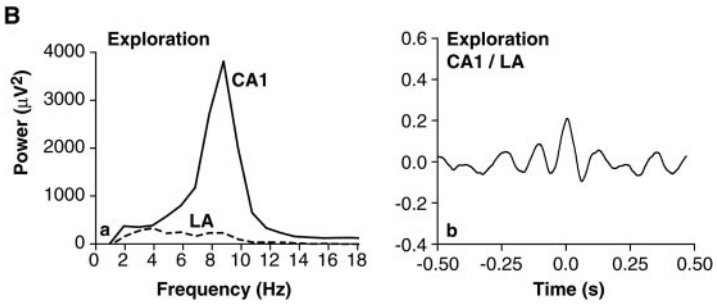


Fig. 3. (**A**) Averaged cross-correlograms of CA1/LA activity in the population of fear-conditioned animals ($n = 8$; a and c) and control animals ($n = 6$; b and d), during presentation of the first CS^- (a and b) and the first CS^+ (c and d). Synchronization increases as a result of fear conditioning and becomes significant during the presentation of the CS^+. The increase is particularly evident when comparing the predominant behavioral responses: freezing in fear-conditioned animals (e; $n = 5$) and risk assessment in control animals (f; $n = 5$). Asterisks indicate significant differences in the amplitude of the second peak (representing theta activity at about 5 Hz) between the fear-conditioned and control group. (**B**) Power spectra (a) and cross-correlogram (b) of extracellular field recordings in the CA1 and the LA during exploratory behavior. Profound theta activity is apparent in the CA1 but not the LA, and there is a lack of theta synchrony between the two brain areas.

