Cell, Vol. 81, 905–915, June 16, 1995, Copyright © 1995 by Cell Press

Impairment of Spatial but Not Contextual Memory in CaMKII Mutant Mice with a Selective Loss of Hippocampal LTP in the Range of the θ Frequency

Mary Elizabeth Bach, Robert D. Hawkins, Mona Osman, Eric R. Kandel, and Mark Mayford
Howard Hughes Medical Institute
Center for Neurobiology and Behavior
College of Physicians and Surgeons
of Columbia University
New York, New York 10032

Summary

We assessed hippocampal-dependent memory in mice with a Ca^{2+}-independent form of CaMKII generated by the introduction of an aspartate at amino acid 286. The CaMKII-Asp-286 mice show normal LTP at high frequency stimulation, but in the 5–10 Hz range, they show a shift in the frequency-response curve favoring LTD. This range of frequencies is similar to the θ rhythm, which is associated with exploration in rodents. Using the Barnes maze to assess spatial memory, we found the transgenic mice could not learn to navigate to a specific location using spatial cues. In contrast, one line of transgenic mice performed normally in contextual fear conditioning, a task that is also hippocampal dependent. This dissociation between spatial and contextual memory suggests that even though both require the hippocampus, they may be mediated by different synaptic mechanisms.

Introduction

The formation of long-term memories is thought to involve long-term changes in synaptic strength (Hebb, 1949; Kandel and Spencer, 1968). Long-term potentiation (LTP) and long-term depression (LTD) are two different forms of long-lasting synaptic plasticity found in the mammalian brain. LTP and LTD are produced by different frequencies of synaptic activation. A homosynaptic form of LTP is produced in the CA1 region of the hippocampus following high frequency stimulation at 100 Hz, and this LTP has many features that make it an attractive synaptic substrate of memory (see Bliss and Collingridge, 1993, for review). Conversely, low frequency stimulation at 1 Hz produces a homosynaptic form of LTD at the same synapses, which has been proposed in several computational models as a mechanism for erasure or forgetting (Bear and Malenka, 1994; Sejnowski, 1977; Bienenstock et al., 1982; Bear et al., 1987). What happens at the frequencies between 1 and 100 Hz? What role, if any, do these frequencies have for learning and memory storage? Are different frequency-specific forms of synaptic plasticity related to different cognitive forms of learning?

Clear linkages between various forms of hippocampal synaptic plasticity and memory have been difficult to establish for several reasons. First, LTP and LTD are produced experimentally using nonphysiological stimuli. Neither LTP nor LTD has, as yet, been demonstrated to occur in response to environmental stimuli encountered during learning, perhaps because learning leads to changes in only a relatively small number of synaptic connections. Second, although pharmacological or genetic manipulations that interfere with LTP suggest that LTP is required for memory storage, these manipulations generally do not distinguish between LTP and LTD (Silva et al., 1992a, 1992b; Grant et al., 1992; Davis et al., 1992; Stevens et al., 1994; but see Abeliovich et al., 1993a, 1993b). Finally, most experiments have examined synaptic plasticity only at the extremes of the frequency response range (1 and 100 Hz), yet it is the intervening frequencies that are thought to be of particular importance physiologically. When an animal engages in exploratory behavior, as during the acquisition of hippocampally mediated spatial memories, the hippocampus undergoes an oscillation in its electrical activity at frequencies between 4 and 12 Hz, referred to as the θ rhythm (Bland, 1986; O'Keefe, 1993; O'Keefe and Recce, 1993). Disruption of the θ rhythm by lesions of the cholinergic inputs to the hippocampus that drive this rhythm also blocks spatial memory (Winson, 1978). How the θ rhythm affects spatial memory is unclear. One possibility is that the θ rhythm patterns the hippocampal inputs so as to lead to effective LTP. In fact, patterned stimulation in the range of 5 Hz is especially effective in producing LTP (Larson et al., 1986; Arai and Lynch, 1992). Also, induction of the θ rhythm in hippocampal slices by treatment with carbachol produces enhanced LTP in the CA1 region (Huerta and Lisman, 1994).

In the preceding paper, we described two lines of transgenic mice that allow us to assess the role in learning and memory of LTP and LTD produced by θ frequency stimulation (Mayford et al., 1995b [this issue of *Cell*]). The introduction into transgenic mice of a mutant form of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) that is Ca^{2+} independent leads to a shift in the frequency–response curve for the production of both LTP and LTD. The mice carry a mutant form of CaMKII that is Ca^{2+} independent due to the introduction of an aspartate residue at amino acid 286. In adult wild-type mice of both lines, LTP is produced at all frequencies from 5 to 100 Hz, and weak LTD is produced at 1 Hz. In both lines of CaMKII–Asp-286 transgenic mice, LTP is normal at high frequency stimulation of 100 Hz, and LTD is produced at 1 Hz. However, in the transgenic mice, LTP is not produced at frequencies in the range of the θ rhythm. At 5 Hz, LTD is produced, and at 10 Hz, there is no change in synaptic strength. Thus, the transgenic animals maintain the capacity for both LTP and LTD, but the frequency–response function is shifted systematically toward LTD in the range of the θ rhythm.

We took advantage of this shift in the frequency–response function to compare the spatial learning and memory ability of transgenic and wild-type mice on a series of both hippocampal-dependent and -independent tasks. Hippocampal-dependent memory was assessed with the spatial version of the Barnes circular maze (Barnes, 1979)

and contextual fear conditioning (Kim and Fanselow, 1992; Phillips and LeDoux, 1992). In the spatial version of the Barnes maze, the mouse needs to learn and remember the relationship between distal cues in the environment to navigate to an escape tunnel. In contextual fear conditioning, the mouse needs to learn an association between a novel environment and an aversive stimulus. Hippocampal-independent or cued versions of each task were also used. In these tasks the animal needs to recognize and remember only a single environmental cue to perform the task successfully.

The CaMKII–Asp-286 transgenic mice readily acquired the cued version of the Barnes circular maze, but were unable to learn the spatial version despite a lengthy training period. This suggests that it is not only the capacity for LTP and LTD that may be important for spatial memory but also the precise frequency at which each is produced. Although both lines of CaMKII–Asp-286 transgenic mice failed to learn the spatial version of the Barnes maze, one line was able to learn the contextual fear conditioning task, another learning paradigm that is hippocampal dependent. Thus, our results suggest that the ability to form an internal representation of the environment, which is needed for both spatial and contextual memory, can be dissociated genetically from the ability to navigate within that environment, which is necessary only for spatial memory tasks. Furthermore, these results suggest that the two different hippocampally mediated cognitive processes may utilize different synaptic mechanisms.

Results

Transgenic Mice Are Defective on the Spatial Version of the Barnes Circular Maze

The generation and physiological analysis of the CaMKII–Asp-286 transgenic mice is described in the preceding paper (Mayford et al., 1995b). Long-term spatial memory, which is dependent on the functioning of the hippocampus, was assessed in both transgenic lines (referred to as P3 and P15) using the Barnes circular maze. Conceptually, this task is similar to the Morris water maze, since in each case an escape response is required. Moreover, as in the Morris water maze, there are two versions of the task, spatial and cued. We have adapted the Barnes maze, originally used for rats, for studying the learning and memory capabilities of mice because we found it has certain advantages over the Morris water maze. First, the Barnes maze is much less taxing physically than the Morris maze, an important consideration when testing mice, as they are not as large or strong as rats. Second, the circular maze is very easy for mice to traverse and can be managed by both young and aged mice, animals that are poor swimmers. Therefore, the same maze can be employed to assess mice repeatedly across the lifespan. Lastly, the Barnes maze readily reveals the strategies used by the animal to perform the task. In learning the Barnes maze, wild-type mice use a fixed sequence of three search strategies: random, serial, and spatial (Barnes, 1979). Because these strategies are more obvious in the Barnes maze than in other mazes, the strategies can be readily recognized, analyzed, and quantified. As a result, one can not only determine whether an animal learns the task but also gain insight into the behavioral mechanisms used by the animal by seeing which strategies the animals can and cannot use.

