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New excitement in cognitive space: Between place cells and spatial memory

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Abstract

Hippocampal principal neurons ('place cells') exhibit location specific firing. Recent work is addressing the link between place cell activity and hippocampal memory function. New tasks that challenge spatial memory allow the results of mnemonic computations to be recorded in individual neurons as well as ensembles, and insight into the cellular mechanisms is beginning to emerge.

Introduction

The exciting discovery of place cells by John O'Keefe and Jonathan Dostrovsky thirty years ago has sparked much research into hippocampal function and emphasized a role for the hippocampus in spatial cognition [1]. Place cells are hippocampal principal neurons that fire when an animal is in a particular location in its environment (the place field; reviewed in [2, 3]). Their firing is controlled primarily by the configuration of distal visual landmarks and frequently follows these cues if they are rotated in concert. In addition to visual information, olfactory cues and vestibular and kinesthetic input exert a significant influence, particularly when visual input is unavailable or less reliable.

However, place cells do not passively mirror the sensory input that the animal receives. For example, place fields are frequently maintained even if significant landmarks are removed [4, 5]. Moreover, when rats visit two virtually identical environments sequentially, these environments may give rise to very different place representations [6,7], and subtle changes in sensory input may cause complete remapping [8-10]. These observations suggest that place cells signal more than the immediate sensory input.

The hippocampus has a pivotal function in spatial memory (reviewed in [11]). Are place cells responsible for some of the underlying computations? During the past few years, attempts have been made to identify activity in place cells and place cell ensembles that can be related to specific memory operations. Although our understanding of the link between place cells and spatial memory is still incomplete and developing, several new experimental approaches look promising. This review will focus on these developments. The possible involvement of hippocampal neurons in non-spatial memory will not be discussed (reviewed in [12]).

Memory in single hippocampal neurons

Several groups have increased the mnemonic demands during recording from the hippocampus, and report neurons that fire apparently in response to signals from short-term or long-term memory (Fig. 1). First, two groups recorded activity of hippocampal pyramidal cells at the choice point of a modified T-maze (Fig. 1A) [13**, 14**]. Rats were trained to alternate left and right turns in the maze. Many cells with place fields on the central stem exhibited differential activity on traverses that led to left turns as compared to those that led to right turns. It is likely that the rat made a decision at or before the T junction, and that this decision was based on memory from the previous lap. This memory might be represented by firing activity of hippocampal neurons.

In a second series of experiments, activity of hippocampal pyramidal cells was recorded in rats trained to find a hidden platform at a constant location in an annular watermaze (Fig. 1B) [15**]. The number of cells with peak activity around the unavailable platform on probe trials was more than twice the number firing in equally large areas elsewhere in the maze. The accumulation of place fields was not seen in rats that were trained with a variable platform location, and the shape and directional modulation of the firing

fields were independent of training conditions [16]. These results suggest that the set of active neurons at a particular location depends on what the animal associates with that place. An animal that slows down over the platform position has probably recognized the goal location and recalled that the place previously contained an escape platform. The hippocampus is necessary for this process [17*]. In addition, there was probably a mismatch between perceived and recalled information. There is evidence that the hippocampus has a role in the detection of prediction errors [18, 19*]. Thus, it is conceivable that some of the neurons with firing fields in the platform area participated in retrieval of memory as well as mismatch detection.

Several earlier studies have failed to find specific activity at the goal location in a radial or circular maze [20, 21]. One potential reason for this apparent discrepancy is that these spatial tasks involve different cognitive operations. The spatial alternation task and the watermaze task have one important feature in common: The animal performs a retrieval or recognition operation at a distinct location in the environment, either at the decision point in the T maze or over the unavailable platform in the annular maze. In both cases, there are cells whose activity correlates with task-specific memory operations. In a radial maze, on the other hand, choices are made in the center of the maze rather than at the ends of the arms where the rewards are delivered, and in a circular maze, performance does not normally require hippocampus-dependent memory. Expectations and problem solving strategies may vary even within the same task, as demonstrated by the fact that the neuronal circuits and mechanisms that are engaged depend on the animal's training history [22-24]. Clearly, to identify mnemonic computations in single hippocampal neurons, and ensembles of neurons, future studies should be aimed at gaining better understanding and control of the sequence and timing of cognitive operations occurring as the animal performs a spatial task.

Plasticity in single hippocampal neurons

How does plasticity in place representation contribute to spatial memory, and what cellular mechanisms are involved? Long-term potentiation (LTP) remains the best studied example of activity-dependent neuronal plasticity and can be induced in the major excitatory synapses of the hippocampal formation by high frequency afferent stimulation (reviewed in [25]). Most treatments that block hippocampal LTP, including NMDA receptor blockade, also disrupt spatial learning (reviewed in [26]). However, NMDA receptor-dependent synaptic plasticity is not necessary for forming place fields, nor for maintaining them between consecutive test sessions in the same environment on the same day. Following interventions with the NMDA receptor [10, 27], Ca^{2+}/calmodulin-dependent protein kinase II [28] or protein kinase A [29], LTP is blocked or impaired, but place fields develop normally and are preserved for at least 1-2 hours. Only longer-term stabilization of the place fields is seriously affected [10, 28, 29].

How can place fields form and be maintained for hours in the absence of LTP, but not for longer

periods? Perhaps LTP is required for long-term consolidaton of place fields. Such LTP might be induced naturally during hippocampal sharp-wave bursts. Sharp waves are population EPSPs associated with coherent discharge of CA3 pyramidal cells during slow-wave sleep [30, 31]. When spontaneously occurring sharp wave bursts were repeatedly paired with strong depolarisation of individual CA1 cells in anaesthetised rats, a long-lasting potentiation of subsequent sharp-wave-associated intracellular depolarisation was seen [32*]. Although we do not know whether similar strong depolarisation occurs during sharp waves in the behaving rat, these results, together with the poor long-term stability of place fields after disruption of LTP, suggest a role for synaptic plasticity in consolidation of spatial maps in the hippocampus. Such a function would be consistent with the reactivation of spike sequences that occurs in a temporally compressed manner during sharp-wave bursts [33, 34, 35*]. Alternatively, or additionally, synaptic changes might be induced during cholinergically induced oscillations, as seen during REM sleep [36, 37]. This latter view, which is consistent with the established importance of REM sleep for memory processes, has now received more direct support from behavioural experiments in which it was observed that long temporal sequences of patterned multineuronal activity in awake animals were reproduced during REM sleep [38**].

Although place fields may be formed without NMDA receptor-dependent plasticity in the hippocampus, there are subtle differences between fields formed in the presence and absence of NMDA receptor function [10, 27]. A predicted consequence of hippocampal LTP is the expansion of place fields in the direction opposite to the direction of movement when the animal is running several times through a place field [39]. There is now experimental support for this prediction: Place fields in CA1 expand and become asymmetric after repeated unidirectional running through the field [40, 41*], and the process leading to asymmetry is NMDA receptor dependent [42**].

There are probably multiple forms of plasticity in the hippocampus during spatial learning. One way towards understanding them better is to study in more detail the neuronal activity that occurs during learning. Two distinct signals can be seen in place cells when an animal is exploring an environment, single spikes and bursts of spikes (complex spikes) [43]. Recent data suggest that single and complex spikes are associated with slightly different behavioural events [44, 45]. Bursts of spikes are often assumed to have functions distinct from those of single spikes, and it was recently shown that postsynaptic bursts, when paired with single presynaptic stimuli, are able to cause LTP in synapses targeted by the presynaptic afferents, suggesting that one of these functions is the induction of synaptic plasticity [46**].

Complex spikes are themselves subject to plasticity. The progressive attenuation of spike amplitudes during complex spike bursts decreases with repeated running through the same place during a test session [47**]. This effect was specific to the training environment and was blocked by an NMDA–receptor antagonist. Calcium-dependent persistent facilitation of spike backpropagation has been reported *in vitro* [48*], and could underlie this newly discovered form of plasticity. The reduction in spike amplitude attenuation was associated with an increase in pyramidal cell-to-interneuron coupling [47**]. Although the

mechanism of this increase in coupling strength is not yet known, interestingly both NMDA receptor-dependent and –independent components of LTP have now been identified in interneurons *in vitro* [49*, 50*]. These findings together suggest that spatial learning may be associated with an intricate inter-relationship between plasticity of complex spikes, interneuronal control of backpropagating action potentiatials and, possibly, synaptic potentiation in pyramidal cells as well as interneurons.

Hippocampal ensemble coding

While the above results suggest that mnemonic operations and plasticity can be studied in individual hippocampal neurons, several observations indicate that a more complete understanding requires the recording from large neuronal ensembles. A classical example of ensemble function in the hippocampus is the all-or-none 'remapping' of place fields that sometimes occurs after a substantial change in a single defining feature of a spatial environment, e.g. when a square environment is made circular but other spatial reference points remain fixed [8-10]. As the original map is accurately reinstated when the original environment is restored, these place cells are likely to be somehow linked together in a functional ensemble. A second piece of evidence for ensemble coding in the hippocampus is the fact that cells with overlapping place fields also tend to fire together during subsequent slow-wave sleep [33, 34, 35*, 51*] and REM sleep [38**]. Not only the pattern of coactivity, but also temporal sequences are replicated. Together, remapping and reactivation suggest that places or episodes may be represented by patterns of activity in distinct neuronal ensembles.

How are ensembles organized within the hippocampus? It has been suggested that place cells are organized as continuous attractor networks where cells with firing fields at the current or nearby locations are mutually excited whereas cells with fields at other locations are inhibited in a distance-dependent manner [52, 53*, 54*]. Distance between place fields of two pyramidal neurons is thought to be encoded by the strength of the connecting synapses. As the density of recurrent synapses depends on distance between neurons [55, 56], these models might suggest a topographical organisation within the hippocampus. It has indeed been reported that ensembles representing distinct spatial and nonspatial features of a delayed non-matching bar pressing task are organised in alternating, interleaved bands along the length of the hippocampus [57]. However, there was no apparent topographical clustering of place cells when firing was recorded during running on an elevated track or in a wheel [58, 59]. In further research, it might be useful to examine functional differences along the transverse axis of CA3, CA1 and subiculum. Unlike the longitudinal axis of the dorsal hippocampus, these regions are segregated into divisions with highly different cortical input [60].

Ensemble activity enables information to be encoded in forms other than which neurons are firing and at what rate. The temporal relations between activity in different neurons could potentially carry additional information. Network activity in the hippocampus displays a characteristic temporal structure during spatial

learning, namely theta oscillations. It has been suggested that the timing of spikes relative to this external oscillation carries significant information. An interesting example is the phenomenon of phase precession. Phase precession refers to the fact that as an animal actively moves through the place field of a cell, the cell fires progressively earlier relative to the on-going theta activity [61, 62]. How much extra information is carried by such a phase code, and, what is the content of the phase-coded information? Using both phase and rate information from an ensemble of place cells, position could be reconstructed more accurately than with the use of rate information alone [63*] (but see [64]). Firing phase could also encode different information. It has been established that firing rate is influenced by the speed of movement of an animal [65, 66]. When running speed and position were dissociated by letting rats run in a wheel, firing rate was found to correlate strongly to the running speed, whereas the phase of firing relative to the on-going theta activity remained constant [67*].

How does phase precession occur? O'Keefe and Recce [61] originally proposed that phase precession could occur as a consequence of the interference between two theta oscillators with slightly different frequencies. This idea has received support from experiments and modeling recently. Evidence from coherence analysis of EEG signals *in vivo* indicates that there are at least two distinct and partially independent hippocampal theta generators in the CA1, mediated via the entorhinal cortex and the intrahippocampal association circuitry, respectively [68*]. Dual recording from soma and dendrites of individual pyramidal cells *in vitro* has confirmed that two phase shifted independent theta oscillators can cause phase precession of spike generation [69*], and a minimal biophysical model of two neurons, a pyramidal cell and a feedback connected interneuron driven by a pacemaker input at theta frequency, can explain theta phase precession independent of the firing rate of the pyramidal neuron [70*].

The above evidence suggests that CA1 pyramidal neurons may be controlled by the temporal interaction between input from layer III of entorhinal cortex and CA3 (Fig. 2). Recent data indicate that place fields can be established in CA1 in the absence of input from CA3. When the dorsal CA3 is disconnected from the dorsal CA1 by a longitudinally oriented knife-cut between these subfields, place field formation in CA1 is intact, whereas encoding and retention of spatial memory is impaired [71**]. This result suggests that the CA3 signal has a function specifically related to memory. One possibility is that this input signals contextual or episodic information (Fig. 2). The CA1 might store associations between contextual or episodic information from CA3 and sensory information from the entorhinal cortex. Ensembles in CA1 may also perform mismatch detection between these sources of information (reviewed in [72]).

Conclusion

Considerable progress has been made in linking behavioral memory with neuronal activity. It has been shown that the activity of individual place cells as well as ensemble activity can change as a consequence of behavioural challenges that involve mnemonic operations. Moreover, replay of sequences of activity

during sleep suggests that information has been stored in the network. Simple network models might capture some of the fundamental operations of hippocampus, but we are only beginning to understand how the different neuronal elements operate together as an ensemble. To approach this question we need new methods that can resolve better individual neurons and their identity and that can identify extracellular signatures of intracellular processes [73**, 74**], and it will be necessary to design behavioural tasks in which momentary cognitive operations can be monitored in more detail.

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Rats were running in a fixed sequence through a Ш- or U-shaped maze. Neurons with activity on inbound trajectories on the central stem of the Ш-maze were of particular interest. Firing in these cells depended on whether the rat came from the left or right arm, suggesting that place cells are influenced by specific events in

the animal's short-term memory. Cells in the deep layers of the entorhinal cortex fired at analogous locations on different arms, suggesting that such neurons may represent regularities across trajectories.

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Rats were trained to alternate left and right turns from the central stem of a modified T-maze during continuous unidirectional running. Most hippocampal cells with firing fields on the stem exhibited differential activity on traverses that led to left turns as compared to those that led to right turns. Because performance required that the animal recalled from which arm it came, these data suggest that firing in hippocampal neurons can be influenced by distinct episodes retrieved from short-term memory.

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Place cells in CA1 were recorded from rats searching for a hidden platform in a hippocampal dependent annular watermaze task. When the platform location was constant, the percentage of cells with peak activity around the hidden platform on a probe trial was more than twice the percentage firing in equally large areas elsewhere in the maze. The effect was dissociable from ongoing behaviour and was not expressed in rats trained with a variable platform location. The results suggest that subsets of CA1 cells exhibit specific activity during memory-guided search at an unmarked goal location.

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Rats were trained in an annular watermaze in which the hidden platform was kept unavailable until the rat had swum at least one full lap in the corridor. Control rats, but not rats with hippocampal lesions, slowed down when they approached the platform area. Because the corridor walls guided all animals to the goal, regardless of their navigational abilities, these results suggest that the hippocampus is necessary not only for navigating towards a place but also for recognizing the location upon arrival.

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To study the relative contribution of phase-coded and rate-coded information, an animal's position on a linear track was reconstructed using spikes recorded simultaneously from 38 hippocampal neurons. Use of both phase and rate information improved the reconstruction accuracy by >43 % as compared with the use of rate information alone. This result strengthens the hypothesis that information in rat hippocampal place cells is encoded also by the phase of theta at which cells fire.

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Tetrode recordings were made while rats were running in a running wheel. Whereas the discharge rate of

units active during wheel running increased with increasing running velocity, the theta-phase relation was independent of running velocity up to firing rates of 11 Hz. This shows that spike rate and timing might encode different information.

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Using partial coherence analysis of extracellular activity recorded from a 16-site silicon probe in CA1 - dentate gyrus, a highly coherent coupling was found between theta signals recorded from the hippocampal fissure and CA1 stratum oriens on one hand, and between CA1 stratum radiatum and dentate molecular layer on the other. This result suggests the presence of two, relatively independent theta generators in the hippocampus, mediated by the entorhinal cortex and the CA3-mossy cell circuitry, respectively.

[69*] Magee JC: Dendritic mechanisms of phase precession in hippocampal CA1 pyramidal neurons. *J Neurophysiol* 2001, **86**:528-532.

Dual whole-cell patch clamp recordings were made from the soma and apical dendrite of CA1 pyramidal neurons in hippocampal slices from adult rat. Out-of-phase 5 Hz oscillatory currents were delivered through the somatic and dendritic electrode. Increasing the amplitude of the dendritic current injection caused somatic action potential initiation to advance in time, suggesting that increasing distal dendritic excitation can result in phase advancement similar to phase precession seen during theta activity in the intact hippocampus.