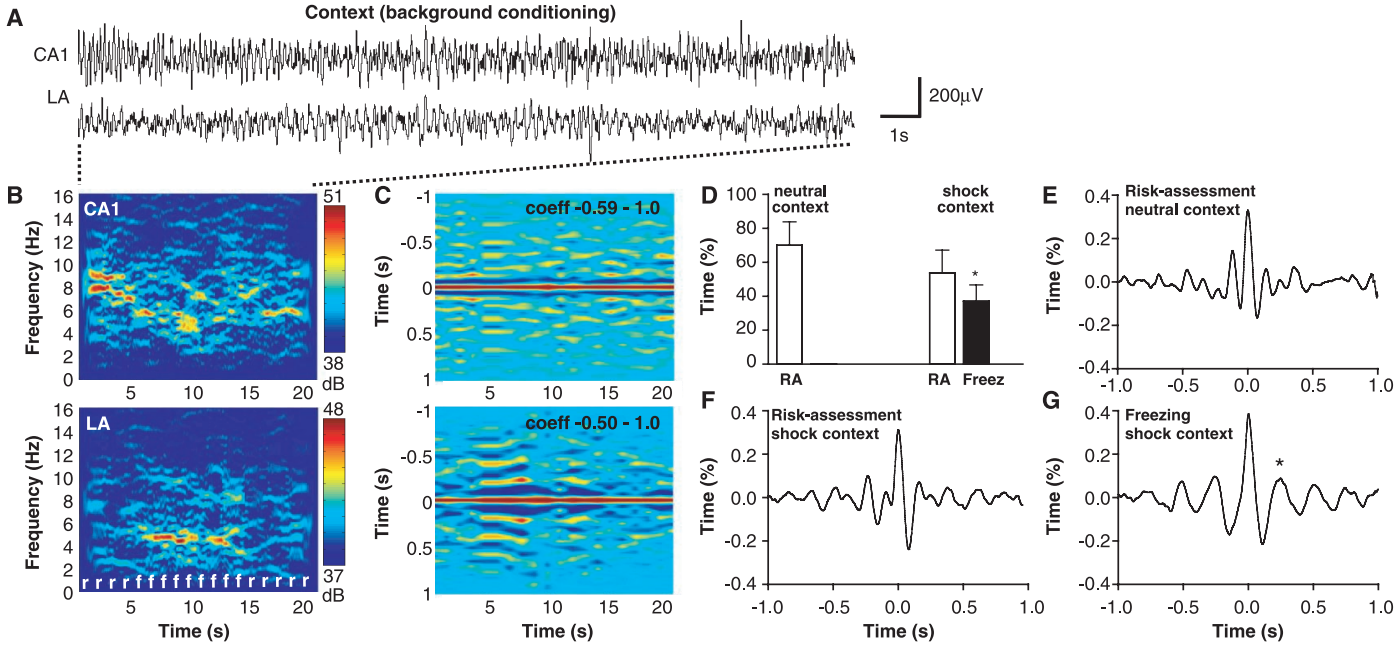


Fig. 4. Neural activity in the CA1 and the LA after background context conditioning. (**A**) Original traces of field-potential recordings in the CA1 (upper trace) and the LA (bottom trace). (**B**) Color-coded power spectrum of the traces shown in (A) [(A) and (B) have different time scales]. Theta activity at 7 to 10 Hz prevails in the CA1 during risk-assessment behavior (r, as indicated near the bottom of the diagrams), and theta activity at 4 to 5 Hz appears in the LA and the CA1 during freezing (f). (**C**) Autocorrelation analysis reveals epochs of rhythmic activities during both risk-assessment and freezing behavior in the CA1 and a high level of rhythmic activity in the LA during freezing. Numbers in the upper right corner indicate correlation coefficients. (**D**) Conditioned mice displayed significant freezing (Freez) and risk-assessment (RA) behavior during reexposure to the training context (mean + SEM) but no freezing behavior in the neutral context. Data of foreground ($n = 2$; fig. S3) and background contextual conditioning ($n = 3$) were similar and were therefore pooled. Lower conditional freezing compared with cued retrieval sessions is probably due to contextual preexposure in all groups. Asterisk indicates significant differences between contexts. (**E** to **G**) Averaged cross-correlograms of electrical neural activity in the CA1 and the LA (pooled from foreground and background conditioning experiments). Asterisk in (G) indicates significant difference in the amplitude of the second peak (representing theta activity at about 4 Hz) between freezing periods in the shock context and periods of risk-assessment behavior in either the neutral or the shock context.

frequencies: The basolateral amygdaloid complex receives synaptic inputs from the hippocampus (*24*), where theta waves have been observed (*17*, *18*), and from the anterior thalamic nuclei (*25*), which could transfer hippocampal theta rhythms to the amygdala. The intrinsic oscillatory properties of LA projection neurons (*26*, *27*), in turn, may provide adequate recurring time windows for the facilitated integration of synaptic inputs at theta frequencies (supporting online material text). Consistent with this notion is the observation that cellular theta activities in the perirhinal cortex and amygdala can be phase locked to entorhinal theta waves (*28*, *29*), and thus most likely also to hippocampal theta waves. Given evidence that theta waves or theta-frequency stimulation facilitates synaptic plasticity, such as long-term potentiation (and depotentiation) in the hippocampus (*30*) or long-term depression in the LA (*31*), the increase in coherent theta activities in amygdalohippocampal circuits may represent an increase in neuronal communication apt to promote or stabilize synaptic plasticity in these areas in relation to the retention of fear memory.

References and Notes

1. J. E. LeDoux, *Annu. Rev. Neurosci.* **23**, 155 (2000).
2. S. Maren, *Trends Neurosci.* **22**, 561 (1999).
3. G. E. Schafe, K. Nader, H. T. Blair, J. E. LeDoux, *Trends Neurosci.* **24**, 540 (2001).
4. D. Paré, D. R. Collins, *J. Neurosci.* **20**, 2701 (2000).
5. D. Paré, D. R. Collins, J. G. Pelletier, *Trends Cogn. Sci.* **6**, 306 (2002).
6. L. Cahill, J. L. McGaugh, *Trends Neurosci.* **21**, 294 (1998).
7. L. Cahill, *Brain Res.* **126**, 29 (2000).
8. M. G. Packard, L. Cahill, J. L. McGaugh, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 8477 (1994).
9. Y. Ikegaya, H. Saito, K. Abe, *Brain Res.* **656**, 157 (1994).
10. Y. Ikegaya, K. Abe, H. Saito, N. Nishiyama, *J. Neurophysiol.* **74**, 2201 (1995).
11. I. Akirav, G. Richter-Levin, *J. Neurosci.* **19**, 10530 (1999).
12. S. Frey, J. Bergado-Rosado, T. Seidenbecher, H.-C. Pape, J. U. Frey, *J. Neurosci.* **21**, 3697 (2001).
13. S. Maren, M. S. Fanselow, *J. Neurosci.* **15**, 7548 (1995).
14. A. Pitkänen, M. Pikkarainen, N. Nurminen, A. Ylinen, *Ann. N.Y. Acad. Sci.* **911**, 369 (2000).
15. Materials and methods are available as supporting material on *Science* Online.
16. Moderate effects upon CS^- presentation may reflect generalization and/or changes in baseline synchronization. These were not significant under the experimental conditions used, corroborating the specificity of conditioning. Similar electrophysiological and behavioral results were also obtained in two mice in which the low-frequency tone had been used as CS^+ and the high-frequency tone as CS^-.
17. G. Buzsaki, *Neuron* **33**, 325 (2002).
18. B. H. Bland, *Prog. Neurobiol.* **26**, 1 (1986).
19. L. Cahill, in *The Amygdala. A Functional Analysis*, J. P. Aggleton, Ed. (Oxford Univ. Press, New York, 2001), pp. 425–445.
20. The possibility that this result was due to volume transmission or other technical artifacts can be excluded, because two groups of animals were compared, and they differed in the temporal order of CS^+ and US presentation during training only but not with respect to any parameters of the electrophysiological recordings. The CS^- was used as a within-subject control. Also, although theta activity was strong in the CA1 during exploratory behavior of the conditioned animals, correlated theta activity with the LA could not be observed (Fig. 3B), providing evidence against a passive transmission of theta oscillations to the LA.
21. S. Maren, *Neurobiol. Learn. Mem.* **76**, 268 (2001).
22. S. Maren, G. Aharonov, M. S. Fanselow, *Behav. Brain Res.* **88**, 261 (1997).
23. R. G. Phillips, J. E. LeDoux, *Learn. Mem.* **1**, 34 (1994).
24. D. B. Willingham, *Neuron* **18**, 5 (1997).
25. R. P. Vertes, Z. Albo, G. Viana Di Prisco, *Neuroscience* **104**, 619 (2001).
26. D. Paré, H.-C. Pape, J. Dong, *J. Neurophysiol.* **74**, 1179 (1995).
27. H.-C. Pape, D. Paré, R. B. Driesang, *J. Neurophysiol.* **79**, 205 (1998).
28. D. Paré, H. Gaudreau, *J. Neurosci.* **16**, 3334 (1996).
29. D. R. Collins, E. J. Lang, D. Paré, *Neuroscience* **89**, 1025 (1999).
30. R. P. Vertes, B. Kocsis, *Neuroscience* **81**, 893 (1997).
31. T. Heinbockel, H.-C. Pape, *J. Neurosci.* **20**, RC68 (2000).

