sites in each county, depending on the popularity of a given mixture with farmers. Plots ranged from 100 to 450 m^2 each, depending on field size.

Survey plots were assessed in late August for the severity of blast symptoms, expressed as the percentage of panicle branches that were necrotic due to the effects of *M. grisea*. Disease was assessed at five sampling points in each plot, distributed in a uniform pattern. Twenty hills resulting from the transplanting process were evaluated at each sampling point, with each hill containing about 10 panicles per hill, to give a total of approximately 1,000 panicles evaluated per plot. Each sampled panicle was visually examined by experienced personnel to estimate the percentage of branches that were necrotic due to infection by *M. grisea*. Each panicle was given a rating[29] from 0 to 5, where 0 is no disease; 1 is less than 5% of panicle branches necrotic; 2 is 5–30% necrotic; 3 is 30–50% necrotic; 4 is greater than 50% necrotic; and 5 is 100% necrotic. Disease severity was summarized within each plot as $\{[(n_1 \times 1) + (n_2 \times 2) + (n_3 \times 3) + (n_4 \times 4) + (n_5 \times 5)]/\Sigma n_0 \ldots n_5\} \times 100$, where $n_0 \ldots n_5$ is the number of culms in each of the respective disease categories. Thus, a disease severity of 0% would indicate no disease and 100% would indicate that 100% of panicle branches were necrotic.

Yield evaluation

Plots were hand-harvested, threshed and weighed to determine grain yield. Individual varieties were evaluated separately in mixtures. Land equivalent ratios[22] were calculated as (yield ha^{-1} of variety A in mixture/yield ha^{-1} of variety A in monoculture) + (yield ha^{-1} of variety B in mixture/yield ha^{-1} of variety B in monoculture).

Statistical analyses

Each survey plot was considered to be an experimental unit, and analyses were based on mean disease severities and grain yield for each plot. Statistical analyses were conducted separately by year and county owing to differences in disease level. One-tailed *t*-tests were used to determine if blast severity for each of the two varieties in each of the four mixtures differed significantly from its corresponding monoculture control.

Received 18 April; accepted 30 June 2000.

1. Browning, J. A. & Frey, K. J. Multiline cultivars as a means of disease control. *Annu. Rev. Phytopathol.* **7,** 355–382 (1969).
2. Wolfe, M. S. The current status and prospects of multiline cultivars and variety mixtures for disease resistance. *Annu. Rev. Phytopathol.* **23,** 251–273 (1985).
3. Mundt, C. C. in *Rice Blast Disease* (eds Zeigler, R. S., Leong, S. A. & Teng, P. S.) 293–308 (CAB International, Wallingford, 1994).
4. Garrett, K. A. & Mundt, C. C. Epidemiology in mixed host populations. *Phytopathology* **89,** 984–990 (1999).
5. Ou, S. H. *Rice Diseases* 2nd edn (Commonwealth Mycological Institute, Kew, 1985).
6. Polis, G. A., Anderson, W. B. & Holt, R. D. Toward an integration of landscape and food web ecology: The dynamics of spatially subsidized food webs. *Annu. Rev. Ecol. Syst.* **28,** 289–316 (1997).
7. Dwyer, G., Elkinton, J. S. & Hajek, A. E. Spatial scale and the spread of a fungal pathogen of gypsy moth. *Am. Nat.* **152,** 485–494 (1998).
8. Schwartz, M. W. Choosing the appropriate scale of reserves for conservation. *Annu. Rev. Ecol. Syst.* **30,** 83–108 (1999).
9. Waide, R. B. *et al.* The relationship between productivity and species richness. *Annu. Rev. Ecol. Syst.* **30,** 257–300 (1999).
10. Matson, P. A., Parton, W. J., Power, A. G. & Swift, M. J. Agricultural intensification and ecosystem properties. *Science* **277,** 504–508 (1997).
11. Tilman, D. The greening of the green revolution. *Nature* **396,** 211–212 (1998).
12. International Rice Research Institute. *IRRI Rice Facts* (International Rice Research Institute, Manila, 1997).
13. Baker, B., Zambryski, P., Staskawicz & Dinesh-Kumar, S. P. Signaling in plant-microbe interactions. *Science* **276,** 726–733 (1997).
14. Staskawicz, B. J., Ausubel, F. M., Baker, B. J., Ellis, J. G. & Jones, J. D. G. Molecular genetics of plant disease resistance. *Science* **268,** 661–667 (1995).
15. Ou, S. H. Pathogenicity and host plant resistance in rice blast disease. *Annu. Rev. Phytopathol.* **18,** 167–187 (1980).
16. Bonman, J. M., Khush, G. S. & Nelson, R. J. Breeding rice for resistance to pests. *Annu. Rev. Phytopathol.* **30,** 507–528 (1992).
17. Kiyosawa, S. Genetics and epidemiological modeling of breakdown of plant disease resistance. *Annu. Rev. Phytopathol.* **20,** 93–117 (1982).
18. Boudreau, M. A. & Mundt, C. C. in *Environmentally Safe Approaches to Disease Control* (eds Rechcigl, J. & Rechcigl, N.) 33–62 (CRC, Boca Raton, 1997).
19. Chin, K. M. & Wolfe, M. S. Selection on *Erysiphe graminis* in pure and mixed stands of barley. *Plant Pathol.* **33,** 89–100 (1984).
20. Calonnec, A., Goyeau, H. & de Vallavieille-Pope, C. Effects of induced resistance on infection efficiency and sporulation of *Puccinia striiformis* on seedlings in varietal mixtures and on field epidemics in pure stands. *Eur. J. Plant Pathol.* **102,** 733–741 (1996).
21. George, M. L., Nelson, R. J., Zeigler, R. S. & Leung, H. Rapid population analysis of *Magnaporthe grisea* by using rep-PCR and endogenous repetitive DNA sequences. *Phytopathology* **88,** 223–229 (1998).
22. Francis, C. A. in *Multiple Cropping Systems* (ed. Francis, C. A.) 1–19 (MacMillan, New York, 1986).
23. Mundt, C. C. in *Plant Disease Epidemiology* (eds Leonard, K. J. & Fry, W. E.) 150–181 (McGraw-Hill, 1989).
24. Mundt, C. C & Browning, J. A. in *The Cereal Rusts* Vol. 2 (eds Roelfs, A. P. & Bushnell, W. R.) 527–560 (Academic, Orlando, 1985).
25. Browning, J. A. & Frey, K. J. in *Strategies for the Control of Cereal Disease* (eds Jenkyn, J. F. & Plumb, R. T.) 37–46 (Blackwell, Oxford, 1981).
26. Wolfe, M. S. in *Barley Genetics VI* (ed. Munck, L.) 1055–1067 (Munksgaard, Copenhagen, 1992).
27. Mann, C. Reseeding the Green Revolution. *Science* **277,** 1038–1042 (1997).
28. Schmidt, R. A. in *Plant Disease: An Advanced Treatise* (eds Horsfall, J. G & Cowling, E. B.) 287–315 (Academic, New York, 1978).
29. *The State Standard of the People's Republic of China, No. GB/T 15790-1995.* 1–13 (China Standard Press, Beijing, 1996).