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A minimal biophysical model of phase precession of place cells in the CA3 region of the hippocampus is presented. Two coupled neurons, a pyramidal cell and an interneuron driven by an external pacemaker at theta frequency, are sufficient to account for phase precession.

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Disconnecting CA3 and CA1 impaired retention of spatial memory in the watermaze but place cell firing in CA1 was preserved. Injections of a fluorescent tracer confirmed that no fibers from the CA3 reached the recording position whereas the direct input from the entorhinal cortex was intact.

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[73**] Harris KD, Henze DA, Csicsvari J, Hirase H, Buzsaki G: **Accuracy of tetrode spike separation as determined by simultaneous intracellular and extracellular measurements.** *J Neurophysiol* 2000, **84**:401-414.

See annotation to [74**].

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By simultaneous intracellular and extracellular measurement it was possible to validate current multiple single-unit recording techniques and determine how much can be revealed about neuronal identity and neuronal processes from extracellular recording. It was shown that the extracellularly recorded spike reveals information about the shape of the intracellular action potential and that the spike width is a useful measure to separate pyramidal cell and interneuron spikes. Behavioural state-dependent changes in spike amplitude and width could be seen, and the dendritic backpropagation of spikes verified. The insights into neuronal operations that can be gained by using such information could have important implications for our view of hippocampal information processing.

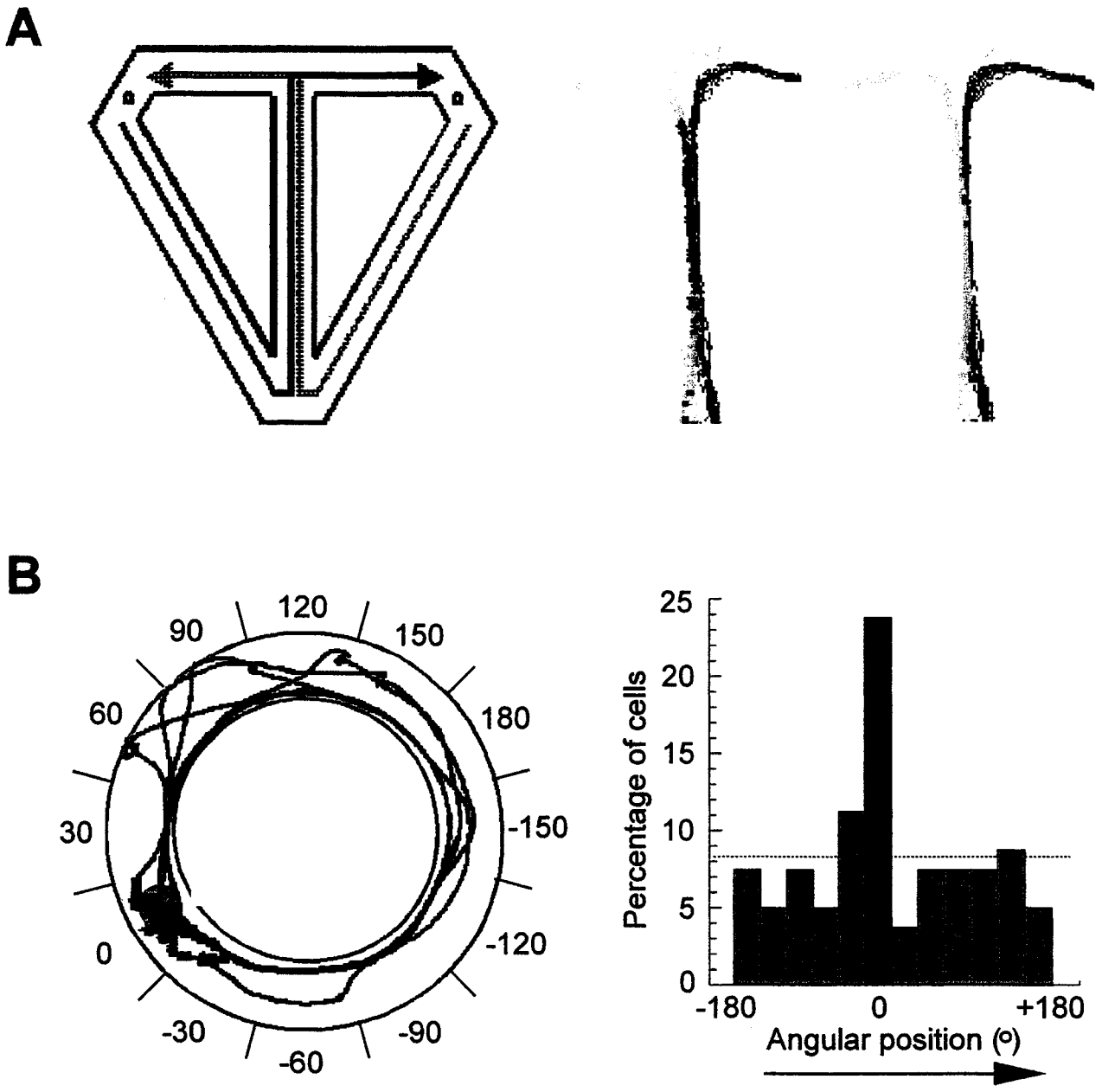
Figure legends

Figure 1. Hippocampal place cell activity in two hippocampus-dependent memory tasks. In both tasks, performance depended on successful retrieval at a certain location in the apparatus. The figure displays the activity of cells with firing fields at these locations. (A) Continuous spatial alternation task. Rats repeatedly traversed the central stem of a modified T maze, alternated between left turns (blue path) and right turns (red path) at the T junction, and returned back to the stem (left panel). Reward for correct alternations was given at the end of each choice arm (small circles). On trials with correct responses, activity in cells with firing fields on the stem depended on whether the rat had previously visited the left (middle panel) or right arm (right panel). (B) Annular watermaze task. Rats were trained to swim in laps through a water corridor, in which a hidden escape platform was made available at a fixed location after the rat had swum one lap or more. Hippocampal activity was measured on probe trials without the platform. Rats slowed down each time they passed the expected platform position, suggesting that they recognized the location and associated it with the platform. Left panel: Cell with firing field at the goal location. Platform location on previous trials is indicated (green). Numbers along the periphery indicate the angular distance from the centre of the platform in degrees. Right panel: The number of cells with activity in the platform segment was significantly larger than the number with firing fields in other equally large segments of the corridor. The swim direction is shown by an arrow. The chance level is indicated by a dashed line. (A) and (B) are modified from [14**] and [15**], respectively.

Figure 2. Model for information processing in area CA1. Pyramidal neurons receive information from at least two distinct sources, layer III of entorhinal cortex (EC, left) and area CA3 of hippocampus (right). These inputs show oscillations during performance of memory tasks (~). The output, encoded as the rate or phase of firing in CA1 pyramidal neurons (vertical arrows), results from the rates and phase relationships of signals from the entorhinal cortex and CA3 [61, 67*, 68*, 69*]. The direct pathway from the entorhinal cortex carries highly processed sensory information about location, whereas the CA3 input might contribute perceived or stored contextual or episodic information [71**]. Thus, neurons in CA1 could associate place (WHERE?) and event (WHAT?) (blue). Alternatively, the CA1 network could detect mismatches between actual sensory information from entorhinal cortex and expectation from memory in CA3 (red) [18, 19*, 72].

Key words:

Rat, hippocampus, learning, memory, spatial, navigation, LTP, plasticity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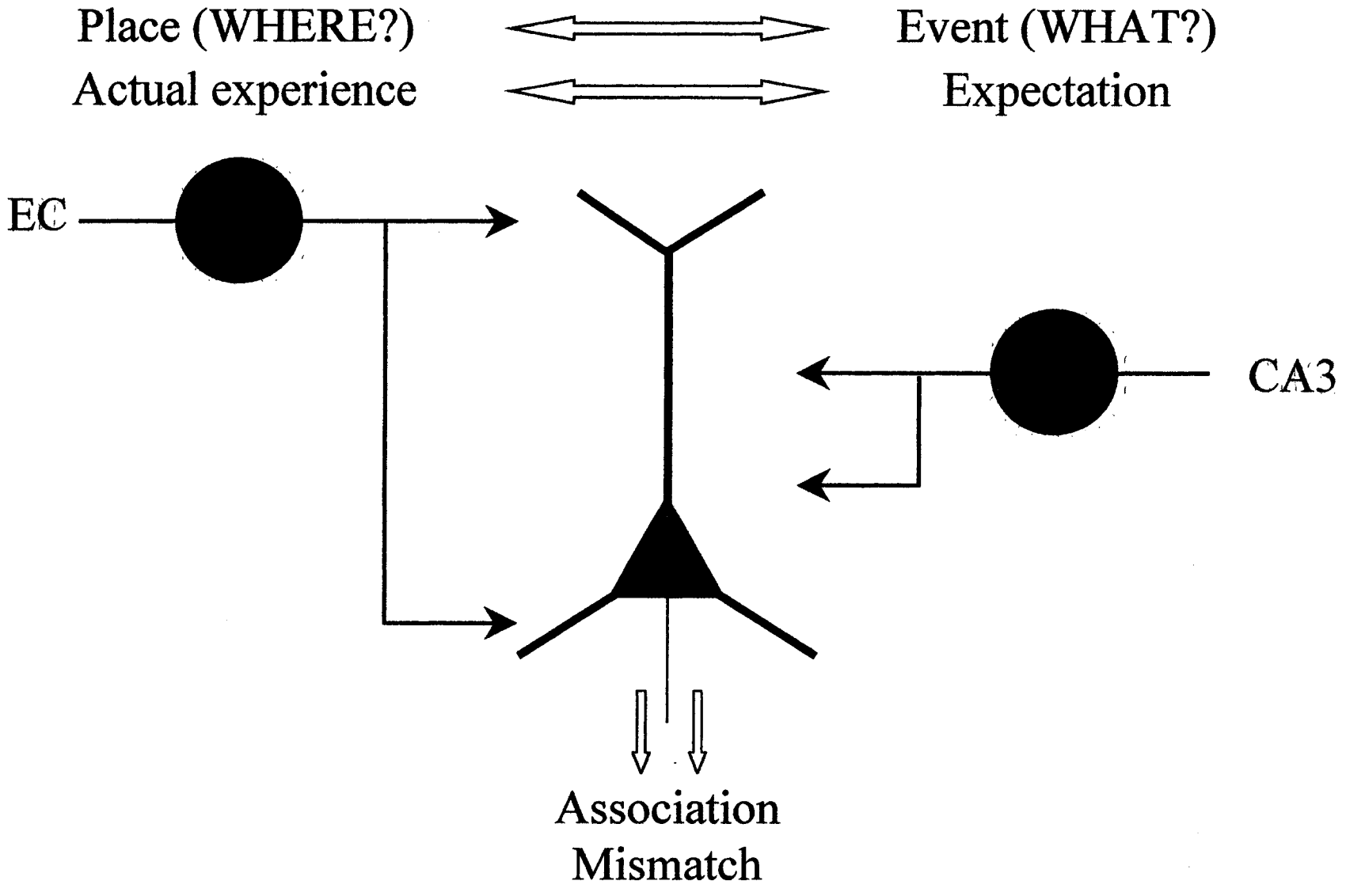
Place (WHERE?)
Actual experience
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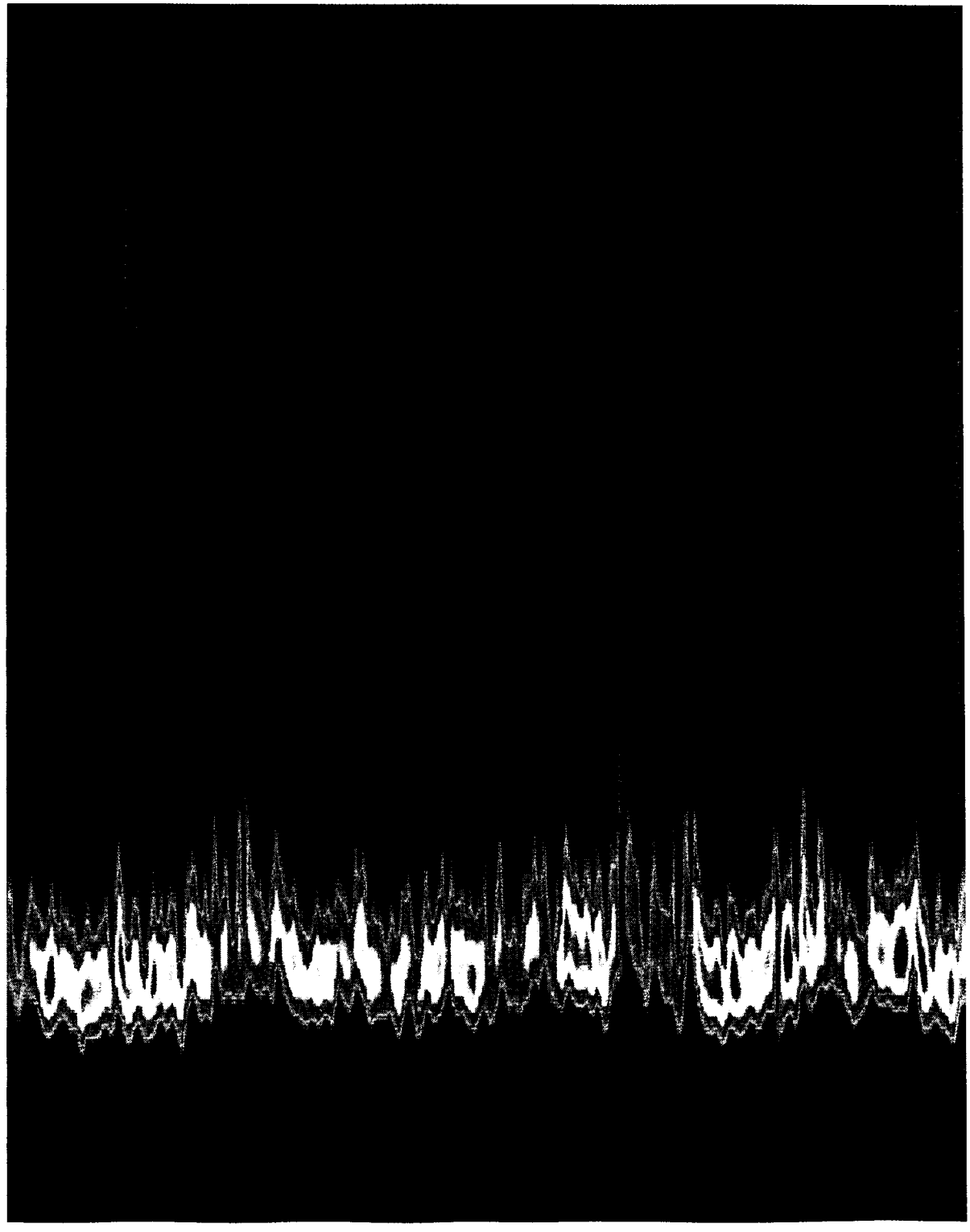

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Accumulation of Hippocampal Place Fields at the Goal Location in an Annular Watermaze Task

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To explore the plastic representation of information in spatially selective hippocampal pyramidal neurons, we made multiple single-unit recordings in rats trained to find a hidden platform at a constant location in a hippocampal-dependent annular watermaze task. Hippocampal pyramidal cells exhibited place-related firing in the watermaze. Place fields tended to accumulate near the platform, even in probe trials without immediate escape. The percentage of cells with peak activity around the hidden platform was more than twice the percentage firing in equally large areas elsewhere in the arena. The effect was independent of the actual position of the platform in the room frame. It was dissociable from ongoing motor behavior and was not related to linear or angular speed, swim direction, or variation in hippocampal theta activity. There was no accumulation of firing in any particular region in rats that were trained with a variable platform location. These training-dependent effects suggest that regions of particular behavioral significance may be over-represented in the hippocampal spatial map, even when these regions are completely unmarked.

Key words: hippocampus; place cells; learning; memory; recognition; spatial; plasticity; rat

The hippocampus appears to be necessary for several types of memory (Squire, 1992; Morris and Frey, 1997; Eichenbaum et al., 1999), but its mnemonic function is particularly clear in tasks for which subjects are required to remember spatial location (O'Keefe and Nadel, 1978). Rats with hippocampal lesions exhibit impairments in both encoding and retrieval of spatial memory (Olton et al., 1978; Morris et al., 1982, 1990). During recall of food locations in a radial maze task, large parts of the hippocampus are activated (Bontempi et al., 1999). A similar activation takes place when humans recall routes in a spatially complex environment (Maguire et al., 1997, 1998).