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Supporting Online Material

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Materials and Methods

Figs. S1 to S3

References

17 April 2003; accepted 8 July 2003

Detection of Sweet and Umami Taste in the Absence of Taste Receptor T1r3

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The tastes of sugars (sweet) and glutamate (umami) are thought to be detected by T1r receptors expressed in taste cells. Molecular genetics and heterologous expression implicate T1r2 plus T1r3 as a sweet-responsive receptor, and T1r1 plus T1r3, as well as a truncated form of the type 4 metabotropic glutamate receptor (taste-mGluR4), as umami-responsive receptors. Here, we show that mice lacking T1r3 showed no preference for artificial sweeteners and had diminished but not abolished behavioral and nerve responses to sugars and umami compounds. These results indicate that T1r3-independent sweet- and umami-responsive receptors and/or pathways exist in taste cells.

The *sac* gene in mice is the major genetic determinant regulating behavioral and nerve responses to artificial sweeteners, such as saccharin, and to several sugars (*1*–*6*). Recently, the taste receptor T1r3 was identified as the *sac* gene product (*7*–*12*). Heterologously expressed T1r3 appears not to function on its own. However, in combination with T1r2 it responds to many sweet compounds, and in combination with T1r1 it responds to glutamate and other umami compounds (*11*, *13*, *14*). To determine the role of T1r3 in vivo, we produced knockout (KO) mice lacking the entire T1r3 coding region by homologous recombination in C57BL6 (B6) embryonic stem (ES) cells and then injected the targeted stem cells into blastocysts (Fig. 1, A and B). T1r3 protein was absent in T1r3 KO mice, as demonstrated by indirect immunofluorescence (Fig. 1, C and D). The T1r3 KO mice were healthy and fertile with no obvious anatomical or behavioral abnormalities. The gross anatomy of the taste tissue and number of taste buds appeared normal in the T1r3 KO mice (Fig. 1F). Knocking out the *T1r3* gene did not alter expression of T1r1 (*15*) or T1r2 (Fig. 1, E and F).

Behavioral tests (*16*) were conducted to examine the responses of T1r3 KO mice to tastants representing five taste qualities (sweet, bitter, salty, sour, and umami). In two-bottle preference tests, the T1r3 KO mice displayed indifference to sucrose and three artificial sweeteners (sucralose, acesulfame K, and SC45647) at concentrations that elicited maximal preference in B6 wild-type littermate controls (Fig. 2). At concentrations that were 5 to 10 times as high as those needed to elicit a strong preference in B6 wild-type mice, the T1r3 KO mice preferred sucrose, but avoided all three artificial sweeteners. The response of the T1r3 KO mice to glucose was slightly reduced as compared with that of the B6 wild-type controls (Fig. 2) but was not significant at the $P < 0.05$ level ($P = 0.074$; Table 1). There was no difference between the responses of T1r3 KO mice

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