Acknowledgements

This work was supported by the Asian Development Bank, the Yunnan Province Government, The Ministry of Science and Technology of China, the International Rice Research Institute (IRRI), and a scientific agreement between IRRI and Oregon State University. We thank the personnel of the provincial and county Plant Protection Stations and participating farmers for their contributions to this project, and M. Hoffer for computer assistance and graphics.

Correspondence and requests for materials should be addressed to C.C.M. (e-mail: mundtc@bcc.orst.edu).

Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval

Karim Nader, Glenn E. Schafe & Joseph E. Le Doux

W. M. Keck Foundation Laboratory of Neurobiology, Center for Neural Science, New York University, New York, New York 10003, USA

'New' memories are initially labile and sensitive to disruption before being consolidated into stable long-term memories[1–5]. Much evidence indicates that this consolidation involves the synthesis of new proteins in neurons[6–9]. The lateral and basal nuclei of the amygdala (LBA) are believed to be a site of memory storage in fear learning[10]. Infusion of the protein synthesis inhibitor anisomycin into the LBA shortly after training prevents

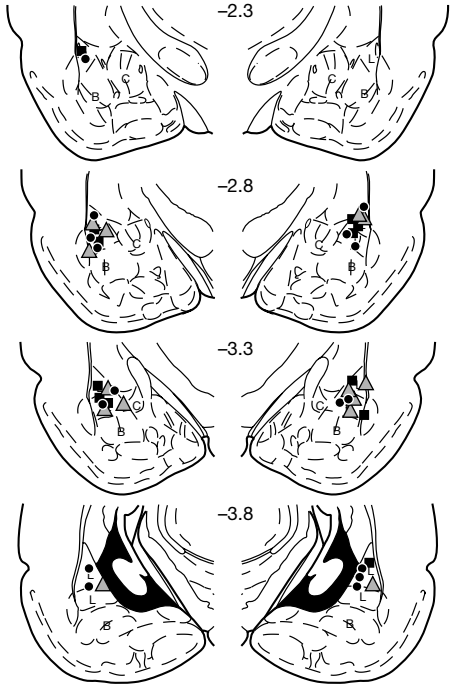

Figure 1 Schematic representation of the amygdala at four different rostral–caudal planes. The numbers represent the posterior coordinate from bregma. Injector placements in the LBA are represented by the filled symbols; black filled squares represent ASCF group placements, grey filled triangles represent the low-dose anisomycin, and black filled circles represent high-dose group. L, lateral nucleus; B, basal nucleus; C, central nucleus. The placements for subsequent experiments all demonstrate similar distributions as in this experiment and therefore are not shown.

consolidation of fear memories[11]. Here we show that consolidated fear memories, when reactivated during retrieval, return to a labile state in which infusion of anisomycin shortly after memory reactivation produces amnesia on later tests, regardless of whether reactivation was performed 1 or 14 days after conditioning. The same treatment with anisomycin, in the absence of memory reactivation, left memory intact. Consistent with a time-limited role for protein synthesis production in consolidation, delay of the infusion until six hours after memory reactivation produced no amnesia. Our data show that consolidated fear memories, when reactivated, return to a labile state that requires de novo protein synthesis for reconsolidation. These findings are not predicted by traditional theories of memory consolidation.