Despite these advances in our understanding of hippocampal function, the neuronal correlates of memory in the hippocampus remain elusive. It is well established that pyramidal cells have location-specific firing correlates (place fields) and fire in distinct regions of the experimental arena (O'Keefe and Dostrovsky, 1971; Muller et al., 1987; O'Keefe and Speakman, 1987; Wilson and McNaughton, 1993). Such place cells appear to participate in a distributed and nontopographic map-like representation of the spatial environment (O'Keefe and Nadel, 1978), and it is often assumed that they are involved when an animal learns to find its way from one place to another. However, we have yet no clear idea of how hippocampal cells could contribute to goal-directed navigation and goal recognition during spatial learning.

One outstanding issue is whether the distribution of place fields matches the behavioral significance of locations along a remembered trajectory. For example, we do not know whether the goal location is represented differently from off-target areas and whether such differential representation, if it exists, develops in parallel with spatial learning. One way to approach such issues is to record single-unit activity from ensembles of hippocampal neurons while rats solve a spatial memory task such as the watermaze. The watermaze is a homogeneous water-filled pool in which rats are trained to find a concealed escape platform by remembering geometric relationships between distal landmarks (Morris et al., 1982). The watermaze is unique in that there are no salient proximal cues, that the goal location is inconspicuous, and that much of the systems approach to understanding hippocampal function is based on this task. However, studies of place cells in the watermaze would be constrained by the fact that a well-trained animal rarely visits all regions of the arena once it has learned the location of the hidden escape platform. We have circumvented this sampling problem by training rats in an annular version of the task, in which the animals must swim one or several laps through the environment before the platform is made available either at a constant or a variable location inside the corridor. There are still no proximal landmarks and the goal is disguised, but the rat covers the entire arena. We used this new task to examine whether the distribution of hippocampal place fields is influenced by what is learned by the animal.

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MATERIALS AND METHODS

Subjects. Thirteen naive male Long–Evans rats (400–600 gm at implantation and testing) were housed individually in large transparent Plexiglas cages (54 × 44 × 35 cm) with food and water available *ad libitum*. The rats were kept on a 12 hr light/dark schedule and tested in the dark phase.

Electrode implantation. Tetrodes (O'Keefe and Recce, 1993) were made of twisted 17 μm polyimide-coated platinum–iridium (90%–10%) wire. Two or four of these were mounted in a cannula connected to a lightweight microdrive (Axona Ltd., Herts, UK) and were then implanted in the dorsal hippocampus.

The animals were anesthetized with Equithesin (1 ml/250 gm). A trephine bit was used to drill a 2 mm hole in the skull overlying the left dorsal hippocampus, and the dura was removed gently. The microdrive and tetrodes were mounted to the stereotaxic frame and lowered slowly until the electrode tips reached the deep layers of the neocortex (1.7 mm below the dura) at 4.0 mm posterior to bregma and 3.2 mm lateral to the midline. An outer protecting cannula on the microdrive was then lowered down to the dural surface, and gel foam was placed around the cannula to protect the electrodes. Finally, the foot of the microdrive and seven anchoring screws were encased in dental acrylic. One screw served as an electrical ground. The tip of one tetrode was 0.3–0.5 mm above that of the others. The animals were given 1–2 weeks of recovery before cell screening started.

Cell screening. Screening for hippocampal units took place in a dedicated room (2.5 × 4 m). Over the course of 4–8 d, the electrodes were advanced in steps of ≤50 μm until multiple complex-spiking cells of >100–150 μV amplitude were identified in the hippocampus. The animal rested on a pedestal or walked in an open field during these screening trials. The pedestal was a 30 × 30 cm square of stainless steel with a 5 cm vertical edge on each side. The pedestal was filled with sawdust. The open field is described below. Screening for cells and testing in the watermaze were always conducted in separate rooms.

Recording procedure. The animal was connected to the recording equipment (Axona Ltd.) via cables of hearing-aid wire, one for each channel. The cables were counterbalanced by a pulley and weight system. Signals from each electrode were passed through AC-coupled, unity gain operational amplifiers close to the head of the rat and were later amplified 15,000–25,000 times and bandpass-filtered between 600 Hz and 6 kHz. Each channel on the deep tetrodes was recorded differentially with respect to an electrode on the shallow tetrode. Unit spikes were identified when the signal amplitude crossed a preset threshold manually adjusted to approximately three times the noise level of the channel. Waveforms of identified spikes were sampled at 48 kHz (50 points per channel, 10 points before trigger, 40 points after trigger), time-stamped, and stored for off-line analysis. An EEG was recorded single-endedly from one of the shallow electrodes, low-pass filtered at 117 Hz, sampled at 234 Hz, and stored with the unit data. The position of the rat in the apparatus was obtained by a video-tracking system (Axona Ltd.) that extracted the *x--y* coordinates of a small light-emitting diode on the headstage (in the open field) or of the black head of the rat on the white pool surface (in the watermaze). Positions were sampled at 47 Hz at a resolution of 4 mm/pixel. The position data were stored with the unit data.

Open field. The rats were trained to chase sweet rice grains that were scattered individually into a black open field (100 × 100 × 50 cm) at 10–15 sec intervals. The box was made of stainless steel and electrically grounded. A white cue card (30 × 20 cm) was centered on one of its walls. Data from the open field were generally recorded before and after testing in the water task.

Annular watermaze. The experiments took place in a watermaze consisting of a white circular polyvinylchloride tank (198 cm diameter, 50 cm deep) filled to a depth of 40 cm with water at 28 ± 2°C. The pool was located in a room (4 × 7 m) with multiple distant cues on all sides. Swimming in the watermaze was constrained by two circular, transparent Perspex walls of 75 and 95 cm diameter, respectively, placed around the center of the tank. The water was made opaque with latex liquid. A 10 cm diameter escape platform was located within the corridor, either northeast (NE), northwest (NW), southwest (SW), or southeast (SE). The platform could be regulated remotely between an available and an unavailable level (1.5 and 22 cm below the surface, respectively). A wall (2 × 2 m) separated the pool from the experimenter during the trials.

Training in the annular watermaze. Animals were trained to escape onto a hidden platform. The active platform was either at a constant location on all trials (nine rats) or the location varied according to a pseudorandom schedule (four rats). In the former condition, the platform was NE for 15 cells, NW for 15 cells, SW for 24 cells, and SE for 26 cells. The platform was always in the submerged (unavailable) position at the start of the trial and was not raised until the rat had swum at least one full lap in the corridor. Start positions were varied between north, south, east, and west in a pseudorandom order. Maximum trial length was 120 sec, and the animal rested 30 sec on the platform after each trial. All animals were trained before surgery (four trials in the morning and four trials in the evening for ≥5 d). After surgery, training continued until the rat was familiar with the recording equipment.

Only the probe trials were analyzed systematically. The corridor was divided into six 60° segments, with the submerged platform in the center of one of these segments. We measured dwell time as well as swim velocity in each segment.

Unit recording in the annular watermaze. Once complex spikes were identified during the screening trials, the rat received at least 3 hr of rest to allow the stability of the recorded potentials to be checked. If the potentials were stable, the rat was given seven trial pairs at an interblock interval of 30 min in the watermaze. On standard trials, the platform was made available after the rat had swum one full circuit. For every fourth trial, a probe trial occurred in which the platform was kept in the unavailable position for the first 60 sec, regardless of the number of laps that the animal swam. Start positions varied between south, west, north, and east, except for the probe trials, in which the rat was always released 180° off the platform position.

Before each test, the headstage was shielded by Vaseline. The cable was counterbalanced by a pulley and weight system, which allowed the rat to swim freely. On all trials, position and spike data were sampled in parallel. Recording was terminated at 120 sec. The data were stored on the hard disk and analyzed off-line. After swimming, the rat was placed under an infrared heating lamp.

Spike isolation and analysis. A "cluster-cutting" program allowed the spikes to be identified as belonging to individual cells according to voltage and temporal criteria (McNaughton et al., 1983a; O'Keefe and Recce, 1993; Harris et al., 2000). Clustering was performed manually in two-dimensional projections of the cluster space (Fig. 1). Pyramidal cells were identified and distinguished from interneurons by the duration of the extracellular action potential (>0.3 msec), firing pattern (complex spikes), and low average firing rate in the watermaze (Ranck, 1973; Harris et al., 2000; Henze et al., 2000). Spikes within the same cluster were autocorrelated to check the quality of the isolation and to identify complex spikes (Fig. 1*C*), whereas spikes from adjacent pairs of clusters were cross-correlated to make sure that the early and late spikes of a complex-spike burst were not mistakenly assigned to different clusters (Fee et al., 1996; Quirk and Wilson, 1999; Harris et al., 2000).

Average firing rate was expressed as the total number of spikes divided by the total length of the recording period. The peak firing rate was found as follows. First, an array of 48 × 48 bins was placed over the sampling arena. The number of spikes in each bin was normalized by the dwell time of the rat within the space represented by the bin, and the values in this array were smoothed by replacing each value with the average of this value and those of the adjacent eight neighbors (those that had been visited). The peak rate was then taken as the maximum smoothed firing rate in any bin. The size of a place field was estimated by counting the number of adjacent bins with a firing rate of >20% of the peak rate. Bins were also counted as adjacent when only their corners touched. A cluster of bins was considered a "place field" if ≥15 adjacent bins exceeded this threshold.

All spike data reported from the water task are from the first 60 sec of the probe trials, when the platform was unavailable to the rat. The firing rate distribution during these trials was examined by dividing the annular corridor into equally large segments (6 segments of 60° or 12 segments of 30°). We determined the firing rate of each cell in each segment and averaged these firing rates across probe trials. Field location was defined as the segment in which the cell had its maximum averaged firing rate. Unless otherwise specified, the data were sorted with respect to swim direction, and only those trial segments that were sampled in the preferred swim direction were retained.

The electrophysiological data were evaluated with nonparametric statistics (Hollander and Wolfe, 1999) because the firing-rate distributions were asymmetric and deviated significantly from normality. Median values are Hodges–Lehmann estimates. Variation is expressed by Tukey–Wilcoxon confidence intervals.

Spectral analysis. Fourier power spectra were calculated using Thompson's adaptive multitaper method, with NW equaling 4 (Percival and Walden, 1993). Time-frequency analysis (Fig. 1) was performed by wavelet transformation (Morlet continuous wavelet transform, $\omega_0 = 8$). Wavelet power was normalized by the signal variance. The wavelet "scales" were converted to the corresponding "harmonic frequencies" and linearized before plotting (Torrence and Compo, 1998).

Histology. The rats were killed with an overdose of Equithesin and perfused intracardially with saline and 4% formaldehyde. The brains were extracted and stored in formaldehyde, and frozen sections (30 μm) were cut coronally, mounted, and stained with cresyl violet. All sections in the electrode area were retained. The sections were subsequently examined under a light microscope to identify the electrode traces.

Approvals. The experiments were conducted in accordance with na-

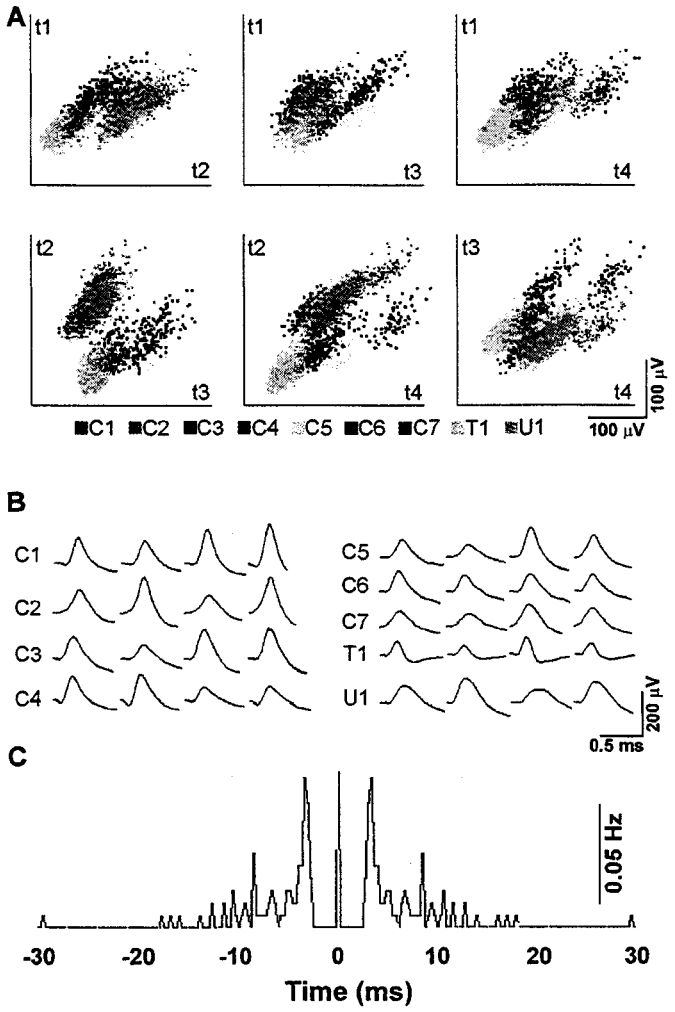

Figure 1. Isolation of single units during recording in water. *A*, Scatter diagrams illustrating the relationship between peak-to-peak amplitudes of spikes recorded simultaneously from electrodes *t1* to *t4* of one tetrode. All electrode combinations are shown. Each *point* represents one sampled signal. Nine clusters were isolated and assigned unique colors. Clusters *C1–C7* were identified as complex spike cells, *T1* was a theta cell, and *U1* remained unclassified. *B*, Average waveforms of the units in *A*. *C*, Autocorrelation analysis of cell *C2* showing frequent interspike intervals of 3–5 msec (complex spikes) and absence of spikes at <2 msec (refractory period). These interspike intervals would be expected if the spikes arose from the same pyramidal cell. *C1* and *C3–C7* had similar profiles and were also classified as pyramidal cells.

tional and European guidelines and approved by the National Animal Research Authority.

RESULTS

Cell sample

Units were isolated by a cluster-cutting procedure (Fig. 1*A,B*). We isolated a total of 139 hippocampal units in 13 rats, all with average firing rates of >0.3 Hz. The rats were tested both in water and in an open field. If a cell fired >0.3 Hz in one task and <0.3 Hz in the other, it was included for analysis only in the former. Place-related firing was not used as a selection criterion. The mean number of isolated units recorded at one time was 7.2.

The units were categorized as pyramidal cells (n = 132) or interneurons (n = 7) depending on spike duration (0.3–0.5 vs <0.3 msec), interspike intervals (Fig. 1*C*), and firing rates. The median peak-to-trough amplitudes (±95% confidence intervals) were 212 ± 6 μV (pyramidal cells) and 131 ± 15 μV (interneurons). The noise level during recording in the watermaze was <30 μV. Movement artifacts were nearly absent during swimming in pretrained animals.

Examination of the electrode traces revealed the electrodes to be positioned in the CA1 layer of the dorsal hippocampus approximately in the middle between CA3 and subiculum in all rats.

Behavior

The rats typically swam four to five circuits in the annular watermaze during the 60 sec of a probe trial. Most animals swam in one direction only. In 12 of 13 rats, the preferred swim direction was the same on all probe trials (usually counterclockwise). One animal swam in the opposite direction on a single trial. All implanted rats swam slower and spent more time in the segment surrounding the platform than in the rest of the corridor on the probe trials (Fig. 2), indicating that they had formed a memory of where the platform used to be positioned. The spatial bias developed gradually during the course of training; there was no preference for the platform region on the first session (Fig. 2*C*).

Constant platform location

All analyses of neuronal activity in the water task were limited to the first 60 sec of the probe trials. The platform was unavailable during this period. Trials in which the platform was raised after the first lap were not included in the present analyses.

Nine of the rats were trained with the platform at a constant position. In these animals, we recorded 80 units in the water task and 47 units in the open field, 23 of which were active in both environments. Seventy units in the water task and 44 units in the open field were spatially selective according to our criteria.

The distribution of average pyramidal cell-firing rates in the water task was asymmetric and skewed toward low values. The estimated median rate on the probe trials was 1.46 Hz, which was slightly, but not significantly, higher than the estimated median rate of cells recorded in the same rats in the open field (Mann–Whitney U test; Z = 1.8; $p > 0.05$) (Table 1). The median peak rate in the water corridor was 9.0 Hz (Table 1). The interneurons fired at an estimated median rate of 12.1 Hz and a peak rate of 25.9 Hz (n = 7).

Most complex-spiking neurons with activity in the corridor (70%) had stable single-peaked place fields (Fig. 3). Twenty percent had more than one firing field in at least one of the probe trials. Place fields were present in all parts of the corridor. On average, the fields covered 18.2% of the total search arena and were thus larger than place fields recorded in the same rats in the open field (Z = 6.7; $p < 0.001$) (Table 1). The interneurons fired all over the search arena, with low peak-to-average firing ratios.

