Behavioral Neuroscience
1996, Vol. 110, No. 5, 1177–1180

0735-7044/96/

BRIEF COMMUNICATIONS

Hippocampal Lesions Impair Contextual Fear Conditioning in Two Strains of Mice

Chong Chen
Massachusetts Institute of Technology

Jeansok J. Kim and Richard F. Thompson
University of Southern California

Susumu Tonegawa
Massachusetts Institute of Technology

Two different strains of mice, C57BL/6J and BALB/c, with hippocampal, cortical, or sham lesions, underwent contextual fear conditioning. In both strains, contextual fear, as measured by the freezing response, was significantly impaired in hippocampus-lesioned animals compared with sham control animals. Fear conditioning was not affected in the cortical-lesioned group. Moreover, there was a strain difference in fear conditioning: The C57BL/6J mice exhibited freezing more frequently than the BALB/c mice. Consistent with previous hippocampal lesion studies in rats, these results indicate that contextual fear conditioning in mice also requires the intact hippocampus. This study provides a basis for evaluating hippocampal synaptic mechanisms in relation to contextual fear conditioning in widely available gene knockout or transgenic mice.

The hippocampus is critical for learning and memory of spatial, contextual, temporal, and declarative information (for reviews, see Eichenbaum, Otto, & Cohen, 1992; Squire, 1992). Lesion studies indicate that the integrity of the hippocampus is essential for a variety of memory tasks in many species, including delayed nonmatching to sample in monkeys (Zola-Morgan & Squire, 1990), spatial learning in rats (Gallagher & Holland, 1992; Morris, Garrud, Rawlins, & O'Keefe, 1982), and trace eyeblink conditioning in rabbits (Kim, Clark, & Thompson, 1995; Moyer, Deyo, & Disterhoft, 1990; Solomon, Vander Schaaf, Thompson, & Weisz, 1986). In contextual fear conditioning, an animal is placed in a novel experimental chamber and presented with mild footshocks. The animal rapidly forms an association between the footshock and the contextual cues of the chamber and reacts by freezing (Blanchard & Blanchard, 1969; Bolles, 1970). Hippocampal lesions significantly disrupt acquisition and retention of contextual fear in rats (Blanchard & Fial, 1968; Kim & Fanselow, 1992; Kim, Rison, & Fanselow, 1993; Phillips & LeDoux, 1992).

Many recent studies have applied the paradigm of contextual fear conditioning to genetically engineered mice; interpretations of those studies have to rely on the evidence generated in rat or monkey lesion studies for establishing the relevance of hippocampal deficiency to this memory task (for reviews, see Chen & Tonegawa, 1995; Mayford, Abel, & Kandel, 1995). For example, various degrees of impairments in contextual fear conditioning are found in various gene knockout mice including PKCγ (Abeliovich et al., 1993), mGluR1 (Aiba et al., 1994), αCaMKII (Chen, Rainnie, Greene, & Tonegawa, 1994), and CREB (Bourtchuladze et al., 1994). However, it has not been determined whether hippocampal lesions per se affect this learning task in mice. In this study, we investigated the effect of hippocampal lesions on contextual fear conditioning in two widely used strains of laboratory mice, C57BL/6J and BALB/c.

Method

Subjects and Surgery

A total of 52 adult mice (weighing between 20 and 30 g) were used. The C57BL/6J mice consisted of 14 males and 14 females, whereas all 24 BALB/c mice were males. Animals were individually housed with ad-lib access to food and water and maintained on a 12-hr light–dark cycle at the MIT mouse colony. All test procedures were conducted during the light phase of the cycle.

Under sodium pentobarbital (65 mg/kg) anesthesia, hippocampus ($n = 10$ for C57BL/6J and $n = 8$ for BALB/c), overlying cortex ($n = 10$ for C57BL/6J and $n = 8$ for BALB/c), or both were aspirated bilaterally using a curved glass pipette. Gelfoam sponges soaked with thrombin were placed on the brain to stop the bleeding. Sham controls ($n = 8$ for both strains) underwent an identical procedure except for the aspiration. In C57BL/6J mice, each group consisted of equal numbers of males and females. All mice were given 10 days of postoperative recovery. Five C57BL/6J mice and 2 BALB/c mice died of anesthesia or surgery. One week before conditioning, mice were handled daily to reduce stress.

Chong Chen and Susumu Tonegawa, Howard Hughes Medical Institute, Center for Learning and Memory, Massachusetts Institute of Technology; Jeansok J. Kim and Richard F. Thompson, Neuroscience Program, University of Southern California.

Correspondence concerning this article should be addressed to Chong Chen, Center for Learning and Memory, E17-358, Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139. Electronic mail may be sent via Internet to cchen@wccf.mit.edu.