The idea that new memories go through an initial labile period before being consolidated into stable long-term memories is an entrenched part of psychological and neurobiological models of memory[12]. For example, there is considerable evidence that the formation of a long-term memory can be disrupted by certain treatments, such as systemic drug injections or electroconvulsive shock, given shortly after training, but that the same treatments given several hours or days later have no effect. One of the most commonly used drug manipulations involves the administration of drugs that block the translation of RNA into protein. Studies of this type indicate that memory consolidation involves protein synthesis[5–9].

It has also been reported that electroconvulsive shock or systemic drug administration given after memory reactivation (retrieval) can cause an amnesia for the original learning[13–16], which indicates that consolidated memories might become labile when retrieved, and might even require reconsolidation. Here we examine whether reconsolidation involving protein synthesis is required for retrieved memories to persist. We use a behavioural paradigm, auditory fear conditioning, for which the neural circuit underlying memory formation is well characterized[17–19]. This allows us to manipulate memory at its presumed locus of storage, in contrast to past studies in which drugs were administered systemically. Specifically, we target infusions of anisomycin, an inhibitor of protein synthesis, to the LBA, a region implicated in fear learning by lesion, pharmacological and physiological findings[17–19]. Previously, we showed that infusions of anisomycin given after training block long-term but not short-term memory of auditory fear conditioning[11]. Here we examine the effects of similar manipulations administered after retrieval.

Rats were given a single pairing of a tone (conditioned stimulus, CS) and foot-shock (unconditioned stimulus, US). On test days, immobility (freezing) was used as an index of fear learning[20]. Twenty-four hours later, the rats received a single CS presentation (test 1) immediately followed by bilateral infusions of anisomycin or vehicle (artificial cerebrospinal fluid; ACSF) into the LBA. Freezing in test 1 was specific to the CS and comparable across groups. An analysis of variance (ANOVA) that compared freezing during the pre-CS or CS periods across groups indicated that there was no interaction between these two variables ($F(2, 18) = 1.6$), nor an effect of group ($F(2, 18) = 1.4$). However, there was a significant effect of period ($F(1, 18) = 160$, $P < 0.01$). Twenty-four hours after test 1, the rats were presented with three CSs (test 2). Anisomycin produced a dose-dependent decrease in freezing in response to the CS in test 2 (Figs 1, 2a–c). An ANOVA revealed a main effect of group ($F(2, 18) = 12$, $P < 0.01$). A Newman–Keuls post hoc test revealed that the low-dose anisomycin and ACSF groups were similar to each other ($P > 0.05$), but both were significantly different from the high-dose group (P values < 0.01). Extinction was observed over the three CS presentations (main effect of trial ($F(2, 36) = 7$, $P < 0.01$), but there was no interaction between trials and group ($F < 1$). This effect of anisomycin requires that the memory be actively retrieved, as omission of the CS before anisomycin infusion in test 1 led to normal conditioned fear responses in test 2 (Fig. 2d, e; no main effect of group ($F < 1$). The latter finding