The firing fields of the pyramidal cells were not homogeneously distributed during the probe trials. The number of units with firing fields in the platform segment was larger than in the other regions of the corridor (Fig. 4*A*). Twenty-seven pyramidal cells had firing fields in the platform segment; in the remaining segments, the number ranged from 8 to 14 (expected value, 13.3; χ^2_5 = 18.4; two-sided test; $p < 0.005$). The second largest number (14) was in the segment preceding the platform segment. The number of cells with firing fields in the platform segment was significantly larger than expected by chance (binomial test; Z = 5.4; $p < 0.001$).

Firing rates in linear environments are modulated by the direction of movement of the animal (McNaughton et al., 1983b; Muller et al., 1994). Because the rats faced the nonpreferred direction more frequently in the platform segment (21.7% of the

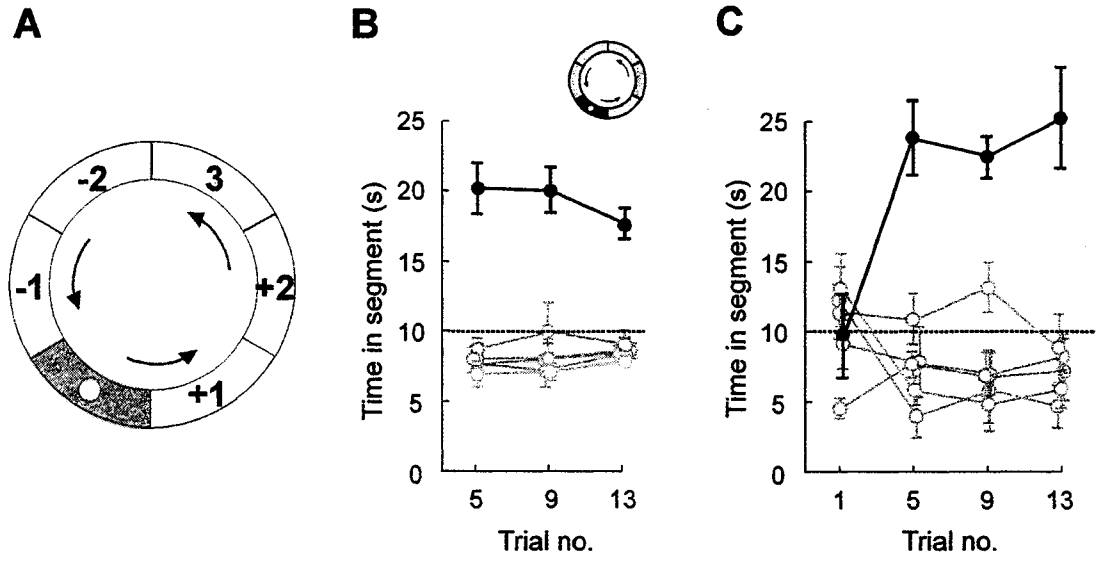

Figure 2. Spatial learning in the annular watermaze task. *A*, Division of the corridor into six equally large segments (60° each), with the platform in the center of *segment 0*. Segments were numbered with respect to preferred swim direction (*arrows*), so that *segment −1* was entered before the platform segment and *segment +1* was entered after the platform segment. *B*, Time in the platform segment (*filled circles*) and each of the remaining segments (*open circles*) on three probe trials (means ± SEM). Unit activity was recorded on all trials. The rats had received >40 trials in the corridor at this stage. *C*, Time distribution on four probe trials in rats that had not been exposed to the environment before. Preference for the platform segment developed gradually. *Symbols* are as described in *B*.

Table 1. Discharge characteristics of pyramidal cells during spatial and nonspatial training (estimated median values and 95% confidence intervals)

	Average firing rate (Hz)	Peak firing rate (Hz)	Relative field size (%)	Absolute field size (m^2)
Rats trained with constant platform				
Water corridor	1.46 (1.23–1.77)	9.0 (7.9–10.3)	18.2 (16.6–20.3)	0.19
Open field	1.16 (0.93–1.44)	13.3 (11.2–15.6)	7.8 (6.8–9.6)	0.08
Rats trained with variable platform				
Water corridor	1.29 (0.97–1.62)	10.7 (8.4–13.8)	15.6 (13.2–17.9)	0.17
Open field	1.06 (0.77–1.66)	12.2 (9.8–15.7)	8.3 (5.5–13.5)	0.08

swim time) than in the other segments (7–17%), more cells could have been detected at the goal location simply as a result of better bidirectional sampling. To control for this possibility, we sorted the data with regard to swim direction and retained only those trial fragments that were sampled in the preferred direction of movement (87.6% of the total data set). The sorting procedure did not attenuate the over-representation of the platform segment. The number of cells with peak activity in the goal segment was now 24 compared with between 9 and 14 in the other segments. Again, the second largest number (14) was in the segment preceding the platform segment (Fig. 4*B*). Statistical analysis showed that the distribution of the unidirectional data remained nonuniform ($\chi^2_5 = 11.7$; two-sided test; $p < 0.05$) and that the number of cells with firing fields in the goal segment was larger than expected by chance (binomial test; $Z = 4.4$; $p < 0.001$). The number of cells with fields in the preceding segment was not significantly larger than in the succeeding segment in this analysis (binomial test; $Z = 0.9$).

The accumulation of firing fields in the platform segment was maintained in a separate analysis of those cells that had single fields according to our criteria (platform segment, 20 cells; nontarget segments, from six to eight cells). The distribution was nonuniform ($\chi^2_5 = 15.1$; two-sided test; $p < 0.01$), with more fields in the platform segment than expected by chance (binomial test; $Z = 3.8$; $p < 0.001$). Cells with no distinct field or with multiple fields were not associated with any particular segment of the corridor (no field, one cell in the platform segment and zero to three cells in the other segments; multiple fields, three cells in the platform segment and one to five cells in the other segments).

To determine how exactly the accumulation of firing fields corresponded to the location of the platform, we doubled the number of segments. Segment boundaries were defined so that the platform position was in the middle of one of the segments. After directional sorting, 19 of the 80 cells exhibited peak activity within the 30° of arc that now defined the platform segment (Fig. 4*C*). The corresponding numbers in the remaining 11 segments ranged from three to nine, with nine in the segment that preceded the platform position and three in the segment that succeeded it (the others ranged from four to seven). The distribution was clearly nonuniform ($\chi^2_{11} = 29.2$; two-sided test; $p < 0.001$), with a sharp increase in the number of cells that had firing fields just where the platform used to be located. The increase in the number of fields in the platform segment was highly significant (binomial test; $Z = 5.0$; $p < 0.001$). In addition, with the increased resolution added by finer segmentation, there was now a larger

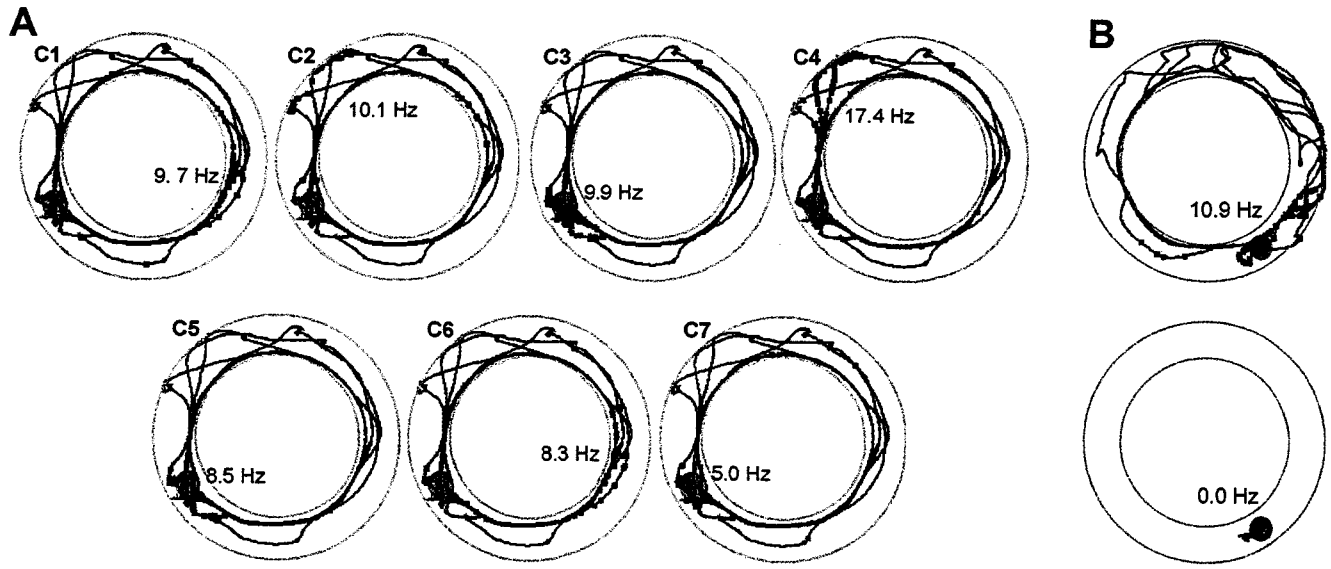

Figure 3. Place fields in the annular watermaze. *A*, Firing correlates of seven pyramidal cells (Fig. 1, *C1–C7*) during the first 60 sec of a probe trial in the annular watermaze. The platform was unavailable to the rat during this period. The rat had been trained with the platform set SW (*green*). Spikes (*red squares*) are superimposed on the swim path (*black*). *Numbers* indicate peak firing rates and location of peak activity. *B*, Firing in the platform area before and after escape on the platform. The cell had a field at the goal location during swimming (0–81 sec) but ceased firing after the rat climbed onto the platform (81–100 sec). *Symbols* are as described in *A*.

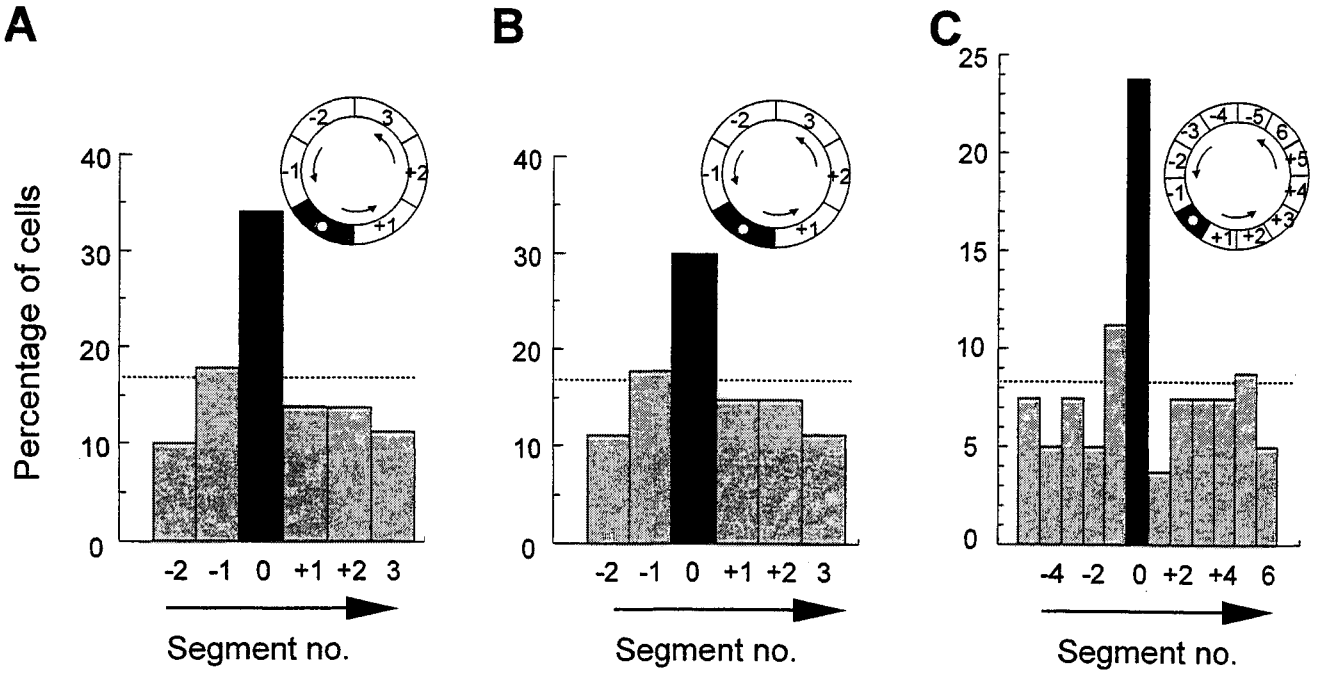

Figure 4. Distribution of firing fields after training with a constant platform location. *A*, Percentage of firing fields in each 60° segment of the corridor (80 cells; average of 3 probe tests). Field location was defined as the segment with the maximal averaged firing rate. Firing fields accumulated in the platform segment (*segment 0, black*). The chance level was at 16.7%. *Inset*, Diagram of the corridor. *Arrows* indicate swim direction. *B*, Percentage of firing fields in each 60° segment after directional sorting (same trials and same *symbols* as described in *A*). Only data sampled during swimming in the preferred direction are retained. *C*, Percentage of firing fields in segments of 30° after directional sorting. The platform was in the middle of *segment 0*.

number of units with fields in the segment preceding the goal than in the segment after it (binomial test; $Z = 2.5$; $p = 0.01$).

The bias was independent of the actual position of the platform in the room. Fifteen of the 80 cells in our sample were recorded with a NE platform, 15 with a NW platform, 24 with a SW platform, and 26 with a SE platform. In all four cases, the largest percentage of place fields was found in the platform segment (30°). The respective percentages were 33.3% (NE), 20.0% (NW), 29.2% (SW), and 15.4% (SE). The chance level was at 8.3%.

Most cells with peak activity at the platform position during the probe trial (12 of 19 units) became less active as the rat entered the platform. In eight units (42%), the firing rate in the goal segment was reduced to <30% of the rate at this location during

swimming. One unit almost stopped firing (the firing rate decreased from 5.6 to 0.07 Hz) (Fig. 3*B*). However, there were also cells that maintained or increased their activity after the rat climbed the platform ($n = 7$).

The size of place fields with peaks within the platform segment was comparable with that of fields in other segments. Place fields in the platform segment covered 18.4% (15.3–22.2%) of the visited area, whereas the fields of the remaining cells covered 18.2% (16.4–20.6%) (estimated medians and 95% confidence intervals, respectively). The background firing of cells with firing fields in the goal segment was not different from that of cells with fields at other places (0.49 vs 0.50% of average rate; background defined as >45° off the center of the segment with peak activity).

Variable platform location

In four rats, the platform was varied according to a pseudorandom schedule. We isolated 28 cells in the water task and 17 in the open field in these animals. Ten of the cells were active in both conditions. Twenty-three of the cells in the water task and 15 cells in the open field satisfied the criteria for spatial selectivity.

Average and peak firing rates during the probe trials were not significantly different from those recorded in the same rats in the open field or those recorded in different rats in the constant platform condition (Table 1). There was no significant effect of training condition on the shape of the firing fields in the water task. The fields were not sharper when the platform location was constant compared with when the location was varied randomly (Fig. 5). The ratios between in-field and background firing rates were 12.9 and 11.1, respectively (estimated median values; field defined as the 30° segment with peak activity; background defined as all areas >45° off the center of the field segment; Mann–Whitney U test; $Z = 1.0$; $p > 0.30$).

Place fields were also present in all regions of the corridor when the platform location was varied. The fields did not cluster in any particular region (Fig. 6). When we defined the segments by room coordinates (relative to the external cues), the number of units with firing fields in each segment ranged from three to six after directional sorting (expected value, 4.7; $\chi^2_5 = 1.88$; $p > 0.50$) (Fig. 6*A*). When the segments were defined relative to the position of the platform on the preceding trial, six cells had firing fields in the previous platform segment, whereas the number of fields in the other segments was between three and six ($\chi^2_5 = 1.88$; $p > 0.50$) (Fig. 6*B*).

Place fields or instantaneous behavior?