The Barnes circular maze is a white disc with 40 holes cut in the perimeter. In both the spatial and cued versions of the task, the mouse is motivated to escape the open, brightly lit maze that is associated with aversive noise from a buzzer. To escape the aversive stimuli, the mouse needs to locate a darkened escape tunnel that is placed underneath 1 of the holes. When the mouse enters the escape tunnel, the buzzer is turned off and the mouse is allowed to remain in the darkness for 1 min. For the spatial version, which we shall consider first, the tunnel was always located underneath the same hole, which was randomly determined for each mouse. To locate the tunnel efficiently in this version of the task, the mouse needs to remember and use the relationships among the distal cues in the room where the maze was placed. The mice were tested once a day until they met a criterion consisting of three errors or less on 7 of 8 consecutive days. Errors were defined as searching any hole that did not have the tunnel beneath it.

The majority of wild-type mice (89%) acquired the task with a median time to criterion of 22 days. By contrast, only 6% of the transgenics met criterion (transgenic versus wild-type: $\chi^2 = 23.139$, $p < .0001$; Figure 1). All but 1 of the 17 transgenic mice failed to reach criterion, even though they were trained for 45 days. Figure 2A presents the mean number of errors made by transgenic and wild-type mice across blocks comprised of five sessions. In this and the following figures, the data were collapsed across gender and line if an ANOVA did not reveal a significant effect of either variable. Transgenic mice made significantly more errors than wild-type mice across all session blocks (overall $F[1,33] = 18.2931$, $p < .001$). Although the transgenic mice showed an initial decrease in errors, this was followed by a leveling off in performance, after which no further improvement occurred.

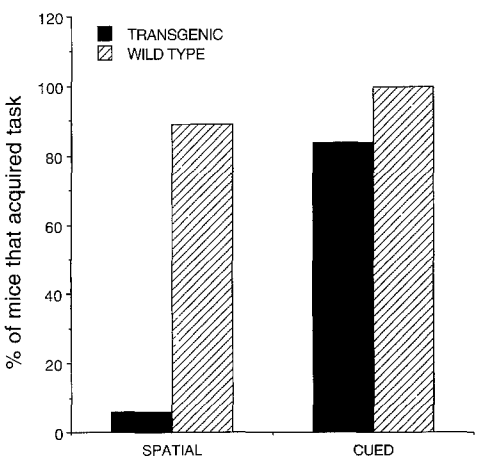

Figure 1. Percentage of CaMKII Transgenic and Wild-Type Mice That Acquired the Spatial and Cued Versions of the Barnes Circular Maze Task

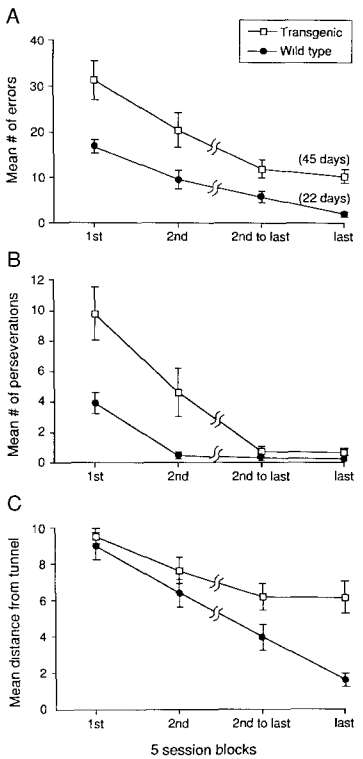

Figure 2. Performance of Transgenic and Wild-Type Mice across Session Blocks on the Spatial Version of the Barnes Circular Maze Task

The data were collapsed across gender and line if an ANOVA did not reveal a significant effect of either variable. Values represent group means ± SEM.

(A) Errors. The ANOVA revealed that errors significantly decreased across session blocks in both CaMKII–Asp-286 transgenic and wild-type mice ($F[3,31] = 21.7276$, $p < .0001$).

(B) Perseverations. In both transgenic and wild-type mice, perseverations significantly decreased across sessions ($F[3,31] = 22.7045$, $p < .0001$). The genotype by session blocks interaction was also significant ($F[3,31] = 3.5576$, $p < .05$).

(C) Distance from tunnel during initial search. A repeated measures ANOVA revealed that distance significantly decreased across session blocks ($F[3,31] = 25.1441$, $p < .0001$). The genotype by session block interaction was also significant ($F[3,31] = 4.7954$, $p < .01$).

Transgenic Mice Learn Random, Serial, but Not Spatial Search Strategies

Many of the differences in performance of wild-type and transgenic mice can be explained by differences in the three search strategies used in the maze. These search strategies were originally described by Barnes (1979). Figure 3 illustrates the representative search strategies employed by a wild-type mouse (Figure 3A) and a transgenic mouse (Figure 3B) from the P15 line. The random search strategy entails exploring many holes in an unsystematic fashion, with many center crossings and some perseverations. Perseverations are defined as repeatedly searching the same hole or alternately searching 2 adjacent holes. The serial search strategy is characterized by the mouse running to the perimeter and then exploring consecutive holes in a clockwise or counterclockwise fashion. The spatial search strategy is the most efficient and results in mice taking a direct course to the escape tunnel.

Both wild-type and mutant mice initially employed the random search strategy, spending ~55% of the time using this strategy during the first session block (Figure 4A). However, the initial performance of the transgenic mice differed from that of the wild type in that they made significantly more perseverations during the first and second session blocks (overall $F[1,33] = 10.3978$, $p < .01$; see Figure 2B). This increase in perseverations accounts for ~50% of the initial difference in errors (see Figure 2A). Both wild-type and mutant mice exhibited a similar decrease in use of the random search strategy across session blocks, using this strategy <10% of the time by the last session block (Figure 4A).

The wild-type and transgenic mice next shifted to the

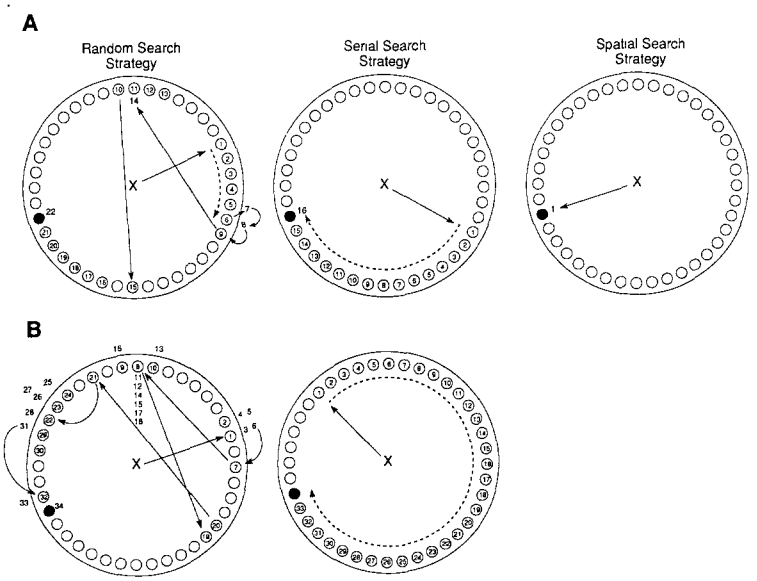

Figure 3. Representative Examples of the Search Strategies Employed on the Spatial Version of the Barnes Circular Maze Task

(A) Wild-type mice.