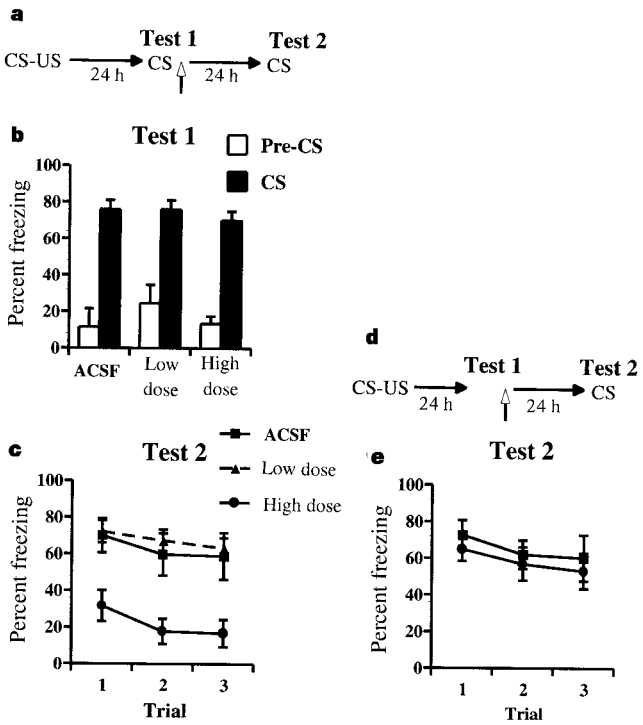

Figure 2 A test of whether consolidated fear memories can become labile when reactivated. **a**, The behavioural procedure used for experiment 1A. **b**, Freezing to the CS on test 1 was comparable across groups and was specific to the CS. **c**, Intra-LBA anisomycin infusions after reactivation of a consolidated fear memory produce amnesia for the original learning, as measured on test 2. **d**, **e**, Rats demonstrated normal memory if the CS was omitted before anisomycin. **d**, The behavioural procedure used for experiment 1B. Rats were placed in the test chamber and received infusions of anisomycin. **e**, Percent freezing on test 2. Figure legend is applicable to both **c** and **e**. Vertical open-headed arrows represent infusions. All data points represent group means ± s.e.m.

rules out the possibility that the deficit we observed is due to a disruption of late waves of protein synthesis[21] that may be necessary for the consolidation of the original learning, or to nonspecific effects such as damage to the amygdala by the drug. It is unlikely that the high dose of anisomycin damaged the amygdala after CS transmission, as there was no histological evidence of amygdala damage. Furthermore, rats relearn fear conditioning normally when the same dose of anisomycin is infused into the amygdala after initial learning. The fact that anisomycin infusions after reactivation of the fear memory produced amnesia for the original learning indicates that reactivation of a consolidation fear memory may place it in a labile state, one that has to be reconsolidated by protein synthesis to remain usable to the organism in future situations.

Protein synthesis inhibitors typically impair the consolidation of new memories when they are administered during a specific time window (which varies from minutes to hours) after learning[8]. Administration of such drugs after this time window does not affect memory. We next asked whether a time window also exists for reconsolidation, by delaying anisomycin infusions for 6 h after retrieval. Freezing in test 1 was specific to the CS and comparable across groups. ANOVAs revealed no period × group interaction ($F < 1$) and no effect of group ($F = 1$) but a significant effect of period ($F(1, 13) = 124$, $P < 0.01$). In contrast to anisomycin infusion immediately after retrieval, infusion 6 h after retrieval had no effect (Fig. 3). ANOVAs revealed that in test 2 there was no main effect of group ($F(1, 13) = 4$), no trial × group interaction ($F(2, 26) = 2.7$) and no main effect of trial ($F(2, 26) = 3$). Note that the nonsignificant impairment produced by delaying anisomycin is much smaller than that seen when anisomycin is given immediately after CS reactivation (compare with Fig. 2c). Thus, both consolidation and reconsolidation have time windows within which protein synthesis is required if a memory is to persist.

In all of the previous experiments, the time between training and CS presentation for test 1 was about 24 h. We next investigated whether reconsolidation has a temporal gradient. For example, older memories may be more thoroughly consolidated and thus may be resistant to becoming unstable when retrieved. To test this, we waited for 14 days between conditioning and test 1 (Fig. 4). Postponing test 1 by 14 days produced an incubation effect[22] such that freezing in test 1 was higher than in the previous experiment. Freezing during test 1 was specific to the CS and was comparable across groups. There was no period × group interaction ($F(1, 9) = 1.1$) and no effect of group ($F = 1$), but a significant effect of period ($F(1, 9) = 440$, $P < 0.01$). However, the performance of the groups in test 2 diverged—the group receiving intra-LBA infusions of anisomycin showed significantly less freezing than the controls. ANOVAs revealed a main effect of group ($F(1, 9) = 20.6$, $P < 0.01$) and trials ($F(2, 18) = 7$, $P < 0.01$), but no interaction between these variables ($F < 1$). Thus, blockade of protein synthesis in the LBA after memory reactivation caused amnesia of the original learning, even though the learning took place 14 days before the reactivation and drug treatment. Even well consolidated memories are labile and subject to disruption when reactivated.

Although anisomycin blocks reconsolidation, it is possible that the impairment is due to nonspecific effects that render the amygdala temporarily dysfunctional for reasons other than protein synthesis inhibition. To provide compelling evidence that any manipulation acts specifically on the molecular mechanisms mediating consolidation, as opposed to producing nonspecific effects, at a minimum it is necessary to show that memory is intact shortly after training but impaired later[23–26]. For example, rats freeze normally 4 h but are impaired 24 h after receiving intra-LBA anisomycin infusions immediately after training[11]. Using the same logic and applying it to reconsolidation, if the effects of anisomycin infused into the LBA shortly after memory reactivation are specific to reconsolidation, then freezing should be normal at 4 h, but impaired at 24 h, after CS presentation. We refer to these two time points are post-reactivation short-term memory (PR-STM) and post-reactivation long-term memory (PR-LTM), respectively.