Enhanced activity during swimming in the platform area could reflect changes in state or behavior. First, silent cells frequently turn on during large-amplitude irregular activity (LIA) in the hippocampal EEG (Thompson and Best, 1989; Buzsáki et al., 1992). Enhanced firing at the platform location could reflect brief epochs of LIA. LIA is associated with sharp waves of 40–100 msec duration (Buzsáki et al., 1983). These are positive (reversed) in the pyramidal cell layer (Buzsáki et al., 1983) and should appear as increases in power between 10 and 25 Hz. There was no change in this frequency band as the rat swam over the platform on the probe trials (Fig. 7). Theta oscillations were maintained throughout the trial, even when the rat slowed down in the target area. Fourier power spectra showed a sharp peak in the theta frequency band (6–9 Hz) both inside and outside the platform segment (estimated medians, 0.175 and 0.177 mV^2, respectively). The difference was not significant (robust R regression; Jaeckel–Hettmansperger; $F_{(1,15)} = 1.8$; $p = 0.20$).

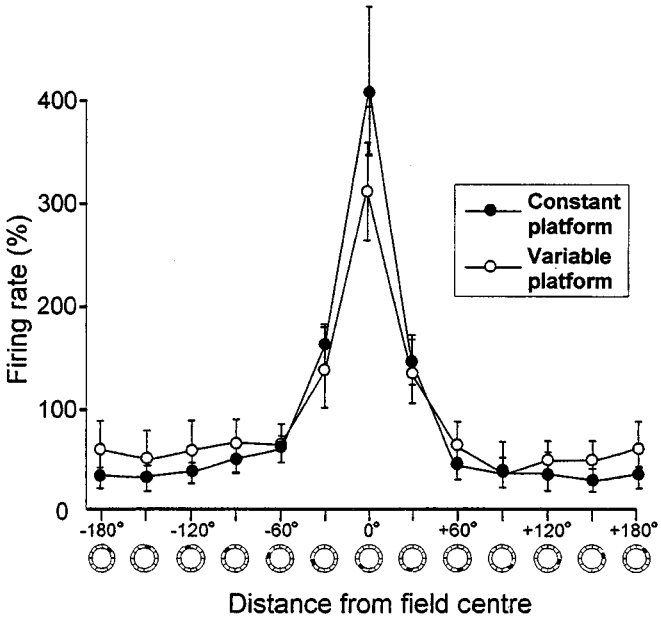

Figure 5. Similar shape of hippocampal place fields after training with a constant or a variable platform location. The diagram shows unidirectional median firing rates (Hodges–Lehmann estimates ± upper and lower 95% Tukey–Wilcoxon confidence intervals) as a function of distance from the segment in which the cell fired at the highest rate (0°). Segments cover 30° of arc each.

Second, the recorded neurons may have been more active in the target region simply because the rats swam more slowly there. However, slow swimming was not accompanied by enhanced firing when it occurred outside the platform segment (Fig. 7*A*, *right*). To quantify the relationship between annular speed and firing rate, we used data from animals trained with a variable platform location and calculated mean firing rate above and below median swim velocity with kernel density estimation (essentially as in McNaughton et al., 1983b). The weighted geometric mean of the ratio (above/below median) was 0.9 (95% bootstrap confidence intervals of 0.2–4.5), indicating no difference in firing rates.

Third, firing rates may have increased in the platform segment because the angular velocity of the rat increased (Wiener et al., 1989). However, firing rates were not elevated when the rat turned outside the platform segment (Fig. 7*A*, *right*). We divided the swim paths into consecutive, staggered segments and calculated how much the length of each segment deviated from the shortest possible line between the start and end points of the segment. Mean firing rates above and below the median were calculated for the variable platform data in the same way as for linear speed. The weighted geometric mean of the ratio (above/below median) was 1.0 (95% bootstrap confidence intervals of 0.2–6.5), indicating that firing rates did not increase as a function of frequent turning per se.

A fourth caveat is that enhanced activity near the platform could reflect more efficient sampling of place fields. Because of deviations from strict clockwise or counterclockwise movement, the area visited was slightly larger in the platform segment than elsewhere (40 vs 32–35 pixels; average values of three probe tests; segments of 60°). We analyzed a subset of the data, using from each rat only the trial with the poorest coverage in the platform segment. This eliminated the differences in coverage (34.4 vs 31–39 pixels) but failed to change the disproportionately large

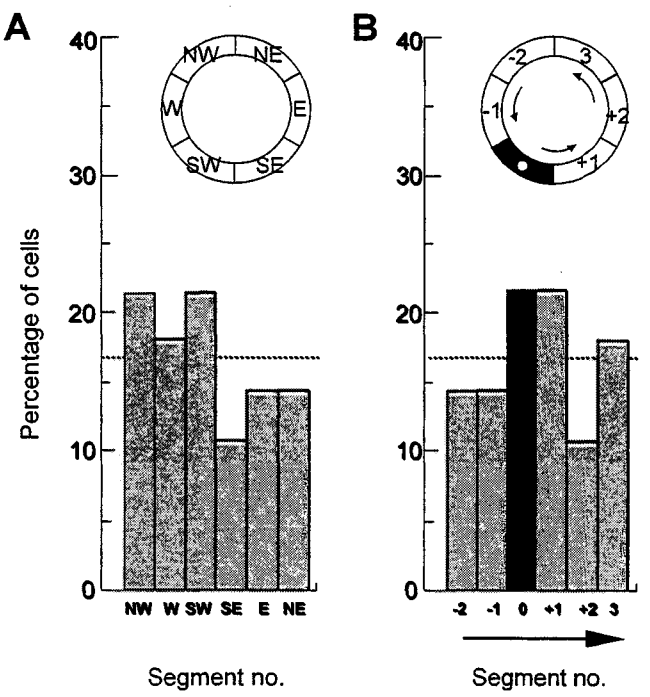

Figure 6. Distribution of firing fields after training with a variable platform location. *A*, Segments (60°) defined relative to external cues (room frame). *B*, Segments defined relative to platform location on the preceding trial (platform in *segment 0*). *Symbols* are as described in *A* and Figure 4.

number of place fields in the platform segment compared with the other segments (23 vs 4–13 units, respectively; χ^2 test, $\chi^2_5 = 14.6$; two-sided test, $p = 0.01$; binomial test, $Z = 3.4$; two-sided test, $p < 0.001$). Similar results were obtained with 30° segments (15 vs 2–8 units; $Z = 3.9$; $p < 0.001$).

DISCUSSION

The main finding is that spatial training in the annular watermaze led to a nonrandom distribution of hippocampal place fields. The area around the platform was over-represented, despite the fact that the escape platform was unavailable. The effect appeared only in animals that expected to find the platform at that location.

Place fields or behavioral modulation?

Although position is the primary determinant of firing in hippocampal pyramidal cells, firing rates are modulated by the instantaneous behavior of the animal. We were concerned that the hippocampal theta activity was interrupted by brief periods of LIA as the animal swam over the platform. During LIA, the out-of-field firing rate may increase and previously silent cells may start to fire (Thompson and Best, 1989; Buzsáki et al., 1992). However, there was no reduction in the power within the theta frequency range, and there was no enhancement at frequencies that are expected to increase during sharp waves. A second concern was the linear and angular speed of the animal, which changed over the platform. There was no relationship between speed and firing rate. Previous research has shown that hippocampal firing rates decrease rather than increase during slow movement (McNaughton et al., 1983b; Wiener et al., 1989; Czurkó et al., 1999). Finally, the disproportionate number of firing fields in the platform area was not an artifact of better coverage of the platform segment, and the effect was not attenuated when the preferred swim direction was analyzed separately. Thus, none of the examined behavioral changes accounted for the clustering of place fields in the platform region.

Representation of the goal area

The increased number of place fields in the platform zone challenges the idea that place fields are evenly distributed. There are several possible reasons why firing fields were more abundant near the target. One relates to familiarity. The rat spent more time in the platform segment and might be more attentive as it passes through this area than at other places, and so more cells may have been recruited to represent this location. However, prolonged exposure does not appear to change the number of cells with place fields in a given environment. Place fields are usually established within the first few minutes that a rat spends in a novel arena (Hill, 1978; Bostock et al., 1991; Wilson and McNaughton, 1993) and change little thereafter (Muller and Kubie, 1987; Thompson and Best, 1990). Because all regions of the swim corridor had been visited at least once during each of >40 pretraining trials, the off-target segments were probably not under-represented because of insufficient exposure.

Firing fields may accumulate at some places because these places contain particularly salient stimuli. In enclosed environments, place fields appear to be more common near edges and walls than in the center (Muller et al., 1987; Hetherington and Shapiro, 1997). Peripheral stimuli exert strong and distance-dependent control over the activity of place cells (O'Keefe and Burgess, 1996), but few place fields are influenced by landmarks inside the experimental arena (Cressant et al., 1997). It is unlikely that such geometrical constraints are responsible for the uneven distribution in the annular water task. The shape of the corridor was homogeneous, and the only salient proximal stimulus (the platform) was absent during the reported trials.

However, firing fields may also reflect the behavioral significance of stimuli at particular locations. Previous research has shown that place fields tend to accumulate at reward locations when these consist of prominent landmarks (Eichenbaum et al., 1987; Breese et al., 1989; Kobayashi et al., 1997), but it remains elusive whether the location-specific firing is controlled by the sensory or the incentive-related properties of the goal object. Firing fields sometimes follow salient stimuli, even when these are not goal objects (Young et al., 1994; Wood et al., 1999), suggesting that, in some cells, the sensory characteristics of the goal may provide sufficient input to control firing in a place-independent manner (Gothard et al., 1996). Attempts to find nonsensory goal-related place activity have so far been unsuccessful (Speakman and O'Keefe, 1990).

The present experiment differs from previous ones in that the goal object was completely unmarked. All analyses were limited to the first minute of probe trials when the platform was unavailable to the rat's senses, implying that the sensory environment was identical for rats trained with the platform at different locations. Despite this fact, the goal location attracted a disproportionate number of firing fields, regardless of its location. The only difference between locations in which fields accumulated and locations in which they did not was whether the animal expected to find the platform there. The fact that place fields were more abundant in the segment preceding the goal than in the succeeding segment further suggests that expectancy may have contributed to the firing in the platform area. This possibility is consistent with data showing that activity in the place field is frequently influenced by where the animal comes from or is going next (Frank et al., 2000; Wood et al., 2000).

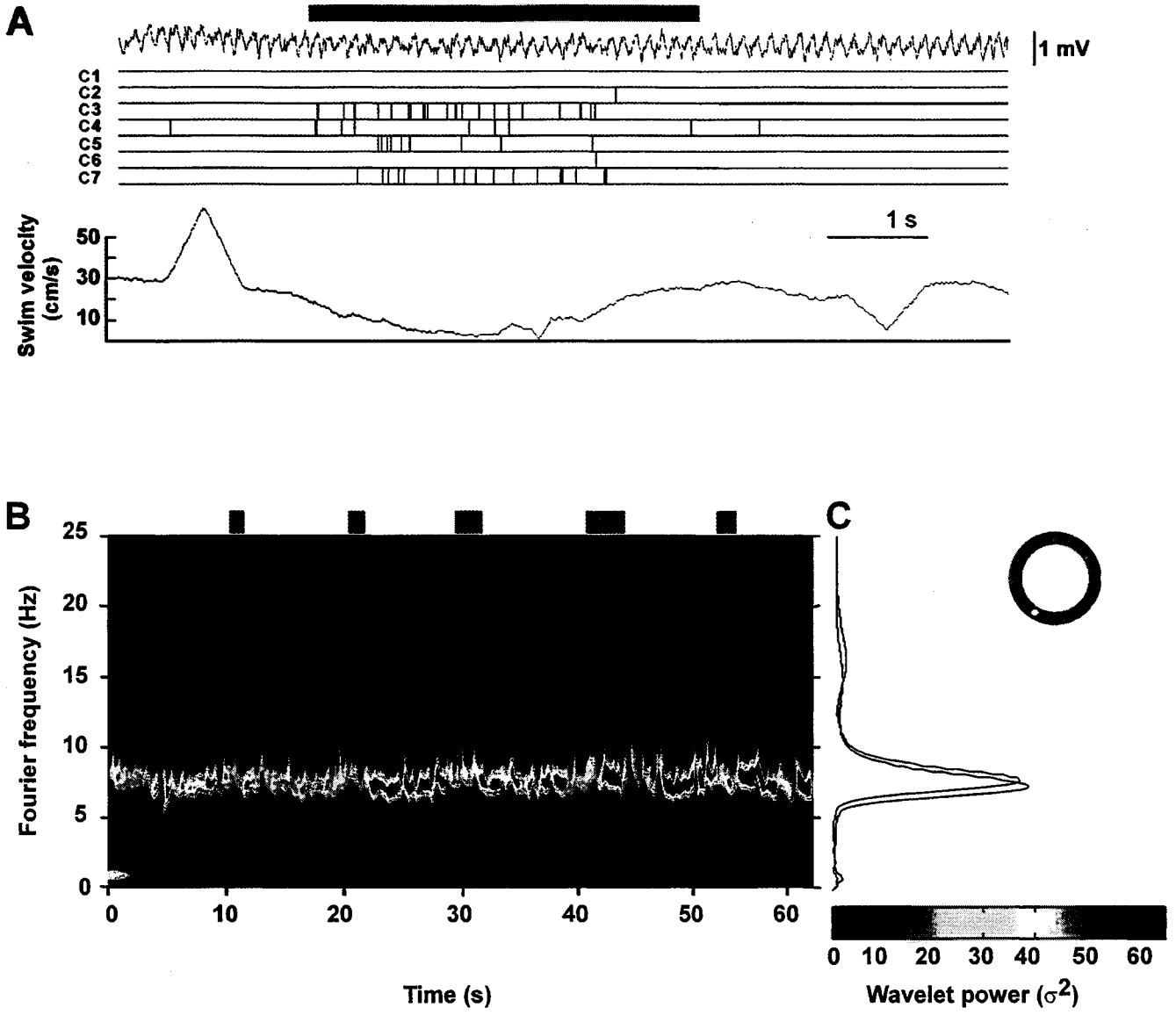

Figure 7. Independence of platform-related firing from instantaneous behavior (same cells as in Figs. 1 and 3). *A*, Hippocampal EEG (*top*), activity of pyramidal cells *C1–C7* (*middle*), and swim speed (*bottom*) during 10 sec of clockwise swimming from SE toward NE. Swimming over the platform (±10 cm) is indicated (*horizontal gray bar*). As the rat passed over the target, there was a significant increase in the activity of several, but not all, recorded units (not *C1*, *C2*, and *C6*). Swim speed decreased, but theta oscillations were maintained. Reduced speed (turning) outside the platform region (*right*) was not accompanied by increased firing. *B*, Multiresolution color-coded spectrogram of hippocampal EEG during the same probe trial. Note the maintenance of theta activity and the absence of other frequencies in the 0–25 Hz range as the animal searched over the platform (*horizontal gray bars*). Low-frequency waves at 0–1 and 59–60 sec reflect electrical noise during release and platform elevation, respectively. *C*, Time-averaged wavelet spectra of EEG sampled inside (*red*) and outside (*blue*) the platform segment. The frequency axis is as described in *B*.

It is possible that some of the cells with firing fields in the platform segment are identical to the "misplace cells" previously reported to respond to the absence of expected objects (Ranck, 1973; O'Keefe, 1976). A few units clearly fired less after the rat had found the platform. The effect was sometimes so strong (Fig. 3*B*) that it cannot only be attributed to changes in behavior or hippocampal EEG. However, there were other cells that maintained a high firing rate after the rat escaped onto the platform, suggesting that the population of cells with firing fields in the target area may be functionally heterogeneous.

The extent to which cells with firing fields at the platform location represent the goal as such can only be established conclusively by moving the platform to a new position and observing whether the firing follows. Preliminary data suggest that some cells with platform-related activity exhibit a partial shift in firing after reversal of the platform position (Moser et al., 1999), but the sample was too small to determine whether the shift was specific for these cells or reflected a more general remapping within the ensemble. The possibility of nonselective remapping, as well as functional heterogeneity among cells with firing fields at the remembered goal location, suggests that multitetrode recording from large ensembles may be necessary to demonstrate goal-related activity specifically and unequivocally.