(B) CaMKII–Asp-286 transgenic mice.

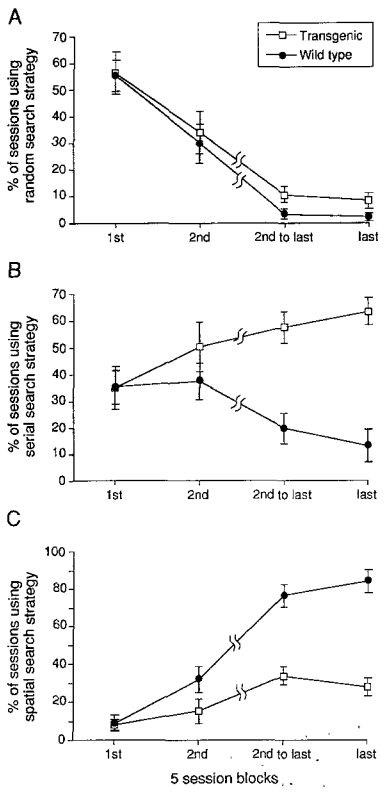

Figure 4. Use of Search Strategies on the Spatial Version of the Barnes Circular Maze

The percentage of sessions each of the three search strategies was employed by transgenic and wild-type mice across session blocks on the spatial version of the Barnes circular maze (values represent group means ± SEM). The data were collapsed across gender and line if an ANOVA did not reveal a significant effect of either variable.

(A) Random search strategy. The percentage of time the random search strategy was employed across session blocks for both groups significantly decreased ($F[3,31] = 45.669$, $p < .0001$).

(B) Serial search strategy. There was a significant genotype by session block interaction ($F[3,31] = 9.6184$, $p < .0001$).

(C) Spatial search strategy. The percentage of time the spatial search strategy was employed significantly increased across session blocks ($F[3,31] = 52.6299$, $p < .0001$), and the genotype by session block interaction was also significant ($F[3,31] = 17.477$, $p < .0001$).

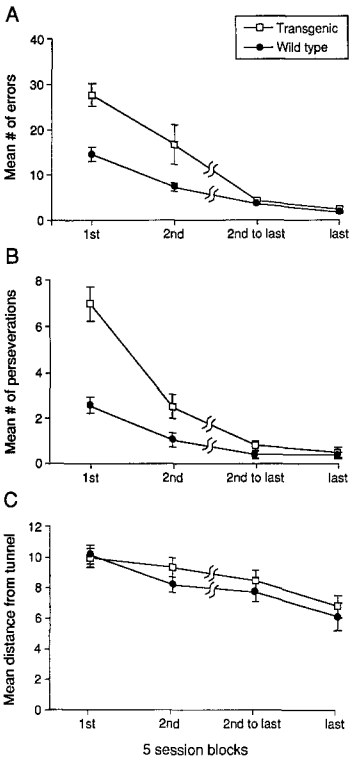

Figure 5. Performance of Transgenic and Wild-Type Mice across Session Blocks on the Cued Version of the Barnes Circular Maze Task

The data were collapsed across gender and line if an ANOVA did not reveal a significant effect of either variable. Values represent group means ± SEM.

(A) Errors. The ANOVA revealed that errors significantly decreased across session blocks in both CaMKII–Asp-286 transgenic and wild-type mice ($F[3,33] = 65.2362$, $p < .0001$). The genotype by session blocks interaction was also significant ($F[3,33] = 5.205$, $p < .005$).

(B) Perseverations. In both transgenic and wild-type mice, perseverations significantly decreased across session blocks ($F[3,33] = 33.7849$, $p < .0001$), and the genotype by session block interaction was also significant ($F[3,33] = 7.9853$, $p < .0005$).

(C) Distance from tunnel during initial search. A repeated measures ANOVA revealed that distance significantly decreased across session blocks ($F[3,33] = 12.2933$, $p < .0001$).

more efficient serial search strategy (Figure 4B). This shift decreased the amount of time the mouse was exposed to the aversive contingencies of the task and also decreased the number of errors made (see Figure 2A). During the first two session blocks, both wild-type and transgenic mice spent a similar percentage of time using this strategy (Figure 4B). During the last two session blocks, the transgenics employed the serial search strategy a majority of the time, whereas the wild types did not (overall $F[2,33] = 15.47$, $p < .0001$).

While the transgenic mice were able to shift from the random to the serial search strategy in a manner similar to the wild types, they failed to use the spatial search strategy consistently, using this strategy <30% of the time during the last two session blocks (Figure 4C). By contrast, the wild-type mice employed the spatial strategy >80% of the time during the last two session blocks (overall $F[1,33] = 29.1153$, $p < .0001$).

Our operational definition of the spatial search strategy was finding the escape tunnel with error and distance scores of ⩽3. The distance score is determined by counting the number of holes between the first hole searched and the escape tunnel. The maximum distance possible is 20; chance is 10. During the first session block, the distance from the tunnel was at chance level for both transgenic and wild-type mice (see Figure 2C). This was expected, since both groups were unfamiliar with the maze and its relation to the spatial cues within the room. During the last session block, the mean distance for the wild-type mice was <2, indicating that the initial search was very close to the tunnel. By contrast, during the last session block, the mean distance for the transgenic mice was 6.2, indicating that they showed some improvement but significantly less than the wild types (overall $F[1,33] = 11.3$,

$p < .001$). An inspection of the data revealed that some transgenic mice had learned the approximate area where the tunnel was located, which might be achieved by associating an extramaze cue with the tunnel area. Such a nonspatial strategy would produce a modest decrease in errors and distance (see Figures 2A and 2C) and a modest increase in the percentage of trials classified as showing the spatial strategy (Figure 4C), but would not enable a mouse to meet the acquisition criterion (7 out of 8 days with three or fewer errors)

Transgenic mice of both lines exhibited significant impairment on the spatial version of the Barnes circular maze. Transgenic mice made significantly more errors and perseverations, were significantly farther away from the tunnel during the initial search, were unable to employ a spatial search strategy, and, lastly, were unable to reach criterion despite lengthy training (~3 weeks over that of wild types). The failure of transgenic mice to learn the spatial strategy could in principle be explained in one of two ways. The mice could have a basic deficit in spatial memory. Alternatively, the impairment in the transgenic mice could be due to a performance deficit as a result of a sensory, motivational, or motor deficit. To examine these alternative explanations, a second group of transgenic and wild-type mice of both lines were assessed on two additional tasks: the cued version of the Barnes maze, to examine their performance capabilities including sensory and motor skills, and a measure of anxiety, to determine their level of motivation.

Transgenic Mice Perform Normally on the Cued Version of the Barnes Maze

The contingencies of the cued task were the same, except that a visible cue was now placed immediately behind the hole with the escape tunnel beneath it and the position of the escape tunnel varied from day to day. Thus, to escape the aversive contingencies of the task, the mouse just needed to associate the proximal cue with the tunnel. This cued version of the Barnes maze does not require hippocampal function. The majority of the transgenic mice (84%) met the criterion (7 out of 8 consecutive days with three or fewer errors) and were not significantly different from wild types (100%) in acquisition of the cued version of the task (see Figure 1). The median sessions to acquisition were 31 and 25 for the transgenic and wild-type mice, respectively (Mann-Whitney $U = 81$, $p < .01$). The number of errors made by both transgenic and wild-type mice decreased significantly across session blocks (Figure 5A). Although transgenic mice made significantly more errors (overall $F[1,35] = 12.2878$, $p < .01$) during the first two session blocks, they were not significantly different from wild types during the last two session blocks. The elevation in errors during the first two session blocks in the transgenic mice can be partially accounted for (~50%) by a significantly greater number of perseverations (overall $F[1,35] = 22.9760$, $p < .001$; Figure 5B).