The two groups exhibited comparable freezing during test 1

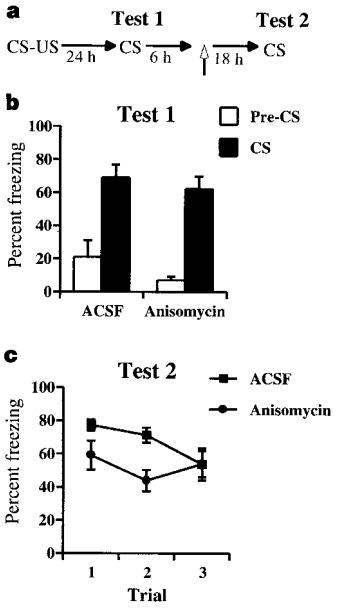

Figure 3 Intact memory if anisomycin infusions are delayed by 6 h. **a**, The behavioural procedure used for experiment 2. Vertical open-headed arrows represent infusions. **b**, Freezing on test 1 was specific to the CS and comparable across groups. **c**, Percent freezing during test 2. The groups are not significantly different. All data points represent group means ± s.e.m.

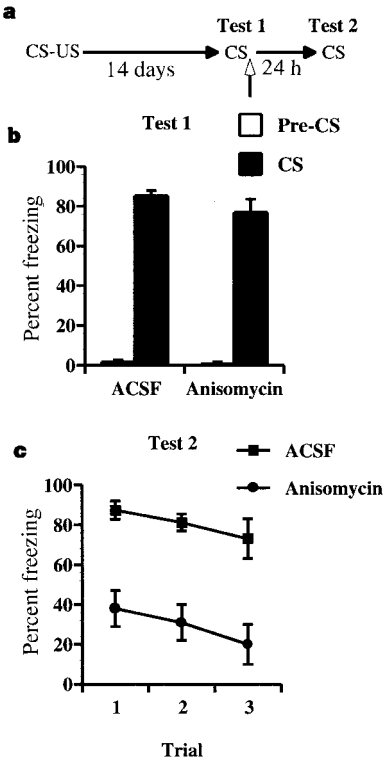

Figure 4 Fourteen days after training, anisomycin infusions after reactivation of the memory still produce amnesia. **a**, The behavioural procedure used for experiment 3. Vertical open-headed arrows represent infusions. **b**, Freezing during test 1 was specific to the CS and was comparable across groups. **c**, Percent freezing on test 2. All data points represent group means ± s.e.m.

(Fig. 5). An ANOVA revealed a significant effect of period ($F(1, 10) = 147$, $P < 0.01$), no effect of group ($F < 1$) and no interaction between these two variables ($F < 1$). Rats treated with anisomycin immediately after test 1 exhibited intact PR-STM but deficient PR-LTM. ANOVAs on the scores of the PR-STM test showed that there was no main effect of group ($F < 1$), no interaction between trial × group ($F < 1$) and no effect of trial ($F < 1$). Conversely, a similar analysis on the PR-LTM scores did reveal a significant effect of group ($F(1, 14) = 14$, $P < 0.01$). Furthermore, there was no interaction between group and trial ($F < 1$) but there was a main effect of trial ($F(2, 28) = 5$, $P < 0.05$). The fact that animals can accurately perceive, evaluate and respond to the CS 4 h after anisomycin infusion shows that the amygdala is functionally intact at the time of the PR-STM test, and thus that anisomycin did not affect reconsolidation by producing nonspecific effects. This pattern of findings, intact PR-STM and impaired PR-LTM, localizes the effects of anisomycin on fear behaviour to the molecular processes mediating reconsolidation.

The conventional view of memory consolidation predicts that blockade of protein synthesis should block new learning in test 1 of these experiments, which is an extinction test. The only new learning that occurs is about the failure of the CS to predict the US. Blockade of protein synthesis should therefore block extinction, and thus enhance memory, according to the conventional view. In contrast, however, blockade of protein synthesis had the opposite effect—it eliminated the memory rather than making it stronger.

These results provide evidence that fear memories, once retrieved, must undergo protein-synthesis-dependent reconsolidation in the LBA or nearby areas to remain accessible at later times. The full implications of this finding for fear and other memories are not understood at present. It is possible that not all memories require reconsolidation. There may be a range of parameters within which reactivation of a memory converts it into a labile state, possibly involving the extent of experience with the particular learning situation, the kind of learning system engaged and the motivational state of the subject at the time of learning and retrieval.

Reconsolidation may reflect the dynamic nature of the process by which new information is added to existing stores. It has long been believed that memory retrieval is an active or constructive process by which old information is integrated with the current knowledge base of the organism[27]. Reconsolidation may be part of the neural mechanism through which constructed memories are stored for later constructions.