Experience-dependent plasticity and memory

Previous studies suggest that hippocampal place cells not only respond to immediate sensory information but also express information stored in the animal's memory. First, when a rat is kept in

the recording apparatus, place cells continue to fire after the surrounding landmarks are concealed (Muller and Kubie, 1987; O'Keefe and Speakman, 1987; Quirk et al., 1990). Second, rats develop different hippocampal representations of two visually identical parts of an environment that probably are distinguishable only on the basis of recent memory (Sharp et al., 1990; Skaggs and McNaughton, 1998; Tanila, 1999). Third, some hippocampal place cells (misplace cells) respond primarily during mismatches between what an animal is likely to expect at a place and what it actually experiences there (Ranck, 1973; O'Keefe, 1976). Finally, some hippocampal neurons appear to respond specifically during recall of task-relevant information, such as during the presentation of the conditioned stimulus in a classical conditioning task (Berger et al., 1976) and in the matching phase of a delayed-matching short-term memory task (Wood et al., 1999). Collectively, these studies suggest that hippocampal neurons can express information that is retrieved from memory. The contribution of direct sensory input is often hard to eliminate entirely, however. Removing distal cues does not cancel the contribution of proprioceptive or kinesthetic stimuli, and comparing activity in two versions of the same environment does not guarantee that the animal perceives the environments as identical. In the present study, place fields accumulated at the goal, even when the goal object was completely unavailable to any of the rat's sensory systems. There was probably no other way that the platform could influence firing than through an association between particular landmarks and the remembered platform.

The abundance of place fields in the goal area may have contributed to the maintenance of spatial memory in the annular task. The number of goal-associated place fields was increased only when the animal knew where the platform was located. However, was this plasticity necessary for the performance of the animal? Many tasks used to demonstrate changes in hippocampal firing during recall are hippocampal-independent (Schmaltz and Theios, 1972; Dudchenko et al., 2000). Successful performance in the annular watermaze task does require an intact hippocampus (S. Hollup, K. G. Kjelstrup, J. Hoff, M. B. Moser, and E. I. Moser, unpublished observations). Rats with hippocampal lesions learn to swim in laps but fail to slow down when they pass the platform location on the probe trials, suggesting that they do not recognize the platform region. It will be important in future research to determine whether the observed plasticity in the ensemble representation of the goal location represents an essential link in the chain of events culminating in recognition of this area.

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COMMENTARY

Functional Differentiation in the Hippocampus

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ABSTRACT **The hippocampus is critically involved in certain kinds of memory. During memory formation, it may operate as an integrated unit, or isolated parts may be responsible for different functions. Recent evidence suggests that the hippocampus is functionally differentiated along its dorsoventral (septotemporal) axis. The cortical and subcortical connections of the dorsal and ventral hippocampus are different, with information derived from the sensory cortices entering mainly in the dorsal two-thirds or three-quarters of the dentate gyrus. Rats can acquire a spatial navigation task if small tissue blocks are spared within this region, but equally large blocks at the ventral end are not capable of supporting spatial learning. In primates, the posterior hippocampus (corresponding to the dorsal hippocampus of rodents) appears to be more important than anterior areas for encoding of spatial memory and certain forms of nonspatial memory. The ventral (or anterior) hippocampal formation is to some extent disconnected from the rest of the structure both in terms of intrahippocampal and extrahippocampal connections and may be performing functions that are qualitatively different from, and independent of, those of the dorsal hippocampal formation.** ***Hippocampus 1998;8:608–619.***

KEY WORDS: **spatial learning; memory; dorsal; ventral; septotemporal**

INTRODUCTION

It has been known for a while that the hippocampus and related temporal-lobe areas are important for memory formation. Attention to the mnemonic functions of the temporal lobe was raised by the human case H.M., who suffered severe anterograde and retrograde amnesia following bilateral surgical removal of the medial temporal lobe (Scoville and Milner, 1957). Extensive testing showed that H.M. was impaired on a broad range of memory functions, including recognition of previously presented words or figures, free recall of noun pairs, and memory for the position of objects (Milner, 1965; Milner et al., 1968). Similar findings were later made with patients with damage restricted to the hippocampus (Zola-Morgan et al., 1986; Rempel-Clower et al., 1996), suggesting that the hippocampus is responsible for many of the mnemonic operations of the medial temporal lobe. Functional imaging in normal humans has confirmed this view, demonstrating activation in the hippocampal area during encoding of verbal and visual information (Squire et al., 1992; Nyberg et al., 1996; Stern et al., 1996; Rombouts et al., 1997; Fernandez et al., 1998).

Even though there is converging evidence for hippocampal participation in learning and memory, less is known about the intrinsic operations allowing the hippocampus to lay down memory traces. Does the hippocampal formation operate as a unitary structure, or do different regions perform different functions? Is there more than one type of hippocampus-dependent memory? If so, to what extent are specific mnemonic functions restricted to specific regions of the hippocampal formation? How are cells recruited to mnemonic circuits: are specific neuronal ensembles dedicated to specific functions, or are the networks created dynamically? Questions like these have recently been approached by a variety of techniques. The results suggest that the hippocampal formation may be a functionally heterogeneous structure.

ARE THERE MULTIPLE FORMS OF HIPPOCAMPUS-DEPENDENT MEMORY?

It is still controversial whether the hippocampus participates in a single type of memory or whether several distinct memory circuits are embedded within the structure. If the hippocampus deals with a single form of memory, it is likely to operate as an integrated unit. If there are multiple forms of hippocampus-dependent memory, they may be organized in two fundamentally different ways. They may be subtypes of a single, more general type of memory, with the entire hippocampus being dedicated to this type of memory, or, alternatively, diverse hippocampus-dependent forms of memory may depend on separate intrahippocampal circuits. These circuits may be segregated or overlaid.

The most consistent evidence for hippocampal involvement in memory functions has perhaps been obtained in tasks in which animals or humans use spatial memory

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for navigation (O'Keefe and Nadel, 1978; Nadel, 1991). First, in rats, a significant proportion of the pyramidal cells of the hippocampus have spatial firing correlates (O'Keefe and Dostrovsky, 1971; Muller, 1996). These "place cells" discharge only at certain positions of an experimental arena. Their spatially restricted firing pattern is maintained on memory trials after the controlling spatial cues have been removed (O'Keefe and Conway, 1978; O'Keefe and Speakman, 1987). Second, specific lesions of the hippocampal formation in rodents severely disrupt acquisition of spatial navigation tasks. In radial mazes, rats with hippocampal damage fail to learn which arms are baited with food and which are not, and they re-enter arms from which they have already collected the food (Jarrard, 1978; Olton et al., 1978). Similarly, rats with complete hippocampal lesions are unable to find an invisible, submerged platform in a Morris water maze when only distal cues can be used to locate the platform (Morris et al., 1982, 1990). Third, functional imaging studies of the human brain have shown that recall of routes through a complex but familiar environment activates the hippocampal area of the right human hemisphere in a performance-related manner (Maguire et al., 1997, 1998). The activation was specific to navigation tasks; the right hippocampus was not engaged during simple recall of landmarks (Maguire et al., 1997). Taken together, these findings clearly suggest that the hippocampus plays a critical role during encoding and retrieval of spatial memory.

A more controversial question is whether additional mnemonic functions are implemented in the hippocampus. Research on humans suggests that this may be the case. Selective damage to the hippocampus results in a general impairment of declarative memory formation (Zola-Morgan et al., 1986; Rempel-Clower et al., 1996), with episodic memory appearing to be more severely disrupted than semantic memory (Vargha-Khadem et al., 1997). Deficits are observed in a variety of verbal and visual episodic memory tasks, many of which are probably solved without spatial cognition. Functional imaging studies suggest that the hippocampus is activated in such memory tasks also in normal subjects. Although early studies were inconsistent, possibly because the hippocampus was also active in the baseline condition, new approaches have demonstrated more robust hippocampal activation (Nyberg et al., 1996; Stern et al., 1996; Rombouts et al., 1997; Fernandez et al., 1998).

In animals, it is difficult to design associative learning tasks that in no way rely on spatial strategies. Nevertheless, such tasks probably do exist, and it has been proposed that specific hippocampal lesions disrupt performance in several of them (Rawlins, 1985; Rudy and Sutherland, 1995; Eichenbaum, 1996; Wallenstein et al., 1998). Rats with selective neurotoxic lesions of the hippocampal formation fail to acquire socially transmitted food preferences (Bunsey and Eichenbaum, 1995), and they are not able to learn tasks with food deprivation states as discriminative stimuli (Davidson and Jarrard, 1993). They also fail to perform complex associations like those required for solving transverse-patterning, transitivity and symmetry problems (Alvarado et al., 1995; Bunsey and Eichenbaum, 1996). Together with the work on humans, these results imply that the hippocampus may contribute to more than one form of memory. If it does, it will be important to determine whether a common circuit or different neuronal populations are involved.

SEGREGATION IN THE LONGITUDINAL PLANE

The hippocampus consists of a largely unidirectional transverse loop of excitatory pathways through the dentate gyrus, CA3, CA1, and subiculum. Although this intrinsic pattern of connectivity basically repeats itself along the longitudinal axis of the hippocampus (Andersen et al., 1971), afferent and efferent connectivity changes as one moves from one pole to the other. This suggests that the dorsal (septal) and ventral (temporal) parts of the hippocampus may be responsible for dissimilar and dissociable functions.

The hippocampus is connected both to the retrohippocampal cortices and to various subcortical forebrain structures. The major cortical connections are channeled through the entorhinal cortex, which in terms of connectivity can be divided into three parallel zones cutting across cytoarchitectonally defined borders between subfields of the entorhinal area (Witter et al., 1989b). These band-like zones are only sparsely interconnected in the rat (Dolorfo and Amaral, 1998b), suggesting that they represent separate functional units (Fig. 1A). The three zones project in a topographic manner to distinct and partly nonoverlapping regions along the longitudinal axis of the dentate gyrus (Ruth et al., 1982, 1988; Dolorfo and Amaral, 1998a). The caudolateral zone projects to the septal half of the dentate gyrus, the intermediate zone innervates the adjacent quarter, and the rostromedial zone sends fibers to the temporal quarter of the dentate gyrus (Dolorfo and Amaral, 1998a) (Fig. 1B). Interestingly, each band receives, partly via the perirhinal and postrhinal cortices, its specific set of cortical and subcortical inputs (Deacon et al., 1983; Naber et al., 1997; Burwell and Amaral, 1998a, 1998b). Information originating in the sensory cortices is generally channeled to the lateral and intermediate bands in the rat (Witter et al., 1989b; Burwell and Amaral, 1998a); only olfactory information seems to be distributed to all three zones (Kosel et al., 1981). A similar pattern of organization exists in cats and monkeys (Witter and Groenewegen, 1984; Insausti et al., 1987a; Witter et al., 1989a).

The afferent fractionation of the longitudinal axis is preserved on the efferent side. Axons originating along a dorsal-to-ventral axis in the CA1 and the subiculum terminate along a lateral-to-medial axis in the entorhinal cortex (Köhler, 1985; van Groen et al., 1986; van Groen and Wyss, 1990). From the entorhinal cortex, projections reach the perirhinal cortex, which has extensive projections back to the cortical areas from which the afferent information originated (Insausti et al., 1997).

Also, the rostral subcortical connections of the hippocampus are topographically organized along the septotemporal axis. For example, dorsal, intermediate, and ventral regions of the hippocampus project to cytoarchitectonically different sectors of the lateral septum (Swanson and Cowan, 1977; van Groen and Wyss, 1990;

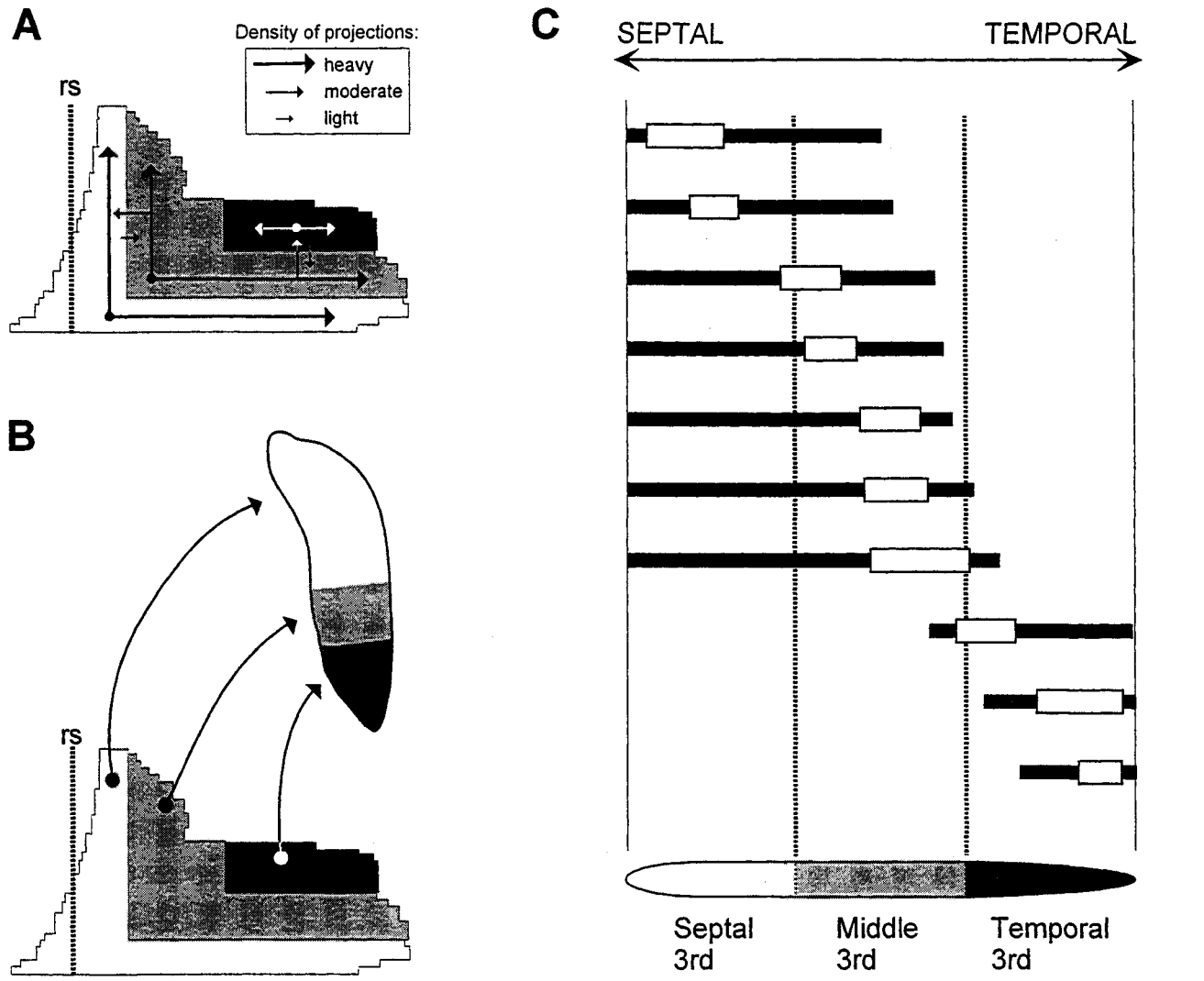

FIGURE 1. Segregation of extrinsic and intrinsic connections in subdivisions of the septotemporal axis of the hippocampal formation, and in associated subdivisions of the entorhinal cortex. (A) Intrinsic connections of the caudolateral (white), intermediate (gray), and rostromedial (black) zones of the entorhinal cortex. Each band has substantial associational connections (large arrows) within the zone of origin. Projections between bands are sparse (small arrows); rs = rhinal sulcus. (B) Projections from these three zones of the entorhinal cortex to the dentate gyrus. The caudolateral (white) zone projects to the septal (dorsal) half of the dentate gyrus, the intermediate zone (gray) to the third quarter, and the rostromedial zone (black) to the temporal (ventral) quarter of the structure. (C) Distribution of associational (ipsilateral) fibers from the dentate hilus along the longitudinal axis of the hippocampal formation (left to right), as determined by autoradiography. White boxes indicate ^{3}H-proline injection sites (in the hilus); black horizontal bars show the longitudinal distribution of label ipsilaterally. Although the cells of origin have widely projecting fibers, axons tend not to cross a border between the septal two-thirds and the ventral one-third of the hippocampal formation. A similar segregation of associational projections exists in CA3 (Swanson et al., 1978). A and B are taken from Dolorfo and Amaral (1998b); C is modified from Fricke and Cowan (1978). (Reproduced with permission of Wiley-Liss Inc., a subsidiary of John Wiley & Sons, Inc. Copyright 1978 and 1998 John Wiley & Sons.)