In the cued version of the Barnes maze, there was no significant difference in the mean distance from the tunnel between transgenic and wild-type mice across any of the session blocks (Figure 5C). Both wild types and transgenics started at a distance equivalent to chance, and in both groups a modest but significant decrease occurred across session blocks. This similarity in performance suggests that both transgenic and wild-type animals used a similar strategy in this cued task. Interestingly, during the last session block, the distance from the tunnel remained relatively high despite the fact that the mice in both groups made few errors during these sessions. Inspection of the raw data revealed that on some days the mice from both groups initially ran to the perimeter and then searched for the cue, resulting in the distance being approximately chance. On other days, the search occurred from the center, and the mouse ran directly to the cue, resulting in a distance of 0.

Transgenic and Wild-Type Mice Show a Similar Light-Dark Preference

Light–dark preference is a classic measure of anxiety in rodents (Costall et al., 1989). We used this task because in the Barnes circular maze the mouse is motivated to escape into a darkened area from a brightly lit area, and therefore a difference in light–dark preference might have affected performance on the Barnes circular maze. A shuttle box was employed to measure light–dark preference across 5 min. Transgenic and wild-type females of both lines were not significantly different from each other and were found to have no preference for either the light or dark side (Figure 6). Male mice of both genotypes had a strong preference for the dark side but were also not significantly different from each other. For both genotypes, females were found to spend significantly more time on the light side than males ($F[1,34] = 9.526$, $p < .005$).

The observation that the transgenic mice acquired the cued version of the Barnes maze and were not significantly different from wild-type mice in light–dark preference sug-

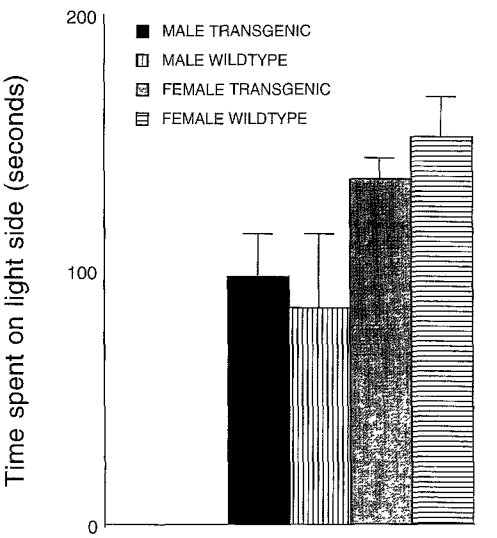

Figure 6. Total Amount of Time Spent on the Light Side for Male and Female Transgenic and Wild-Type Mice during Light–Dark Preference Assessment

Values represent group means ± SEM.

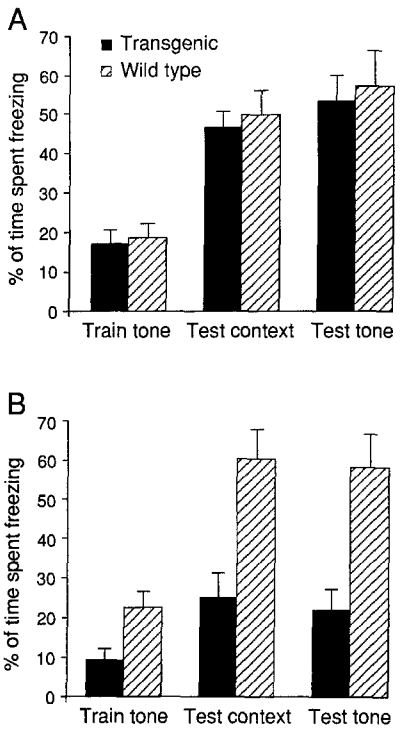

Figure 7. Percentage of Time Spent Freezing to Tone during Training and to Tone and Context during Testing by Transgenic and Wild-Type Mice

(A) P3 line; (B) P15 line. Values represent group means ± SEM.

gests that transgenics do not have severe sensory, motivational, or motor deficits. Therefore, their failure to acquire the spatial version of the Barnes maze is evidently due to their failure to acquire and retain a spatial search strategy that requires the hippocampus.

Transgenic Mice Can Learn Context Conditioning

The finding of a selective defect in one hippocampal-dependent spatial task encouraged us to assess performance on another hippocampal-dependent task, context conditioning. In context conditioning the animal learns to associate an aversive stimulus with the novel environment or context in which it is placed. We assessed both contextual (hippocampal-dependent) and cued (hippocampal-independent) memory in transgenic and wild-type mice from both lines using the conditioned fear paradigm. In this task, a tone (cue) and a novel environment (the distinct new context the animal is placed in) are paired with shock on the training day. On the testing day (24 hr later), if the mouse has learned either the tone–shock or context–shock association, then the tone or context will come to elicit fear. When mice are afraid, they freeze and show a total lack of movement (Blanchard and Blanchard, 1969; Fanselow, 1980). Typically, the mouse assumes a crouched position and shows increased respiration.

We measured the amount of time the mice spent freezing during both training and testing days and found no difference in freezing to either tone or context between transgenic and wild-type mice of the P3 line (Figure 7A). Thus, the CaMKII–Asp-286 mutation did not impair recognition of context in this line of mice, even though it impaired spatial memory. The transgenic mice from the P15 line behaved somewhat differently than those of the P3 line (Figure 7B). They froze significantly less to tone on the training and testing days (genotype by line interaction: $F[1,45] = 5.0411$, $p < .05$) and significantly less to context on the testing day (genotype by line interaction: $F[1,45] = 5.2893$, $p < .05$), suggesting a deficit in both contextual and cued fear conditioning. However, further analysis of the tone data across days did not reveal a significant day by genotype by line interaction, suggesting that the transgenic mice learned the tone–shock association normally. Furthermore, although the P15 transgenics froze significantly less overall, they nevertheless exhibited other species-specific defense reactions, such as tail rattling, jumping, increased respiration, and crouching position, that are consistent with their being afraid (Bolles, 1970). For example, although we did not systematically score tail rattling, we observed that the P15 transgenic animals exhibited more tail rattling than the wild-type animals following training. Thus, this line of animals also seemed to learn and remember both the cue and context shock association, but revealed that memory with the display of other species-specific defense reactions. This difference between the P3 and P15 lines may result from a motivational difference due to slight qualitative or quantitative differences in transgene expression outside of the hippocampus. Nevertheless, our data suggest that both lines of mice can learn about context, whereas they cannot learn a spatial memory task. Consistent with this interpretation, a second group of P15 trangenics behaved normally on the conditioned fear task (data not shown).

Discussion

Genetic and Behavioral Dissection of Discrete Behavioral Subroutines of Hippocampal-Based Spatial Learning

Some time after the initial demonstration by Scoville and Milner (1957) that the hippocampus is essential for certain forms of memory storage, it became clear that memory storage is not a unitary faculty of the mind but rather involves a number of different storage processes, each using its distinctive neural systems (see Squire, 1992; Schacter and Tulving, 1994, for review). In humans the hippocampus, along with the medial temporal lobe, is required for the acquisition of new information about people, things, and places. Both spatial and contextual memory are forms of hippocampal-dependent memory that are particularly easy to study in experimental animals, and there is now strong evidence that the hippocampus is essential for both types of memory (Olton and Werz, 1978; Fanselow, 1980). That both spatial and contextual memory require the hippocampus, in turn, raises the question: Are all forms of hippocampal-dependent memory fundamen-

tally similar in nature, or can hippocampal-dependent memory be subdivided into cognitive subroutines that employ different molecular steps, different aspects of neuronal activity, and perhaps different regions within the hippocampus?