Current models of learning propose that the production of new proteins is necessary for structural encoding of recent experiences in long-term memory[6–9]. In addition, it now appears that new proteins are also required to maintain memories that have been reactivated. It seems unlikely that retrieval reverses the structural changes induced by original learning. Rather, some property of retrieval may destabilize the structural changes such that they now have to be reconsolidated with the aid of new proteins. The fact that animals demonstrate intact freezing 4 h after anisomycin indicates that the structural changes may remain functional for at least 4 h. Particularly important for future work will be the clarification of the physiological basis of memory lability during retrieval and the requirement for reconsolidation. It is possible that some modification of the synaptic tagging hypothesis[28,29], which proposes that active synapses are given molecular markers that help stabilize synapses by capturing proteins made in the cell nucleus, might account for lability and reconsolidation, although this remains to be seen.

We have shown that reactivation changes the status of a consolidated fear memory to a labile one that must be reconsolidated using de novo protein synthesis to persist. Like consolidation itself, reconsolidation has a temporal window during which blockade of protein synthesis is effective, and beyond which it is not. Furthermore the reconsolidation process, like the consolidation process, has a short-term phase that is not dependent on protein synthesis. A definition of consolidation based on 'new' memories is insufficiently broad to describe these data. We propose, in keeping with the original suggestion by Misanin *et al.*[13], that as a first approximation 'active' rather than 'new' memories be viewed as labile, subject to disruption, and requiring protein-synthesis-dependent consolidation processes. □

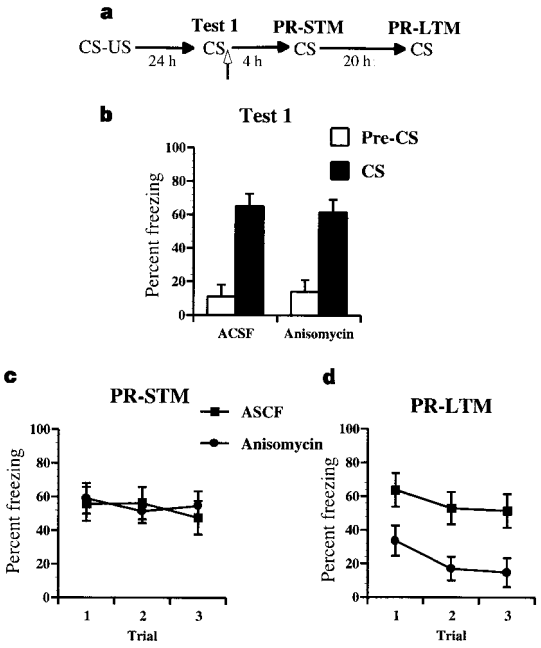

Figure 5 Amnesia following anisomycin is not due to nonspecific effects. **a**, The behavioural procedure used for experiment 4. Vertical open-headed arrows represent infusions. Anisomycin infusions impaired post-reactivation long-term memory (PR-LTM), but had no effect on post-reactivation short-term memory (PR-STM). **b**, All rats demonstrated comparable freezing scores on test 1. **c**, Scores on the PR-STM test 4 h after reactivation and anisomycin. **d**, Scores on the PR-LTM test 24 h after reactivation and anisomycin. The key is applicable to both **c** and **d**. All data points represent group means ± s.e.m.

Methods

Subjects

Subjects were adult male Sprague–Dawley rats from Hilltop Labs. Rats were housed individually in plastic Nalgene cages and maintained on a 12/12 h light/dark cycle. Food and water were provided *ad libitum*.

Surgery and histology

Under Nembutal anaesthesia (45 mg kg^{-1}), rats were implanted bilaterally with 22-gauge stainless steel cannulas into the lateral amygdata. Coordinates were 3.0 mm posterior to bregma, 5.3 mm lateral to the midline and 8.0 mm ventral to the skull surface. Rats were given at least 5 days to recover before experimental procedures. All procedures were in accordance with the NIH Guide, and were approved by the NYU Animal Care and Use Committee. At the end of the experiment, using standard histological methods, animals were perfused and their brains sectioned at 50 μm thickness. The sections were stained using cresyl violet and examined by light microscopy for cannula penetration into the LBA.

Intra-LBA infusions

Drugs were infused slowly using an infusion pump into the LBA at 0.25 μl min^{-1}. Following drug infusion, cannulas were left in place for an additional minute to allow diffusion of the drug away from the cannula tip. Anisomycin (Sigma) was dissolved in equimolar HCl, diluted with ACSF and adjusted to pH 7.4 with NaOH. Although the lateral nucleus was the main target, the 0.5-μl infusions also probably affected the adjacent basal nucleus. We therefore refer to the affected area as the lateral and basal amygdala (LBA).