Apparatus and Procedure

Both training and testing took place in a small rodent chamber (Coulbourn Instruments, Allentown, PA) placed in a sound-attenuating box. A ventilation fan supplied background noise (75 dB). The floor of the chamber was composed of stainless steel rods (5 mm diameter, spaced 1 cm apart), which were connected to a scrambled shock generator. The chamber was cleaned with a 1% solution of acetic acid and dried prior to conditioning and testing each mouse.

On the day of conditioning, subjects were placed in the chamber. After 3 min, mice received three mild footshocks (0.5 mA, 1 s duration, 60 s apart). They were removed 60 s after the last shock and returned to their home cage. On the following day, animals were placed back in the chamber for an 8-min test in the absence of shock. Behavior was videotaped throughout the experiment. For both conditioning and test sessions, freezing response, characterized by a motionless, crouching posture, was assessed using a time-sampling procedure in which an observer blind to mouse treatments scored each animal every 2 s. Percentage freezing per minute was then calculated by dividing the number of freezing episodes by the total number of observations and multiplying by 100.

Histology

At the conclusion of the experiment, mice were deeply anesthetized with metofane and perfused intracardially with 0.9% saline followed by 10% formalin. The brains were sectioned using a cryostat (60 μm thickness), mounted on gelatinized slides, and stained with cresyl violet.

Results

Figure 1 shows a photomicrograph of a transverse brain section stained with cresyl violet from a typical mouse in the hippocampus-lesioned group. Mice in this group sustained substantial bilateral destruction of the dorsal hippocampus. The overlying cortex, the corpus callosum, and the fimbria were also damaged. Lesions in the cortical group consisted of the region of neocortex and corpus callosum, which overlie the hippocampus.

Previously, fear conditioning had been examined in several strains of wild-type or gene knockout mice, and no gender difference had been observed (Abeliovich et al., 1993; Aiba et al., 1994; Chen et al., 1994). Consistent with these studies, we did not detect any difference in freezing response between male and female mice in the present study. Therefore, the fear conditioning data were pooled across gender for the C57BL/6J strain, whereas only male mice were used for the BALB/c strain.

Figure 2A shows the mean percentage of freezing exhibited by sham-, cortex-, and hippocampus-lesioned groups from the C57BL/6J strain. In the conditioning phase (Figure 2A), none of the groups exhibited freezing behavior prior to the footshocks. During the three 1-min intervals following the footshocks, all groups displayed postshock freezing that increased linearly over the number of footshocks. Although hippocampus-lesioned mice appear to show less postshock freezing than sham- or cortex-lesioned mice, a one-way analysis of variance (ANOVA) indicated that there were no reliable group differences in postshock freezing, $F(2, 22) = 3.09$, $p > .05$. In contrast, when behavior was observed the next day (Figure 2B), hippocampus-lesioned mice exhibited significantly lower freezing than either sham- or cortex-lesioned mice, $F(2, 23) = 11.92$, both $ps < .01$ (Newman–Keuls tests). The cortex- and sham-lesioned groups did not differ from each other.

In contrast to C57BL/6J mice, BALB/c mice exhibited very little freezing response (Figures 2C and 2D). Following footshocks, none of the groups displayed appreciable freezing (Figure 2C). When mice were tested 24 hr after conditioning,

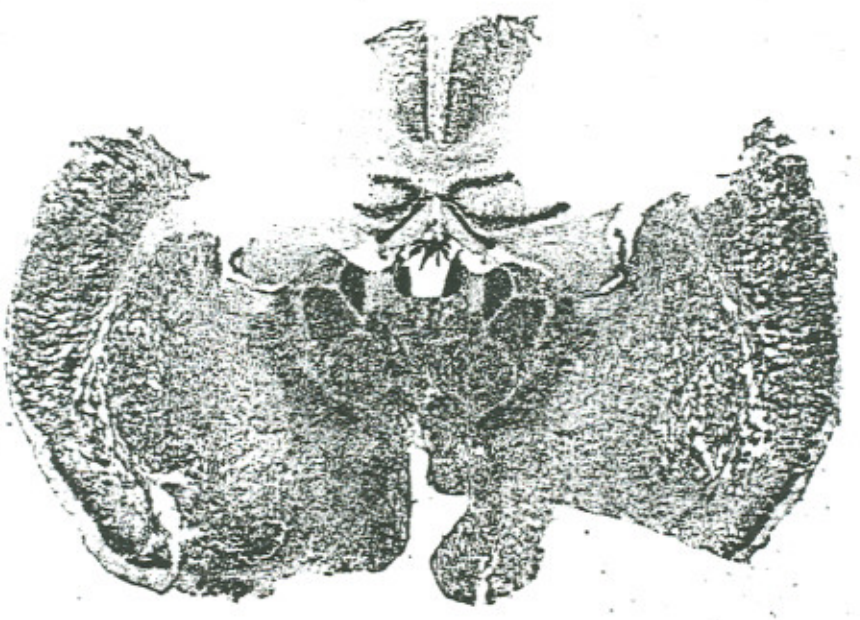

Figure 1. A photomicrograph of transverse brain section stained with cresyl violet from a typical mouse in the hippocampal-aspirated group.