Risold and Swanson, 1996, 1997). Each of these sectors in turn innervates specific sets of nuclei in the hypothalamic region (Risold and Swanson, 1996, 1997). The connections between the amygdala and the entorhinal cortex are also topographically organized (Krettek and Price, 1974, 1977; Room and Groenewegen, 1986; Insausti et al., 1987b; Witter et al., 1989b; Swanson and Petrovitch, 1998). Nuclei receiving sensory input from the thalamus and cortex, such as the lateral amygdaloid nucleus, appear to be interconnected with the perirhinal cortex and the lateral bands of the entorhinal cortex. Nuclei more closely associated with hypothalamic and olfactory areas (cortical, medial, and central amygdaloid nuclei) have stronger relations with the ventromedial entorhinal cortex. There is also a direct projection from the ventral part of area CA1 to the amygdala (van Groen and Wyss, 1990). As for the subiculum, the projection to the mammilary complex originates in the dorsal two-thirds, whereas the connections with the rostral hypothalamus, the amygdala, and the nucleus accumbens are limited to the ventral part (Krettek and

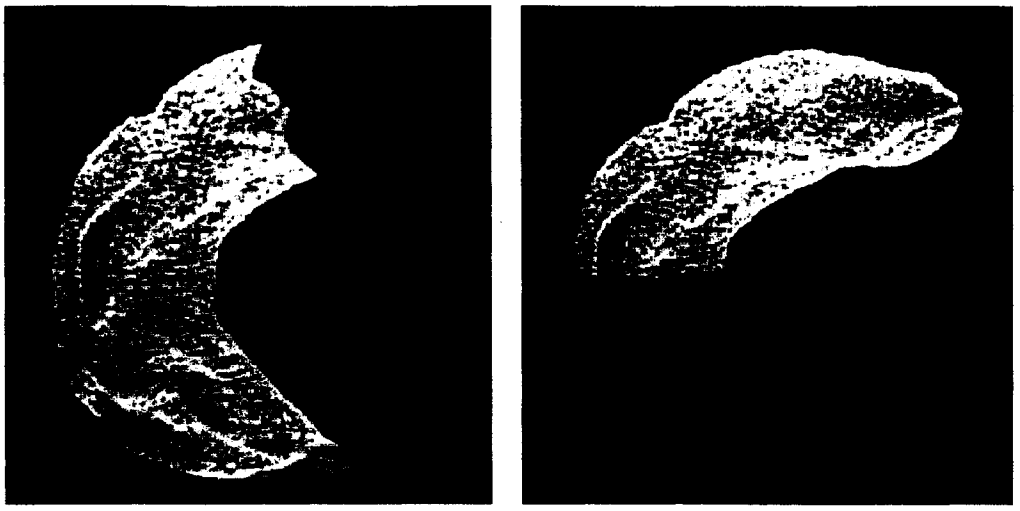

FIGURE 2. Three-dimensional reconstruction of residual hippocampal tissue in two rats with typical excitotoxic partial hippocampal lesions. The lesions spared either the dorsal or the ventral hippocampus (blue and green, respectively). Comparable volumes of tissue were spared (29.5% and 31.2%, respectively). The extreme ventral tip of the hippocampus (blue) was often spared in animals with dorsal tissue blocks, but intrinsic circuitry was not complete in such small "islands." Each remnant is superimposed on an intact hippocampus (grey). Only the right hippocampus is shown. Only the animal with the dorsal remnant showed successful acquisition in a water maze task.

Price, 1977; Swanson and Cowan, 1977; Canteras and Swanson, 1992).

Taken together, these data suggest that dorsal and ventral segments of the longitudinal axis of the hippocampal formation differ in terms of input and output. In addition, there are differences in the neuronal constitution of the networks within the dorsal and the ventral hippocampus. In rodents, the number of calretinin-immunoreactive mossy cells is larger in the ventral part of the structure (Blasco-Ibáñez and Freund, 1997), and the density of dopaminergic, noradrenergic, and serotonergic terminals is also higher (Gage and Thompson, 1980; Verney et al., 1985). Field potentials in ventral CA1, but not in dorsal CA1, commonly display multiple population spikes (Gilbert et al., 1985), and the threshold for epileptiform afterdischarges is lower in ventral than dorsal parts of the CA1 (Racine et al., 1977). Thus, neuronal networks within the dorsal and ventral hippocampus appear to differ in several respects, suggesting that they may not necessarily use the same computational algorithms to process the incoming information.

This pattern of extrinsic and intrinsic connectivity suggests that the hippocampus may consist of two or more rather independent modules. Behavioral experiments are needed to determine whether each putative module is responsible for a specific form of memory.

THE DORSAL HIPPOCAMPUS IS REQUIRED FOR SPATIAL LEARNING

If different kinds of information are processed at different longitudinal levels of the hippocampal formation, it should be possible to identify such circuits by making lesions restricted to specific levels of the longitudinal axis (Fig. 2). Through the topographically organized connections with the entorhinal cortex, information derived from the visual, auditory, and somatosensory cortices is channeled to the dorsal, but not to the ventral, parts of the hippocampus. If this separation is maintained within the hippocampus, one might expect severe deficits in hippocampus-dependent and visually guided spatial navigation in rats following dorsal hippocampal lesions, but not necessarily following equally extensive ventral lesions.

Early work suggested that behavior was indeed affected differentially by dorsal and ventral lesions of the rat hippocampus (Hughes, 1965; Nadel, 1968; Stevens and Cowey, 1973; Sinnamon et al., 1978). Perhaps the most consistent finding was that maze learning was disrupted by dorsal, but not ventral, damage (Hughes, 1965; Sinnamon et al., 1978). Later work with more selective and more comparable dorsal and ventral lesions has confirmed that the dorsal hippocampus has a special role in spatial learning in the rat. Dorsal hippocampal lesions, but not equally large ventral lesions, severely impair memory formation in a water maze (Fig. 3) (Moser et al., 1993, 1995). The impairment was proportional to the damage to the dorsal hippocampus. It has also been reported that spatial learning is inversely correlated to loss of dorsal CA1 neurons following transient forebrain ischemia (Volpe et al., 1992; Olsen et al., 1994). In each case, the threshold volume of damage required to produce a deficit was fairly high. Spatial learning was possible with tissue remnants as small as 20–30%, provided that these were located within the dorsal hippocampus (Moser et al., 1995).

The conclusions from studies comparing effects of dorsal and ventral hippocampal lesions are valid only if there was no damage in the ventral hippocampus in the groups with lesions aimed at the dorsal part of the structure. Although a fiber-sparing toxin was

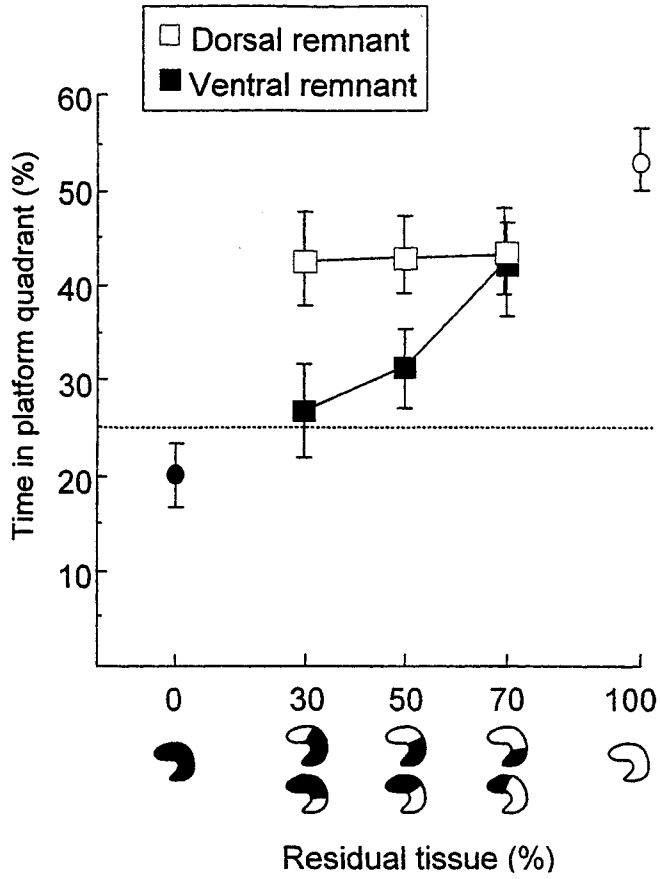

FIGURE 3. Postoperative learning in rats with blocks of spared tissue in either the dorsal or the ventral hippocampus. The rats were trained in a Morris water maze. On the last day, the platform was removed and retention was measured. Time spent in the platform quadrant is expressed as a function of residual hippocampal tissue in animals with dorsal or ventral hippocampal lesions. Chance level is 25% (stippled line). (Reproduced with permission from Moser MB, Moser EI, Forrest E, Andersen P, and Morris RGM. [1995] Spatial learning with a minislab in the dorsal hippocampus. *Proceedings of the National Academy of Sciences USA*, 92:9697–9701. Copyright 1995 National Academy of Sciences, U.S.A.)

used to make the lesions (Moser et al., 1995), fibers of extrahippocampal origin, projecting within or adjacent to the dorsal hippocampus on their way to or from the ventral hippocampus, could have been disrupted mechanically. However, several factors suggest that the ventral hippocampus was functional after dorsal hippocampal lesions. Field potentials with population-spike amplitudes within the normal range could still be recorded from perforant-path synapses of the ventral half of the dentate gyrus in rats with dorsal ibotenic-acid lesions (Moser et al., 1995). Moreover, as in the dorsal hippocampus (Alvarez-Leefmans and Gardner-Medwin, 1975; Fantie and Goddard, 1982), the spike amplitude in the intermediate-to-ventral part of the hippocampus could be modulated by stimulation in the region of the medial septum 5 ms prior to the perforant-path stimulus (E.I. Moser and R.G.M. Morris, unpublished observation), suggesting that modulatory input from the septal region was still present. In addition, acetylcholine esterase staining in the ventral remnant of a dorsally lesioned hippocampus was not strikingly different from that of an intact ventral hippocampus. Finally, rats with tissue remnants including the intermediate and ventral part of the septotemporal axis of the hippocampus, but not the dorsal part, are able to learn a water maze task (Moser et al., 1995). These rats have the septal pole of the hippocampus removed and should have nearly the same proportion of descending fibers transected as those with a lesion on the entire dorsal hippocampus. Altogether, these observations suggest that dorsal fiber damage may not be a sufficient explanation of the poorer performance in rats with dorsal tissue blocks than in rats with ventral blocks.

Whereas rats with excitotoxic lesions restricted to the ventral hippocampus were indistinguishable from sham-operated control rats (Moser et al., 1993, 1995), it has been reported that spatial learning is disrupted by unilateral or bilateral lidocaine injection into the intermediate-to-ventral portion of the hippocampus. This effect appears both in a radial maze and in an exploration task (Thinus-Blanc et al., 1991; Poucet and Buhot, 1994). However, in both cases the injection tracks were probably close to perforant-path fibers projecting along the hippocampus in the angular bundle before they turn into the dentate gyrus and the hippocampus at more dorsal levels (Lømo, 1971; Tamamaki and Nojyo, 1993). Thus, the disruption of spatial navigation in the lidocaine study may, in fact, reflect inactivation of the cortical input to the dorsal hippocampus.

If spatial navigation primarily relies on neuronal processing in the dorsal part of the hippocampal formation, the difference between septotemporal regions of the hippocampus might be reflected in the firing correlates of dorsal and ventral principal cells. Neurons with spatially restricted discharge patterns (place cells) have been reported to exist not only in the dorsal hippocampus, but also in the ventral part of the structure (Jung et al., 1994; Poucet et al., 1994). However, the proportion of cells with spatial correlates is lower in the ventral hippocampus, and place fields are generally wider and less selective there than in the dorsal region (Jung et al., 1994). This suggests that different kinds of analyses may be performed in the dorsal and the ventral portions of the rat hippocampus.

Recent work suggests that hippocampal information processing may be polarized in other species as well. In primates, the orientation of the hippocampus is different from that of rodents, with the posterior hippocampus corresponding to the dorsal hippocampus of the rat and the anterior hippocampus corresponding to the ventral hippocampus of the rat (Amaral, 1987). Monkeys trained in a spatial delayed matching-to-sample task have a higher proportion of neurons with activity in the delay period in the posterior than in the anterior part of the hippocampus (Colombo et al., 1998). There was no regional difference in delay-related activity in a nonspatial version of the task.

The role of the dorsal or posterior hippocampus, at least in primates, may extend beyond spatial learning. Functional imaging of brain activity during successful encoding of word lists has demonstrated significantly more activation of the posterior hippocampus than of the anterior part of the structure (Fernandez et al., 1998). Signal intensity in the posterior hippocampus correlated with the number of successfully encoded words. A similar

polarization of hippocampal activation has been reported during encoding of visual associations (Rombouts et al., 1997) and novel pictures (Stern et al., 1996). Hippocampal degeneration covering the entire rostrocaudal extent of the structure causes anterograde amnesia (Zola-Morgan et al., 1986; Rempel-Clower et al., 1996), but this is not seen following moderate shrinkage in the anterior region of the hippocampus (Sullivan et al., 1995). Taken together, these studies suggest that the hippocampus may be functionally polarized in humans also, with the posterior region being responsible for several nonspatial memory functions as well.

DORSAL AND VENTRAL HIPPOCAMPUS: QUALITATIVELY OR QUANTITATIVELY DIFFERENT?

We do not know whether the differentiation of the hippocampus along the longitudinal axis is graded or discontinuous. On one hand, the hippocampus may work as a single and unitary structure. Both dorsal and ventral subdivisions may be involved in spatial memory formation in rats, but neurons in the dorsal hippocampus may be more effective. Alternatively, the dorsal and ventral parts of the hippocampus are separate, nonoverlapping units processing qualitatively different kinds of information.

The unitary view is consistent with the observation of place cells in both the dorsal and the ventral hippocampus (Jung et al., 1994; Poucet et al., 1994). Although the ventral place cells are proportionally fewer and have less selective firing fields (Jung et al., 1994), their mere existence suggests that the network for spatial navigation may be distributed over most or all of the hippocampal formation. However, this conclusion may be premature. Many of the place cells reported from the ventral half of the structure were probably recorded in the splenial portion of the ventral hippocampus (Jung et al., 1994; their Fig. 2). If the network involved in spatial learning covers more than 50% of the hippocampus (as measured from the septal pole), some of the "ventral" place cells may in fact have been located within this dorsal network. Thus, it will be crucial to map the firing correlates of such cells to their exact splenial-temporal position in the ventral hippocampus.

Observations following partial hippocampal lesions are consistent with a discontinuous organization. If spatial memory processing was continuously graded along the longitudinal axis of the hippocampus, rats with a hippocampal remnant at the intermediate (splenial) level of the longitudinal axis should display a moderate impairment. Their performance should be superior to that of rats with a temporal remnant, but inferior to that of animals with an equally large remnant at the septal pole. This seems not to be the case. Rats with only 30% of hippocampal tissue spared at the intermediate level learn the water maze task just as well as animals with 30% spared at the septal pole (Moser et al., 1997). In contrast, the performance of rats with only 30% intact at the temporal pole was no better than that of animals with all hippocampal tissue removed. This suggests that the circuit required for spatial learning is not graded. It seems to be distributed throughout the dorsal two-thirds of the hippocampus, with septal and splenial parts being equally important.

The discontinuous view is also supported by the pattern of connectivity along the longitudinal axis. First, both the cortical and the subcortical connections of the hippocampus appear to be segregated into several largely nonoverlapping populations (Witter et al., 1989b; Dolorfo and Amaral, 1998a, 1998b) (Fig. 1A,B). There is one projection from the caudolateral entorhinal cortex to the dorsal 50% of the hippocampus, one from the intermediate entorhinal zone to the adjacent 25% of the longitudinal axis of the hippocampus, and one from the rostromedial zone of the entorhinal cortex to the temporal 25% of the hippocampus (Dolorfo and Amaral, 1998a). Input from the sensory association cortices, channeled through the perirhinal and postrhinal areas, is restricted to the two former dorsal populations (Witter et al., 1989b; Burwell and Amaral, 1998a, 1998b). Thus, visual and somatosensory information required for navigation in a water maze may reach as much as 75% of the longitudinal axis, which is only slightly larger than the behavioral estimate of the size of the dorsal spatial learning substrate (Moser and Moser, 1998).

The subcortical projections of the hippocampal formation, particularly those to the lateral septum and the hypothalamus, divide the longitudinal axis into a similar set of discontinuous populations (Risold and Swanson, 1996, 1997). The dorsal half of the hippocampus connects with the mamillary complex, an intermediate region influences various hypothalamic medial zone nuclei, and the temporal quarter is related to the periventricular zone of the hypothalamus. These hypothalamic target regions are associated with distinct classes of innate behavior (Risold and Swanson, 1997; Risold et al., 1997) and may be influenced differentially by lesions at septal, intermediate, and temporal levels of the hippocampus. Functional differences between these areas may not necessarily be expressed in the water maze, the only task that has been related to specific longitudinal subdivisions of the hippocampus so far.