To address these questions, we compared the performance of wild-type and transgenic mice in two different hippocampal-dependent tasks: the Barnes circular maze and context conditioning.

The Navigational Capacity Is Selectively Impaired in CaMKII-Asp-286 Transgenic Mice

Mice expressing the CaMKII–Asp-286 transgene revealed a severe impairment on the Barnes circular maze. They progressed through the first two stages of learning, using random and serial search strategies, but they failed completely to use a spatial strategy consistently, even when overtrained for 3 weeks beyond the point when wild-type animals had acquired the task. Being unable to master a spatial strategy, the mice persist in using the serial search strategy, which is cognitively less demanding because it only requires the mouse to remember to search each consecutive hole. This serial search strategy makes minimal demands on memory compared with the spatial search strategy, which requires the mouse to use the multiple relationships among extramaze stimuli to guide it to the escape tunnel.

A similar reliance on a serial or response strategy instead of a spatial strategy occurs in experimental animals with other types of spatial memory impairments. The use of a serial or response strategy was observed in animals with hippocampal lesions in both the radial arm maze (Yoerg and Kamil, 1982) and the Morris water maze (Gallagher et al., 1993; DiMattia and Kesner, 1988). Moreover, both aged (Gallagher et al., 1993) and young (Staddon, 1983; Devan et al., 1992) animals employ response strategies. Pharmacological manipulations, such as scopolamine administration, also increased serial search strategies (Watts et al., 1981). Finally, animals also use a serial search strategy when there are few or no extramaze cues (Suzuki et al., 1980; Sutherland and Dyck, 1984) or when they have been blinded (Dale and Innis, 1986; Zoladek and Roberts, 1978). In contrast to this learning disability on a spatial task, the transgenic mice perform as well as wild types on a cued version of this task that does not require a spatial strategy and is independent of the hippocampus.

The Barnes maze also provides an easy and objective measure of perseverations. An increase in perseverations can explain some of the increase in errors made by the transgenic mice at the beginning of training on both the spatial and the cued versions of the task. This observation is consistent with an increase in perseverations and, subsequently, errors, which was noted in animals with hippocampal lesions (Olton and Werz, 1978; Devenport et al., 1988), in animals following the administration of N-methyl-D-aspartate (NMDA) antagonists (Shapiro and O'Connor, 1992), and lastly, in mice with hippocampal disruptions due to genetic manipulations (Silva et al., 1992a, 1992b; Grant et al., 1992).

Spatial and Contextual Memory Can Be Dissociated: A Distinction between Forming a Representation of the Environment and Using That Representation for Spatial Navigation

What cognitive processes underlie spatial performance on the Barnes circular maze? In particular, does forming an internal representation of the environment involve a component of a learning and memory system that is distinct from formulating a strategy for successful spatial navigation within that environment?

Various hypotheses have been advanced to explain rodent performance in mazes. The spatial map hypothesis, first developed by Tolman (1948) and later applied to hippocampal-based learning by O'Keefe and Nadel (1978), suggests that the relationship between goal areas and extramaze stimuli is stored, topographically or in a Euclidian representation, and then used to guide behavior to goal areas. By contrast, the list hypothesis, proposed by Olton (1978), suggests that performance on spatial memory tasks results from forming a list of goal areas in working memory, with each goal associated with particular extramaze stimuli. According to this idea, each goal area and its corresponding extramaze stimuli are treated as individual units; locating a goal area can be achieved without reference to other goal areas and their corresponding extramaze stimuli (i.e., a spatial map is not required). The navigational system hypothesis of Müller et al. (1991) incorporates aspects of both the list and spatial map theories. Müller et al. propose that spatial performance requires two components: a spatial map or an internal representation of space and a locale or navigational capacity. The spatial mapping capacity entails the ability to formulate a topographical representation of the environment based on its shape and the distribution of stimuli within the environment. The navigational capacity entails the ability to formulate (or conceptualize) the shortest and most efficient path through the environment based on the relationships between stimuli in that environment. Both the spatial map and navigational capacities are mediated by the hippocampus, although the precise areas involved in each, or how the mechanisms relate to one another, are not known.

Our studies provide evidence that genetic analysis may allow one to distinguish between the ability to form an internal representation of an environment, on the one hand, and the ability to formulate the most efficient path toward a distal goal, on the other. The CaMKII–Asp-286 transgenic mice were unable to learn the spatial version of the Barnes circular maze despite being overtrained for 3 weeks beyond the point when wild-type mice acquire the task. However, these transgenic mice were able to learn and remember context in the conditioned fear task. The recognition of context must involve the formation of some type of internal representation of the environment or context. Thus, we find a dissociation in the transgenic

mice between the ability to form an internal representation of an environment necessary to recognize context, and the ability to use that representation to form a navigation strategy. This deficit is reflected in their inability to take the shortest path to the escape tunnel in the Barnes maze. Our studies suggest that the cognitive processes used in spatial mazes are likely to involve a number of hippocampal-based skills that are distinct from those used in recognizing context, and that different neuronal lesions may produce impairments in some skills without affecting others. An alternative explanation is that the CaMKII–Asp-286 transgenic mice have a subtle hippocampal disruption that is revealed only when performance on a more cognitively demanding task is assessed.

Expression of Transgene in Other Regions of Forebrain and during Development Limits the Ability to Correlate Cellular Physiology with Behavior

The clear and persistent impairment in spatial memory found in both lines of transgenic mice suggests a requirement of hippocampal LTP in the range of 5–10 Hz for this type of memory. There are, however, alternative interpretations of this result. One possibility is that the spatial memory deficit is a function of a developmental or pleiotropic effect of the transgene. For example, the presence of this mutation during a critical period of neuronal development might lead to a secondary effect on LTP and LTD. The interpretation that the spatial memory impairment is a function of the absence of LTP at the θ frequency is limited by the fact that the physiological characterization of the mutation was done only in vitro. The effect on LTP and LTD in the intact animal was not determined. Also, it is possible that the memory deficit arises from functional changes in other regions of the forebrain, since the CaMKII–Asp-286 transgene is also expressed in the neocortex, striatum, and amygdala. The effect of the transgene on these other areas is not known, since the physiological characterization of the mice focused exclusively on the CA1 region of the hippocampus (see Mayford et al., 1995a, for a discussion of some of the problems with transgenics and behavior as well as some possible solutions).

Nevertheless, our results indicate that, even though the expression of the transgene is widespread throughout forebrain structures, its effect on behavior is highly selective. The transgenic mice show a deficit that is limited not only to hippocampal-dependent forms of memory but to a specific subtype of this memory. Moreover, a focus on the CA1 region for spatial and other explicit forms of learning, although incomplete, is not unreasonable. In humans a lesion restricted to the CA1 region of the hippocampus gives rise to a memory loss selective for explicit forms of memory (Zola-Morgan et al., 1986). Similarly, ischemic injury to the hippocampus in experimental animals, which leads to a selective loss of the CA1 pyramidal cells, produces a selective deficit in spatial memory ability (Auer et al., 1989; Davis and Volpe, 1990).