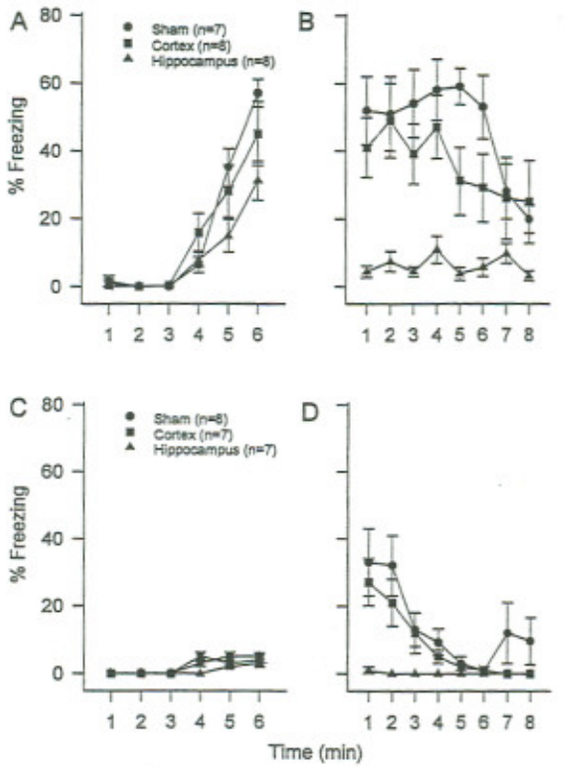

Figure 2. Mean (± *SEM*) percentage of freezing displayed by hippocampus-, cortex-, and sham-lesioned mice of C57BJ/6 strain during (A) and 24 hr after (B) conditioning, and three groups of BALB/c strain (C, D).

hippocampus-lesioned mice showed practically no freezing response (Figure 2D). Both cortex- and sham-lesioned groups displayed small amounts of freezing, but overall they did not differ significantly from the hippocampal-lesioned group, $F(2, 21) = 2.54$, $p > .05$. There was, however, a Group × Time interaction, $F(14, 154) = 2.99$, $p < .01$, suggesting differences in freezing response among the three groups across 8 min of testing. ANOVAs at each time showed that hippocampus-lesioned mice froze significantly less than both cortex- and sham-lesioned mice on the first minute of testing, $F(2, 21) = 4.25$, both $ps < .05$ (Newman–Keuls tests), and considerably less than sham-lesioned mice on the second minute of testing, $F(2, 21) = 4.76$, $p < .05$. No reliable group differences were observed at any other times.

Overall, the BALB/c mice exhibited significantly less freezing than the C57BL/6J mice, both immediately following footshocks, $F(1, 44) = 57.90$, $p < .01$, and 24 hr after conditioning, $F(1, 44) = 13.24$, $p < .01$.

Discussion

Hippocampal lesions, but not overlying cortical lesions, disrupted the freezing response elicited by the contextual cues of the chamber 24 hr after training in both C57BL/6J and BALB/c mice. These results are consistent with findings reported in rat studies (Blanchard & Fial, 1968; Kim & Fanselow, 1992; Kim et al., 1993; Phillips & LeDoux, 1992). The freezing that occurred immediately following the fo shock, however, was not affected by the hippocampal lesi This preservation of immediate postshock freezing has a been reported with both electrolytic and neurotoxic lesions rats (Kim et al., 1993; Young, Bohenek, & Fanselow, 199 Because hippocampal-lesioned mice exhibited postshock fre ing comparable with that of control mice, the deficit in freezi observed 24 hr after training is unlikely due to the anima inability to freeze. Rather, hippocampal lesions seem interfere with the long-term memory (24 hr) of contextual fe Considerable evidence in rats indicates that postshock freezi is entirely a conditioned response that results from an associ tion between the contextual cues of the chamber and t footshock (Blanchard, Fukunaga, & Blanchard, 1976; Fanselo 1986). Thus, the present mouse study supports the notion th the hippocampus is important for the long-term memory contextual fear.

There was a strain difference in the freezing respons Whereas the C57BL/6J mice displayed robust freezing bot immediately and 24 hr after the footshocks, the BALB/c mic exhibited very little freezing at both times (the lack of freezin was more pronounced immediately after the footshocks) Thus, for future studies of genetically engineered mice usin the contextual fear conditioning paradigm, the C57BL/6 strain or hybrids of this and another strain may be more usefu than the BALB/c strain.