Apparatus

Conditioning and tone testing were conducted in different chambers. For conditioning, rats were placed in a Plexiglas rodent conditioning chamber (chamber A) with a metal grid floor (Model E10-10, Coulbourn Instruments) that was enclosed within a sound attenuating chamber (Model E10-20). The chamber was dimly illuminated by a single house light. For tone testing, rats were placed in a different Plexiglas chamber (ENV-001,

MedAssociates), which has been shown to minimize generalization from the conditioning environment[11]. The tone-testing chamber (chamber B) was brightly lit with three house lights and contained a flat black Formica floor that had been washed with peppermint soap. A micro-video camera was mounted at the top of the chamber so that rats could be videotaped during testing.

General behavioural procedures

Rats were placed in chamber A and after a 5-min acclimatizing period, given a single conditioning trial consisting of a 30-s presentation of a 5-kHz, 75-dB tone CS that ended at the same time as a 2.0-mA, 1-s food shock US. Rats were then returned to their home cages. The next day, 24 h later, rats were placed in chamber B and given a single 30-s CS presentation (test 1) to reactivate the memory. Twenty-four hours after test 1, rats were returned to chamber B and given three CS presentations (test 2).

Experiment 1A

Rats were infused with either 62.5 μg per 0.5 μl per side ($n = 8$) or 6.2 μg per 0.5 μl per side ($n = 7$) anisomycin or ACSF ($n = 6$) immediately after CS termination during test 1. The highest dose of anisomycin was chosen based on a previous study showing >90% suppression of protein synthesis in cortex using this concentration[30]. Previous data have shown that post-training intra-LBA infusions of the high but not low dose blocked consolidation of fear conditioning[11].

Experiment 1B

During test 1 no CS was presented while the animals explored chamber B, but rats still received an infusion of vehicle ($n = 6$) or the high dose of anisomycin ($n = 7$) at the end of the exposure to chamber B.

Experiment 2

High-dose anisomycin ($n = 8$) or vehicle ($n = 7$) infusions were performed 6 h after test 1. Animals were transported to the infusion room, received the infusion and were then returned to their home cage.

Experiment 3

Fourteen days were inserted between conditioning and test 1. After CS reactivation, rats received either high-dose anisomycin ($n = 6$) or vehicle ($n = 5$) infusion.

Experiment 4

After test 1 animals received either vehicle ($n = 8$) or high-dose anisomycin ($n = 8$) infusions into the LBA. An extra test was inserted 4 h after test 1 (post-reactivation short-term memory, PR-STM) during which animals received three CS presentations. Test 2 was performed as described above, 24 h after reactivation (post-reactivation long-term memory, PR-LTM). These time points were chosen based on the findings that freezing is intact 4 h, but impaired 24 h, after conditioning[11].

Received 3 April; accepted 18 May 2000.