The Roles of LTP and LTD in Spatial Learning

The synaptic mechanisms that underlie learning and memory in the mammalian brain are not well characterized. Nevertheless, most models of learning and memory invoke some form of use-dependent alteration in synaptic strength like LTP. Indeed, there is a reasonably good correlation between aspects of LTP and spatial memory. Infusion of blockers of the NMDA receptor into rat hippocampus disrupts both LTP and spatial learning in the Morris water and radial arm mazes (Shapiro and O'Connor, 1992; Davis et al., 1992). Similarly, knockout of either the CaMKIIα or the *fyn* gene gives rise to mice that have a defect in LTP and show concomitant deficits in spatial memory (Silva et al., 1992a, 1992b; Grant et al., 1992). However, both blockade of the NMDA receptor and the knockout of CaMKIIα or fyn interfere with LTD as well as LTP (Dudek and Bear, 1992; Stevens et al., 1994; J. Wang, unpublished data). It therefore has proven difficult to assign the memory deficit in these animals solely to the lack of LTP. Indeed, when LTD is spared, as in mice with a knockout of the PKCγ gene, there is only a minor learning impairment, even though high frequency LTP is blocked completely (Abeliovich et al., 1993a, 1993b). Since in these mice the block of LTP can be overcome by priming stimulation at frequencies that produced LTD (1 Hz), Tonegawa and his colleagues have suggested that LTD may serve as a primary synaptic mechanism for the acquisition of spatial information.

The availability of transgenic mice that express the Ca^{2+}-independent Asp-286 transgenic form of CaMKII has allowed us to address this suggestion. Specifically, these transgenic mice have allowed us to investigate two questions. First, is LTD the primary synaptic mechanism for hippocampal-based learning? If so, does enhanced LTD lead to enhanced spatial learning and memory ability? Second, does learning by the animal require that it have the capability to produce LTP at frequencies of 5–10 Hz, or is LTP produced at 100 Hz sufficient for learning and memory?

In our transgenic mice, there is normal LTP produced by high frequency stimulation (100 Hz) and normal LTD produced by low frequency stimulation (1 Hz). However, in the intermediate frequencies, in the range of 5–10 Hz, stimulation in the transgenic animals produces either LTD or no change in synaptic strength, whereas the wild-type animals show LTP at these frequencies. Thus, the transgenic animals retain the capacity to form both LTP at high frequencies and LTD at low frequencies. However, at the frequencies in between, the transgenics favor LTD as compared with wild-type mice. In addition, in one line of CaMKII–Asp-286 mice, the quantitative level of LTD obtainable at 1 Hz is also enhanced.

LTP at the θ Rhythm Frequency May Be Required for Spatial but Not Contextual Memory

The hippocampus is clearly required for spatial memory. Moreover, there is evidence for the involvement of hippocampal LTP in this memory. However, LTP can be produced in a variety of different ways. For example, LTP can

be produced at frequencies that range from 5 to 100 Hz. The precise physiological frequencies that may induce LTP during different forms of learning are not known. There are, however, several reasons to believe that frequencies of about 5 Hz may be particularly important for spatial memory, because these frequencies simulate endogenous firing patterns that seem important for spatial memory. When a rodent explores a new environment, it displays a 4–12 Hz θ rhythm in the hippocampus driven by cholinergic synaptic inputs from the medial septum (Bland, 1986; O'Keefe, 1993; Smythe et al., 1992). Cholinergic activation in turn leads to a depolarizing oscillation at the θ frequency in the membrane potential of the CA3 pyramidal neurons (Bland et al., 1988; MacVicar and Tse, 1989; Leung and Yin, 1991). At the peak of these depolarizations, the CA3 cells fire one or more action potentials that, in turn, might induce θ frequency LTP in the CA1 neurons. Thus, the learning impairment seen in CaMKII–Asp-286 transgenic mice may be due to the lost capacity to form LTP in response to the synaptic activation patterns that occur during learning. Equally interestingly, the enhanced LTD at these frequencies does not seem capable of substituting for LTP in sustaining memory storage in this situation.

The idea that LTP produced by synaptic activation in the θ frequency range is necessary for spatial memory formation is consistent with the observation that lesions of the medial septal area, which destroy the cholinergic input to the hippocampus and thereby disrupt the θ rhythm oscillations, interfere with spatial memory (Winson, 1978). By contrast, septal lesions enhance contextual fear conditioning (Sparks and LeDoux, 1995). This supports the idea that LTP produced by θ frequency stimulation is necessary for the formation of spatial memory but not for contextual memory. This dissociation of these two forms of explicit memory in response to disruption of the θ rhythm parallels the effect we have seen in mice lacking θ frequency LTP. Thus, the ability of CaMKII–Asp-286 mice to learn and remember context in the fear conditioning paradigm, together with the septal lesion data, suggests that LTP produced in the θ frequency range is not utilized for this form of memory.

Different Hippocampal-Based Learning Tasks May Use Different Forms of Synaptic Plasticity

The selective impairment of spatial as opposed to contextual memory in the CaMKII–Asp-286 transgenic mice suggests the possibility that the two hippocampally mediated tasks use different synaptic mechanisms. Just as different regions of the brain control different aspects of animal behavior, including different aspects of memory storage, it is possible that different forms of synaptic plasticity preferentially contribute to different aspects of memory storage in the same brain region. Hippocampal-dependent tasks represent a large variety of learning skills that almost certainly require different neural subsystems and different physiological and synaptic mechanisms. This is certainly the case for hippocampal-independent tasks. For example, long-term memory for habituation of the gill-withdrawal reflex in Aplysia involves a homosynaptic depression in the connections between the sensory and motor neurons (Carew and Kandel, 1973) that seems independent of cAMP, whereas long-term memory for sensitization involves an enhancement of synaptic strength (Frost et al., 1985) that is mediated by cAMP and CREB-induced gene activation (Alberini et al., 1994). Our results suggest that different forms of hippocampal-dependent memory, even for a given class of tasks such as memory for one's environment, can be divided into at least two subcategories, contextual memory and spatial memory, with θ frequency LTP necessary only for the latter. The use of genetically modified mice with subtle deficits in synaptic physiology restricted to limited regions of the brain may help the dissection of different subroutines of memory storage, on the one hand, and the ascription of different forms of plasticity to the cognitive subroutines, on the other.

Experimental Procedures

Barnes Circular Maze Task

Twenty-four mice from the P15 line (transgenic: n = 6 females and 6 males; wild-type: n = 6 females and 6 males) and 11 female mice from the P3 line (transgenic: n = 5 [1 died]; wild-type: n = 6) were assessed on the spatial version. Twenty mice from the P15 line (transgenic: n = 5 females and 5 males; wild-type: n = 5 females and 5 males) and 17 female mice from the P3 line (transgenic: n = 9; wild-type: n = 8 [1 died]) were assessed on the cued version. The Barnes circular maze was an acrylic disc 122 cm in diameter, one-quarter inch thick that was painted with white epoxy paint. The maze was elevated 90 cm above the floor by two movable pedestals. Forty holes, 5 cm in diameter, were located 5 cm from the perimeter, and a black plexiglass escape tunnel, 45 × 11 cm in size, was placed under 1 of the holes. On the first day of testing, a training trial occurred, which consisted of placing the mouse in the tunnel and leaving it there for 1 min. At 1 min following the training trial, the first session commenced. At the beginning of each session, the mouse was placed in the middle of the maze in a 10 cm high cylindrical black start chamber, and a buzzer (80 dB) from a timer (Gradlab model 167) was turned on. The timer was affixed to the ceiling over the maze. After 10 s had elapsed, the chamber was lifted and the mouse was free to explore the maze. The session ended when the mouse entered the escape tunnel or after 5 min elapsed. When the mouse entered the escape tunnel, the buzzer was turned off and the mouse was allowed to remain in the dark for 1 min. In the spatial version of the task, the tunnel was always located underneath the same hole, which was randomly determined for each mouse. In the cued version, the cue (23 cm tall blue, white, and red aerosol can) was placed directly behind the hole with the escape tunnel beneath it. The position of the escape tunnel varied randomly from day to day. In both versions, the mice were tested once a day until they met criterion (7 out of 8 sessions with three or fewer errors) or a maximum number of days had elapsed. The order of holes searched was recorded manually by a blind observer, and from these data the following variables were derived: errors, distance from tunnel during initial search, and perseverations. Errors were defined as searches of any hole that did not have the tunnel beneath it. Searches included nose pokes and head deflections over the hole. Distance was calculated by counting the number of holes between the first hole searched and the escape tunnel. The maximum distance possible was 20; chance was 10. Perseverations were defined as repeatedly searching the same hole or 2 adjacent holes.