In conclusion, our results provide a basis for using genetic mutant mice to study molecular and cellular mechanisms underlying the hippocampus-dependent memory of contextual fear. Our findings of hippocampal functions in mice are consistent with numerous previous studies using rats and other animals.

References

Abeliovich, A., Paylor, R., Chen, C., Kim, J. J., Wehner, J. M., & Tonegawa, S. (1993). PKCγ mutant mice exhibit mild deficits in spatial and contextual learning. *Cell, 75,* 1263–1271.

Aiba, A., Chen, C., Herrup, K., Rosenmund, C., Stevens, C. F., & Tonegawa, S. (1994). Reduced hippocampal long-term potentiation and context-specific deficit in associative learning in mGluR1 mutant mice. *Cell, 79,* 365–375.

Blanchard, R. J., & Blanchard, D. C. (1969). Crouching as an index of fear. *Journal of Comparative and Physiological Psychology, 67,* 370–375.

Blanchard, R. J., & Fial, R. A. (1968). Effects of limbic lesions on passive avoidance and reactivity to shock. *Journal of Comparative and Physiological Psychology, 66,* 606–612.

Blanchard, R. J., Fukunaga, K. K., & Blanchard, D. C. (1976). Environmental control of defensive reactions to footshock. *Bulletin of the Psychonomic Society, 8,* 129–130.

Bolles, R. C. (1970). Species-specific defense reactions and avoidance learning. *Psychological Review, 77,* 32–48.

Bourtchuladze, R., Frenguelli, B., Blendy, J., Cioffi, D., Schutz, G., & Silva, A. (1994). Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. *Cell, 79,* 59–68.

Chen, C., Rainnie, D. G., Greene, R. W., & Tonegawa, S. (1994). Abnormal fear response and aggressive behavior in mutant mice deficient for α-calcium-calmodulin kinase II. *Science, 266,* 291–294.

Chen, C., & Tonegawa, S. (1995). Analysis of synaptic plasticity and

memory in the mammalian brain by the gene knockout technology. In M. Baudry & J. L. Davis (Ed.), *Long-term potentiation: A debate of issues, part 3.* Cambridge, MA: MIT Press.

Eichenbaum, H., Otto, T., & Cohen, N. J. (1992). The hippocampus: What does it do? *Behavioral and Neural Biology, 57,* 2–36.

Fanselow, M. S. (1986). Associative vs. topographical accounts of the immediate shock freezing deficit in rats: Implications for the response selection rules governing species-specific defensive reactions. *Learning and Motivation, 17,* 16–39.

Gallagher, M., & Holland, P. C. (1992). Preserved configural learning and spatial learning impairment in rats with hippocampal damage. *Hippocampus, 2,* 81–88.

Kim, J. J., Clark, R. E., & Thompson, R. F. (1995). Hippocampectomy impairs the memory of recently, but not remotely, acquired trace eyeblink conditioned responses. *Behavioral Neuroscience, 109,* 1–9.

Kim, J. J., & Fanselow, M. S. (1992). Modality-specific retrograde amnesia of fear. *Science, 256,* 675–677.

Kim, J. J., Rison, R. A., & Fanselow, M. S. (1993). Effects of amygdala, hippocampus, and periaqueductal gray lesions on short- and long-term contextual fear. *Behavioral Neuroscience, 107,* 1093–1098.

Mayford, M., Abel, T., & Kandel, E. R. (1995). Transgenic approaches to cognition. *Current Opinion in Neurobiology, 5,* 141–148.

Morris, R. G. M., Garrud, P., Rawlins, J. N. P., & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature, 297,* 681–683.

Moyer, J. R., Deyo, R. A., & Disterhoft, J. F. (1990). Hippocampectomy disrupts trace eyeblink conditioning in rabbits. *Behavioral Neuroscience, 104,* 243–252.

Phillips, R. G., & LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioral Neuroscience, 106,* 274–285.

Solomon, P. R., Vander Schaaf, E. R., Thompson, R. F., & Weisz, D. J. (1986). Hippocampus and trace conditioning of the rabbit's classically conditioned nictitating membrane response. *Behavioral Neuroscience, 100,* 729– 744.

Squire, L. R. (1992). Memory and the hippocampus. *Psychological Review, 99,* 195–231.

Young, S. L., Bohenek, D. L., & Fanselow, M. S. (1994). NMDA processes mediate anterograde amnesia of contextual fear conditioning induced by hippocampal damage: Immunization against amnesia by context preexposure. *Behavioral Neuroscience, 108,* 19–29.

Zola-Morgan, S. M., & Squire, L. R. (1990). The primate hippocampal formation: Evidence for a time-limited role in memory storage. *Science, 250,* 288–290.

Received November 27, 1995
Revision received March 13, 1996
Accepted March 19, 1996 ■