1. Müller, G. E. & Pilzecker, A. Experimentelle beitrage zur lehre vom gedachtnis. *Z. Psychol.* (Suppl. 1) (1900).
2. Burnham, W. H. Retroactive amnesia: illustrative cases and a tentative explanation. *Am. J. Psychol.* **14,** 382–396 (1903).
3. Duncan, C. P. The retroactive effect of electroconvulsive shock. *J. Comp. Physiol. Psychol.* **42,** 32–44 (1949).
4. Hebb, D. O. *The Organization of Behavior* (Wiley, New York, 1949).
5. Dudai, Y. Consolidation: Fragility on the road to the engram. *Neuron* **17,** 367–370 (1996).
6. Goelet, P., Castellucci, V. F., Schacher, S. & Kandel, E. R. The long and short of long-term memory—a molecular framework. *Nature* **322,** 419–422 (1986).
7. Flexner, L. B., Flexner, J. B. & Stellar, E. Memory and cerebral protein synthesis in mice as affected by graded amounts of puromycin. *Exp. Neurol.* **13,** 264–272 (1965).
8. Davis, H. P. & Squire, L. R. Protein synthesis and memory. A review. *Psychol. Bull.* **96,** 518–559 (1984).
9. Agranoff, B. W. in *Basic Neurochemistry* (eds Siegel, G. J., Albers, R. W., Agranoff, B. W. & Catzman, R.) 801–820 (Little, Brown, Boston, 1981).
10. Fanselow, M. S. & LeDoux, J. E. Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. *Neuron* **23,** 229–232 (1999).
11. Schafe, G. E. & LeDoux, J. E. Memory consolidation of auditory Pavlovian fear conditioning requires protein synthesis and PKA in the amygdala. *J. Neurosci.* (in the press).
12. McGaugh, J. L. Memory—a century of consolidation. *Science* **287,** 248–251 (2000).
13. Misanin, J. R., Miller, R. R. & Lewis, D. J. Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace. *Science* **160,** 203–204 (1968).
14. Judge, M. E. & Quartermain, D. Characteristics of retrograde amnesia following reactivation of memory in mice. *Physiol. Behav.* **28,** 585–590 (1982).
15. Sara, S. J. Retrieval and reconsolidation: toward a neurobiology of remembering. *Learn. Mem.* **7,** 73–84 (2000).
16. Mactutus, C. F., Riccio, D. C. & Ferek, J. M. Retrograde amnesia for old (reactivated) memory: Some anomalous characteristics. *Science* **204,** 1319–1320 (1979).
17. Davis, M. Neurobiology of fear responses: the role of the amygdala. *J. Neuropsychiat. Clin. Neurosci.* **9,** 382–402 (1997).
18. Fanselow, M. S. Pavlovian conditioning, negative feedback, and blocking: mechanisms that regulate association formation. *Neuron* **20,** 625–627 (1998).
19. LeDoux, J. E. Emotion circuits in the brain. *Annu. Rev. Neurosci.* **23,** 155–184 (2000).
20. Blanchard, R. J. & Blanchard, D. C. Crouching as an index of fear. *J. Comp. Physiol. Psychol.* **67,** 370–375 (1969).
21. Scholey, A. B., Rose, S. P., Zamani, M. R., Bock, E. & Schachner, M. A role for the neural cell adhesion molecule in a late, consolidating phase of glycoprotein synthesis six hours following passive avoidance training of the young chick. *Neuroscience* **55,** 499–509 (1993).
22. Brady, J. V. The effects of electroconvulsive shock on a conditioned emotional response: the permanence effect. *J. Comp. Physiol. Psychol.* **45,** 9–13 (1952).
23. Abel, T. *et al.* Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus-based long-term memory. *Cell* **88,** 615–626 (1997).
24. Bourtchouladze, R. *et al.* Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. *Cell* **79,** 59–68 (1994).
25. Guzowski, J. F. & McGaugh, J. L. Antisense oligodeoxynucleotide-mediated disruption of hippocampal cAMP response element binding protein levels impairs consolidation of memory for water maze training. *Proc. Natl Acad. Sci. USA* **94,** 2693–2698 (1997).
26. Lamprecht, R., Harzvi, S. & Dudai, Y. cAMP response element-binding protein in the amygdala is required for long- but not short-term conditioned taste aversion memory. *J. Neurosci.* **17,** 8443–8450 (1997).
27. Bartlett, F. C. *Remembering* (Cambridge Univ. Press, Cambridge, 1932).
28. Martin, K. C. *et al.* Synapse-specific, long-term facilitation of aplysia sensory to motor synapses: a function for local protein synthesis in memory storage. *Cell* **91,** 927–938 (1997).
29. Frey, U. & Morris, R. G. Synaptic tagging and long-term potentiation. *Nature* **385,** 533–536 (1997).
30. Rosenblum, K., Meiri, N. & Dudai, Y. Taste memory: the role of protein synthesis in gustatory cortex. *Behav. Neural Biol.* **59,** 49–56 (1993).

Acknowledgements

This research was supported in part by NIMH grants to J.E.L. and a HFSF grant to K.N. The work was also supported by a grant from the W. M. Keck Foundation to N.Y.U. The authors thank A. Schoute for technical assistance.

Correspondence and requests for materials should be addressed to K.N. (e-mail: karim@cns.nyu.edu).

Cortex-restricted disruption of NMDAR1 impairs neuronal patterns in the barrel cortex

Takuji Iwasato*, Akash Datwani†, Alexander M. Wolf‡, Hiroshi Nishiyama*§, Yusuke Taguchi*, Susumu Tonegawa‖, Thomas Knöpfel‡, Reha S. Erzurumlu† & Shigeyoshi Itohara*

** Laboratory for Behavioral Genetics, Brain Science Institute (BSI), RIKEN, 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan*
† Department of Cell Biology and Anatomy and Neuroscience Center, LSUHSC, New Orleans, Louisiana 70112, USA
‡ Laboratory for Neuronal Circuit Dynamics, Brain Science Institute (BSI), RIKEN, 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan
§ Institute for Virus Research, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan
‖ Howard Hughes Medical Institute, Center for Learning & Memory, RIKEN-MIT Neuroscience Research Center, Department of Biology, MIT, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139, USA

In the rodent primary somatosensory cortex, the configuration of whiskers and sinus hairs on the snout and of receptor-dense zones on the paws is topographically represented as discrete modules of layer IV granule cells (barrels) and thalamocortical afferent terminals[1,2]. The role of neural activity, particularly activity mediated by NMDARs (*N*-methyl-D-aspartate receptors), in patterning of the somatosensory cortex has been a subject of debate[3–6]. We have generated mice in which deletion of the *NMDAR1* (*NR1*) gene is restricted to excitatory cortical neurons, and here we show that sensory periphery-related patterns develop normally in the brainstem and thalamic somatosensory relay stations of these mice. In the somatosensory cortex, thalamocortical afferents corresponding to large whiskers form patterns and display critical period plasticity, but their patterning is not as distinct as that seen in the cortex of normal mice. Other thalamocortical patterns corresponding to sinus hairs and digits are mostly absent. The cellular aggregates known as barrels and barrel boundaries do not develop even at sites where thalamocortical afferents cluster. Our findings indicate that cortical NMDARs