A χ^2 test was used to compare the percentage of transgenic and wild-type mice that acquired the spatial and cued versions. In the cued version, a Mann-Whitney U test was employed to compare sessions to acquisition in transgenic and wild-type mice. In both versions, numerical data on errors, distance, and perseverations were analyzed as follows. First, within the P15 line a two factor ANOVA (gender and

genotype) revealed no significant effect of gender, so the data were collapsed across this variable. Similarly, a two factor ANOVA (line and genotype) revealed no significant effect of line, so the data were collapsed across this variable. Lastly, a two factor ANOVA (genotype and block) with one repeated measure was employed to test the effect of genotype.

The search strategy data were obtained by examining each mouse's daily session and classifying the sequence of hole searches into one of three operationally defined categories. The random search strategy was defined as localized hole searches separated by crossings through the center of the maze. The serial search strategy was defined as systematic hole searches (every hole or every other hole) in a clockwise or counterclockwise direction. Lastly, the spatial search strategy was defined as reaching the escape tunnel with both error and distance scores of ⩽3. Two raters, who were blind as to whether the mice were transgenic or wild type, classified each mouse's data independently and attained 100% congruence. For each search strategy, the data were analyzed statistically in the same manner as the data on errors, distance, and perseverations.

Light–Dark Preference Assessment

Twenty-four mice from the P15 line (transgenic: n = 6 females and 6 males; wild-type: n = 6 females and 6 males) and 14 female mice from the P3 line (transgenic: n = 7; wild-type: n = 7) were tested individually in a shuttlebox (Med Associates) for 5 min. One side of the shuttlebox was darkened with black construction paper, and the floor grids were covered with paper. Two halogen lights, each 50 W, were placed 10 cm above the light side. The total amount of time spent in the light and dark sides was recorded. A two factor ANOVA (genotype and sex) was employed to analyze the amount of time spent on the light side of the light–dark box.

Conditioned Fear Paradigm

On the training day, mice were brought to a novel room and placed in a mouse operant chamber (Med Associates) consisting of two plexiglass walls, two metal walls, a metal grid floor, and a clear plexiglass top. The chamber was in a sound- and light-attenuating box illuminated by one 25 W bulb. The experimental contingencies were controlled by a computer via a program written with Med-PC Software (Med Associates). At 3 min after the mouse was placed in the chamber, a 20 s, 75 dB Sonalert tone (Med Associates) was presented. During the last second of tone, a 0.75 mA footshock was delivered through the grid floor. The shock generator was connected to the grid floor through a scrambler (Med Associates). The 1 s footshock was followed by two more tone–shock pairings at 1 min intervals. Memory for either the context–shock or tone–shock association was assessed 24 hr later by measuring the amount of freezing exhibited by the mice in the presence of the old context and then in the presence of the tone in a second novel context. The novel context was also a mouse operant chamber (Med Associates) that was altered by covering the grid floor and walls with construction paper and adding a novel scent. The mice were placed back into the old context for 3 min, and then ~2 hr later they were placed into the novel context for 3 min followed by three 20 s tone presentations, each separated by a 1 min interval. Freezing was defined as a total lack of movement with the exception of respiration. Three factor ANOVAs (genotype, line, and day) with one repeated measure were used to analyze freezing to tone and context.

Acknowledgments

We thank Richard Axel and Tom Jessell for critically reading this manuscript. We also thank Katrina Podsypanina for maintaining and genotyping the mice, Sarah Mack and Chuck Lam for helping with the figures, and Harriet Ayers and Andrew Krawetz for typing the manuscript. This research was supported by the Howard Hughes Medical Institute, the Charles A. Dana Foundation, and NIMH grant MH50733.

Received March 6, 1995; revised April 13, 1995.

References

Abeliovich, A., Chen, C., Goda, Y., Silva, A. J., Stevens, C. F., and Tonegawa, S. (1993). Modified hippocampal long-term potentiation in PKCγ-mutant mice. Cell *75*, 1253–1262.

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

Alberini, C., Ghirardi, M., Metz, R., and Kandel, E. R. (1994). C/EBP is an immediate-early gene required for the consolidation of long-term facilitation in Aplysia. Cell *76*, 1099–1114.

Arai, A., and Lynch, G. (1992). Factors regulating the magnitude of long-term potentiation induced by theta pattern stimulation. Brain Res. *598*, 173–184.

Auer, R. N., Jensen, L., and Whishaw, I. Q. (1989). Neurobehavioral deficit due to ischemic brain damage limited to half of the CA1 section of the hippocampus. J. Neurosci. *9*, 1641–1647.

Barnes, C. A. (1979). Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. J. Comp. Physiol. Psychol. *93*, 74–104.

Bear, M. F., and Malenka, R. C. (1994). Synaptic plasticity: LTP and LTD. Curr. Opin. Neurobiol. *4*, 389–399.

Bear, M. F., Cooper, L. N., and Ebner, F. F. (1987). A physiological basis for a theory of synapse modification. Science *237*, 42–48.

Bienenstock, E., Cooper, L., and Munro, P. (1982). Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. J. Neurosci. *2*, 32–38.

Blanchard, R. J., and Blanchard, D. C. (1969). Crouching as an index of fear. J. Comp. Physiol. Psychol. *67*, 370–375.

Bland, B. H. (1986). The physiology and pharmacology of hippocampal formation theta rhythms. Prog. Neurobiol. *26*, 1–54.

Bland, B. H., Colom, L. V., Konopacki, J., and Roth, S. H. (1988). Intracellular records of carbachol-induced theta rhythm in hippocampal slices. Brain Res. *447*, 364–368.

Bliss, T. V. P., and Collingridge, G. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. Nature *361*, 31–39.

Bolles, R. C . (1970). Species-specific defense reactions and avoidance learning. Psychol. Rev. *71*, 32–48.

Carew, T. J., and Kandel, E. R. (1973). Acquisition and retention of long-term habituation in *Aplysia*: correlation of behavioral and cellular processes. Science *182*, 1158–1160.

Costall, B., Jones, B. J., Kelly, M., Naylor, R. J., and Tomkins, D. M. (1989). Exploration of mice in a black and white test box: validation as a model of anxiety. Pharmacol. Biochem. Behav. *32*, 777–785.

Dale, R. H. I., and Innis, N. K. (1986). Interactions between response stereotypy and memory strategies on the eight-arm radial maze. Behav. Brain Res. *19*, 17–25.

Davis, H. P., and Volpe, B. T. (1990). Memory performance after ischemic or neurotoxin damage of the hippocampus. In The Biology of Memory, L. R. Squire and E. Lindenlaub, eds. (Stuttgart, Germany: Schattauer Verlag), pp. 477–504.

Davis, S., Butcher, S. P., and Morris, R. G. (1992). The NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (D-AP5) impairs spatial learning and LTP *in vivo* at intracerebral concentrations comparable to those that block LTP *in vitro*. J. Neurosci. *12*, 21–34.

Devan, B. D., Blank, G. S., and Petri, H. L. (1992). Place navigation in Morris water maze: effects of reduced platform interval lighting and pseudo random platform positioning. Psychobiology *20*, 120–126.

Devenport, L. D., Hale, R. L., and Stidham, J. A. (1988). Sampling behavior in the radial arm maze and operant chamber: role of hippocampus and prefrontal area. Behav. Neurosci. *102*, 489–498.

DiMattia, B. D., and Kesner, R. P. (1988). Spatial cognitive maps: differential role of parietal cortex and hippocampal formation. Behav. Neurosci. *102*, 471–480.

Dudek, S. M., and Bear, M. F. (1992). Homosynaptic long-term depression in area CA1 of hippocampus and effects of *N*-methyl-D-aspartate receptor blockade. Proc. Natl. Acad. Sci. USA *89*, 4363–4367.

Fanselow, M. S. (1980). Conditional and unconditioned components of post-shock freezing. Pavlovian J. Biol. Sci. *15*, 177–182.

Frost, W. N., Castellucci, V. F., Hawkins, R. D., and Kandel, E. R. (1985). Monosynaptic connections made by the sensory neurons of the gill- and siphon-withdrawal reflex in *Aplysia* participate in the storage of

long-term memory for sensitization. Proc. Natl. Acad. Sci. USA *82*, 8266–8269.

Gallagher, M., Burwell, R., and Burchinal, M. (1993). Severity of spatial learning impairment in aging: development of learning index for performance in the Morris water maze. Behav. Neurosci. *107*, 618–626.

Grant, S. G. N., O'Dell, T. J., Karl, K. A., Stein, P., Soriano, P., and Kandel, E. R. (1992). Impaired long-term potentiation, spatial learning, and hippocampal development in *fyn* mutant mice. Science *258*, 1903–1910.

Hebb, D. O. (1949). The Organization of Behaviour (New York: Wiley).

Huerta, P. T., and Lisman, J. E. (1994). Heightened synaptic plasticity of hippocampal CA1 neurons during a cholinergically induced rhythmic state. Nature *364*, 723–725.

Kandel, E. R., and Spencer, W. A. (1968). Cellular neurophysiological approaches in the study of learning. Physiol. Rev. *48*, 65–134.

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

Larson, J., Wong, D., and Lynch, G. (1986). Patterned stimulation at the theta frequency is optimal for the induction of hippocampal long-term potentiation. Brain Res. *368*, 347–350.

Leung, L.-W. S., and Yim, C.-Y. C. (1991). Intrinsic membrane potential oscillations in hippocampal neurons *in vitro*. Brain Res. *553*, 261–274.

MacVicar, B. A., and Tse, F. W. Y. (1989). Local neuronal circuitry underlying cholinergic rhythmical slow activity in CA3 area of rat hippocampal slices. J. Physiol. *417*, 197–212.

Mayford, M., Abel, T., and Kandel, E. R. (1995a). Transgenic approaches to cognition. Curr. Opin. Neurobiol. *5*, 141–148.

Mayford, M., Wang, J., Kandel, E. R., and O'Dell, T. J. (1995b). CaMKII regulates the frequency–response function of hippocampal synapses for the production of both LTD and LTP. Cell, this issue.

Müller, R. V., Kubie, J. L., Bostock, E. M., Staube, J. S., and Quirk, G. J. (1991). Spatial firing correlates of neurons in the hippocampal formation of freely moving rats. In Brain and Space, Jacques Paillard, ed. (New York: Oxford University Press), pp. 297–333.

O'Keefe, J. (1993). Hippocampus, theta, and spatial memory. Curr. Opin. Neurobiol. *3*, 917–924.

O'Keefe, J., and Nadel, L. (1978). The Hippocampus as a Cognitive Map (New York: Oxford University Press).

O'Keefe, J., and Recce, M. L. (1993). Phase relationship between hippocampal place units and the EEG theta rhythm. Hippocampus *3*, 317–330.

Olton, D. S. (1978). Characteristics of spatial memory. In Cognitive Processes in Animal Behavior, S. H. Hulse, H. Fowler, and W. K. Honig, eds. (Hillsdale, New Jersey: Erlbaum Associates), pp. 341–373.

Olton, D. S., and Werz, M. A. (1978). Hippocampal function and behavior. Spatial discrimination and response inhibition. Physiol. Behav. *20*, 597–605.

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

Schacter, D. L., and Tulving, E., eds. (1994). Memory Systems (Cambridge, Massachusetts: The MIT Press).

Scoville, W. B., and Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. J. Neurol. Neurosurg. Psychiatry *20*, 11–21.

Sejnowski, T. J. (1977). Storing covariance with nonlinearly interacting neurons. J. Math. Biol. *4*, 303–321.

Shapiro, M. L., and O'Connor, C. (1992). *N*-methyl-D-aspartate receptor antagonist MK-801 and spatial memory representation: working memory is impaired in an unfamiliar environment but not in a familiar environment. Behav. Neurosci. *106*, 604–612.

Silva, A. J., Paylor, R., Wehner, J. M., and Tonegawa, S. (1992a). Impaired spatial learning in α-calcium-calmodulin kinase II mutant mice. Science *257*, 206–211.

Silva, A. J., Stevens, C. F., Tonegawa, S., and Wang, Y. (1992b). Deficient hippocampal long-term potentiation in α-calcium-calmodulin kinase II mutant mice. Science *257*, 201–206.

Smythe, J. W., Colom, L. V., and Bland, B. H. (1992). The extrinsic modulation of hippocampal theta depends on the coactivation of cholinergic and GABA-ergic medial septal inputs. Neurosci. Biobehav. Rev. *16*, 289–308.

Sparks, P. D., and LeDoux, J. E. (1995). Septal lesions potentiate freezing behavior to contextual but not to phasic conditioned stimuli in rats. Behav. Neurosci. *109*, 1–5.

Squire, L. R. (1992). Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychol. Rev. *99*, 195–231.

Staddon, J. E. R. (1983). Adaptive Behavior and Learning (New York: Cambridge University Press), p. 378.

Stevens, C. F., Tonegawa, S., and Wang, Y. (1994). The role of calcium-calmodulin kinase II in three forms of synaptic plasticity. Curr. Biol. *4*, 687–693.

Sutherland, R. J., and Dyck, R. H. (1984). Place navigation by rats in a swimming pool. Can. J. Psychol. *38*, 322–347.

Suzuki, S., Augerinos, G., and Black, A. H. (1980). Stimulus control of spatial behavior on the eight-arm maze in rats. Learn. Motiv. *11*, 1–18.

Tolman, E. C. (1948). Cognitive maps in rats and men. Psychol. Rev. *55*, 189–208.

Watts, J., Stevens, R., and Robinson, C. (1981). Effects of scopolamine on radial maze performance in rats. Physiol. Behav. *26*, 845.

Winson, J. (1978). Loss of hippocampal theta rhythm results in spatial memory deficit in the rat. Science *201*, 160–162.

Yoerg, S. I., and Kamil, A. C. (1982). Response strategies in the radial arm: running around in circles. Anim. Learn. Behav. *10*, 530–534.

Zoladek, L., and Roberts, W. A. (1978). The sensory basis of spatial memory in the rat. Anim. Learn. Behav. *6*, 77–81.

Zola-Morgan, S., Squire, L. R., and Amaral, D. G. (1986). Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. J. Neurosci. *6*, 2950–2967.