Finally, if the hippocampus operated as a unitary structure, one would expect incoming information to be integrated equally across various longitudinal domains of the hippocampus. The hippocampal formation has two major longitudinal associational fiber systems which could provide such integration, the longitudinal axon collaterals of the CA3 pyramidal cells and the longitudinally oriented axons of the mossy cells of the dentate hilus (Amaral and Witter, 1989). Each of these two systems consists of two roughly nonoverlapping clusters. Their axons diverge extensively within the dorsal two-thirds and within the ventral one-third of the hippocampus; however, few fibers cross between these subdivisions (Fricke and Cowan, 1978; Swanson et al., 1978; Ishizuka et al., 1990; Li et al., 1994). This suggests that cortical information entering the dorsal two-thirds and the ventral one-third remains segregated within the hippocampus.

It remains to be determined how many longitudinal subdivisions there are within the hippocampus. Both cortical and subcortical connections seem to segregate into three longitudinal subdivisions: one large dorsal part, one smaller intermediate region, and the remaining ventral pole. On the other hand, there

are extensive intrahippocampal connections between the dorsal and intermediate regions. Both of these regions receive input derived from the sensory association cortices, and both are part of the spatial learning substrate of the hippocampus.

THE SIZE OF THE DORSAL HIPPOCAMPAL MEMORY CIRCUIT

The intrinsic connectivity of the hippocampal formation suggests that the dorsal hippocampus operates in a unitary manner rather than as a collection of parallel but independent units oriented in the transverse or lamellar plane of the hippocampus (Amaral and Witter, 1989). Most pathways between subfields of the hippocampal formation are highly divergent, and there are strong longitudinally oriented associational connections within the dorsal CA3 and the dorsal dentate hilus (Amaral and Witter, 1989; Ishizuka et al., 1990; Li et al., 1994). Together, these divergent and collateral systems provide a powerful substrate for associating inputs entering at distributed sites along the septotemporal axis of the hippocampus during spatial memory formation (Marr, 1971; McNaughton and Morris, 1987; Treves and Rolls, 1994; Hasselmo et al., 1995).

In spite of the longitudinal divergence of the intrinsic hippocampal fibers, rats can learn a water maze task with as little as a quarter of the hippocampus when the tissue remnant is within the dorsal hippocampus (Moser et al., 1995). This does not necessarily imply that the ensemble of hippocampal neurons performing the necessary computations is equally small in a normal rat. We investigated how much hippocampal tissue was used for encoding in normal animals by first training rats in a water maze task and then making variably sized lesions of parts of the hippocampus (Moser and Moser, 1998). Whereas partial hippocampal lesions, all sparing more than half of the dorsal hippocampus, did not interfere with the ability to acquire a new water maze task, retrieval of the task learnt before surgery was disrupted (Fig. 4). Successful retrieval was observed only if the entire dorsal two-thirds of the hippocampus was spared. A similar dissociation was observed when parts of the dorsal hippocampus were temporarily inactivated by the $GABA_A$ receptor agonist muscimol and retention was tested 20 min later. Again, retrieval was disrupted whereas new learning was not. Retrieval was normal 48 h after the injection, implying that access to spatial memory reappeared when the drug disappeared from the synapses. These observations suggest that spatial memory depended on an ensemble of neurons distributed across the entire dorsal two-thirds of the hippocampus.

The data further suggested that the amount of hippocampal tissue required for retrieval of a spatial task is not fixed, but rather reflects the volume of hippocampal tissue with which the task was acquired. In a normal rat, a widespread neuronal ensemble was needed for retrieval, probably because the entire length of the dorsal hippocampus was used for encoding. However, if only a small block of the dorsal hippocampus was intact during training,

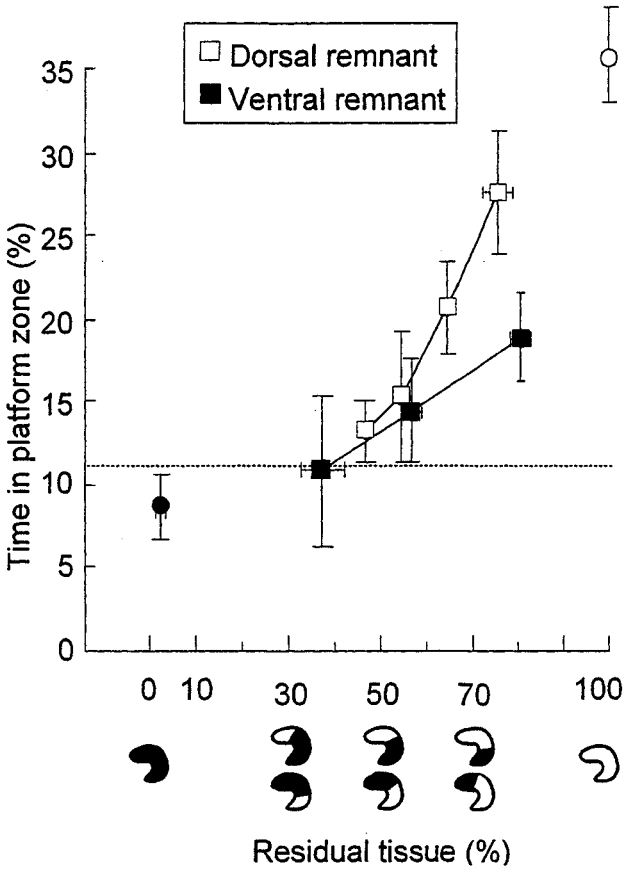

FIGURE 4. Retrieval in pretrained rats with spared tissue in either the dorsal or the ventral hippocampus. After training in a Morris water maze, the rats received a small hippocampal lesion sparing a block at either the dorsal or the ventral pole of the hippocampus. In both lesion groups, the remnants usually extended into the intermediate (splenial) part of the hippocampus. A week later, retrieval was tested by letting the rats swim in the absence of the platform. Time spent around the previous platform position is shown as a function of remaining hippocampal volume. Chance level is 12.5% (stippled line). (Reproduced with permission from Moser MB and Moser EI. Distributed encoding and retrieval of spatial memory in the hippocampus. *The Journal of Neuroscience* 1998;18:7535–7542. Copyright 1998 Society for Neuroscience.)

that block turned out to be sufficient also for later retrieval, probably because all the relevant modifications took place within it. Thus, the recruitment of neurons for encoding of a spatial representation is a dynamic process, but once a representation is formed, the selected neurons must be activated for subsequent recall of the information. This is consistent with human imaging data showing that overlapping portions of the hippocampal formation are activated during encoding and retrieval (Maguire et al., 1996, 1997). Contrasting results have been reported by Gabrieli et al. (1997), but encoding was not dissociated from novelty, and the material presented during encoding was different from the material used during retrieval.

With spatial learning taking up nearly the entire volume of the dorsal hippocampus in the normal rat, hippocampal size may become an important variable for spatial navigation. Certain

species of birds store food items in hundreds or thousands of scattered places and successfully retrieve their food from these cache sites. The avian hippocampus is larger, and contains more neurons, in food-storing species than in closely related nonstoring species (Krebs et al., 1989; Sherry et al., 1989). In mammals, polygamous voles have larger hippocampi than closely related monogamous voles (Jacobs et al., 1990; Sherry et al., 1992). The difference in the voles is specific to males. Male polygamous voles have larger range territories and probably depend more on spatial abilities than the monogamous species and the females of their own species. A similar sex-specific relation between hippocampal volume and spatial memory demands has been reported in species of kangaroo rats (Jacobs and Spencer, 1994). These and related findings suggest that the need for exact recall of extensive amounts of spatial information may correlate with the size of the hippocampal formation. It would be interesting to know whether the growth of the hippocampus with increased spatial demands affects all regions of the structure or only those which receive visuospatial input (assuming a connectivity pattern similar to that of the rat). Although little is known about structural differentiation in the avian hippocampus, it has been reported that food-storing is associated with a preferential enlargement of the rostral part of the hippocampus (Clayton, 1995).

WHAT IS THE ROLE OF THE VENTRAL HIPPOCAMPUS?

We have argued that the longitudinal axis of the hippocampal formation may consist of several functionally independent domains, with spatial memory formation taking part only in the dorsal two-thirds of the structure. If this is the case, a major task will be to determine what kind of information is processed in the ventral one-third of the hippocampus.

The ventral hippocampal formation has strong and specific efferent connections with several subcortical forebrain structures, including the rostral hypothalamus and the amygdala (Amaral, 1987; Witter et al., 1989b; Canteras and Swanson, 1992; Risold and Swanson, 1996, 1997; Risold et al., 1997). These connections suggest that the ventral hippocampus may interact with a variety of autonomic, endocrine, defensive, social, reproductive, and emotional control systems. Information about the motivational and homeostatic status of the animal is likely to reach the ventral hippocampal formation through hypothalamic connections with nuclei in the thalamus and the amygdala, as well as by direct connections (Risold et al., 1997).

One of several hypothalamic functions likely to be influenced by the hippocampal formation is the control of neuroendocrine and autonomic responses. The periventricular zone is particularly important for this control (Swanson, 1987; Loewy, 1991). By way of the lateral septum, the ventral pole of the hippocampus projects specifically to neuroendocrine and preautonomic cell groups of this zone as well as to various hypothalamic nuclei with massive projections to these ensembles (Risold and Swanson, 1997). It has been reported that nonselective hippocampal lesions lead to increased adrenal glucocorticoid secretion in rats (Murphy et al., 1979) and monkeys (Sapolsky et al., 1991). Hippocampal lesions also exacerbate restraint-induced gastric ulcer formation (Kim et al., 1976; Murphy et al., 1979). These effects are largely reproduced with lesions restricted to the ventral part of the hippocampal formation. Nonselective lesions of the ventral, but not the dorsal, hippocampus aggravate gastric erosion following restraint stress (Henke, 1990). Neurotoxic lesions of the ventral subiculum, through which the ventral hippocampus exerts much of its influence on the hypothalamus, cause glucocorticoid hypersecretion following restraint stress and open field exposure (Herman et al., 1998). Glucocorticoid secretion following respiratory stress was not different from that of control animals. Thus, it is possible that the hippocampus, and particularly its ventral pole, is involved in the adaptation of hypothalamo-pituitary-adrenocortical activation to environmental information. The role of memory in this tuning effect remains elusive. Autonomic responses to restraint stress are often attenuated by previous experience with the stressor (Glavin et al., 1994). It is conceivable that the hypothalamic connections of the ventral hippocampal formation convey such mnemonic influences.

The periventricular zone is also part of a set of interconnected hypothalamic nuclei intimately involved in the control of ingestive functions (Leibowitz, 1992; Hoebel, 1997). Rats with hippocampal damage are unable to learn a conditioning task in which food deprivation state serves as the conditioned stimulus (Davidson and Jarrard, 1993). Animals receiving foot-shock on days when they were food-deprived and not on days when they were fed (or vice versa) failed to display more freezing on shock-associated days following ibotenic-acid induced complete hippocampal lesions, whereas sham-operated rats readily discriminated between the deprivation states. It has been reported that selective lesions of the ventral hippocampus cause a similar disruption of internal-state discrimination (Hock and Bunsey, 1998). Surprisingly, however, impairment was also observed in rats with dorsal hippocampal lesions. One possible interpretation of this is that the rats did not use visceral cues to solve the discrimination task. The task might be solved by episodic memory (remembering whether there was food in the home cage), which could depend on the dorsal hippocampus. Although this issue was not addressed specifically, previous work has shown that rats are indeed able to learn a discrimination task based on interoceptive signals. Freezing was successfully conditioned to insulin-induced reductions of plasma glucose levels; no freezing was observed following saline administration (Davidson, 1987). A second and more likely interpretation is that hippocampal circuits involved in visceral associative learning occupy a large portion of the longitudinal axis of the hippocampus, extending beyond the ventral half of the hippocampus. Insulin receptors are abundant in the hippocampus (Unger et al., 1991), and signals from nutrient-sensitive neurons in the hypothalamus and in the brain stem may reach both dorsal and ventral parts of the hippocampal formation via a variety of multisynaptic pathways. Finally, the deprivation-state conditioning task also involves conditioning to a context. (Davidson and Benoit, 1996). State-dependent shocks are always

given when the animal is in a particular chamber. It is possible that this contextual element depends on circuits in the dorsal hippocampus. If this is true, it will be essential to vary hippocampal lesion size systematically to determine the exact location of the hippocampal substrate for conditioning to interoceptive or motivational signals.

FUNCTIONAL DIFFERENTIATION IN THE TRANSVERSE PLANE

The transverse circuit of hippocampal excitatory pathways is often conceived of as a single functional unit. Interruption of the transverse loop at almost any level is sufficient to attenuate acquisition of novel spatial tasks in rats. Damage to the dentate gyrus by colchicine (Sutherland et al., 1983) or adrenalectomy (Conrad and Roy, 1993) impairs spatial learning in this species. Similar deficits are observed following kainic-acid induced lesions of area CA3 (Handelmann and Olton, 1981; Sutherland et al., 1983) and ischemic lesions of area CA1 (Volpe et al., 1984, 1992; Olsen et al., 1994). Ischemic damage to CA1 in humans causes profound impairments in declarative memory formation (Zola-Morgan et al., 1986; Rempel-Clower et al., 1996). Thus, the disruption of learning following selective damage to each of the major subfields appears similar to, and no less severe than, a total lesion of the transverse circuit.

These results suggest that within all the major subdivisions of a transverse section through the hippocampal formation, there are cells that are essential to produce memory. However, this does not imply that the transverse circuit is functionally homogeneous. First, the formation of a memory trace is a complex process involving distinct but interdependent computations which each may take place serially in different neuronal populations of the transverse circuit (e.g., Buzsáki, 1989; Treves and Rolls, 1994). Second, within each subdivision of the transverse axis, there may be multiple neuronal ensembles responsible for different types of memory. Such ensembles may even be linked across subfields. Anatomical evidence suggests that there are several parallel transverse circuits through the hippocampus (Ishizuka et al., 1990; Amaral et al., 1991). One of the circuits consists of CA3 cells near the CA2 border, which mostly innervate the proximal part of CA1. From there, projections mainly reach the distal portions of the subiculum. A second circuit consists of CA3 cells close to the dentate gyrus, which preferentially connect with distal CA1 cells. The distal CA1 cells in turn project to the proximal part of the subiculum. A third distinct population is made up of intermediately located neurons. These three populations are differentially connected to the medial and lateral entorhinal cortices (Steward, 1976; Witter and Amaral, 1991), which in turn receive unequal amounts of input from the perirhinal and postrhinal cortices (Naber et al., 1997; Burwell and Amaral, 1998a). Thus, different segments of the CA1 and the subiculum are likely to process information originating in different sensory areas of the brain (Burwell and Amaral, 1998b). These segments also influence highly different regions of the brain through their distinct subicular output connections (Witter et al., 1990). This pattern of segregation, in addition to the transverse topographical organization of the hippocampal output to the lateral septum (Swanson and Cowan, 1977), suggests that several overlaid but functionally dissociable circuits may be embedded within the transverse circuit. Conventional lesions of entire subfields would not be capable of identifying such subsystems.

CONCLUSION

Evidence from several sources suggests that the hippocampus is functionally heterogeneous, with different portions of the longitudinal axis having different functional roles. Functional segregation may arise as a consequence of differential connectivity, with each subdivision receiving input from a specific set of brain regions. Alternatively, or additionally, functional differentiation may reflect variations in neuronal circuitry within the hippocampus. If so, it is conceivable that some of the algorithms by which incoming information is processed in dorsal and ventral portions of the structure are different as well.

The hippocampus may consist of more than two functional units. Distinct modules could be embedded in the transverse as well as the longitudinal plane. Although some of them are likely to be segregated, mosaic patterns are also conceivable. The same hippocampal cells may even be part of multiple, overlapping submodules. The fact that individual pyramidal cells contribute to representations in multiple and apparently unrelated environments (O'Keefe and Conway, 1978; Muller and Kubie, 1987) is consistent with the latter proposal.

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Distributed Encoding and Retrieval of Spatial Memory in the Hippocampus

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To determine whether memory is processed in a localized or distributed manner by the hippocampus, we inactivated small regions of the structure in pretrained rats before a retention test. Ibotenic acid-induced lesions removing 40% of the hippocampal tissue disrupted retrieval of spatial memory in a water maze but failed to affect new learning or retrieval of a task that was acquired postoperatively. Partial inactivation of the hippocampus by local intrahippocampal 5-aminomethyl-3-hydroxyisoxazole muscimol infusion also impaired retrieval but not new learning. This impairment was temporary; infusions had no effect on retrieval of predrug performance when the test was conducted 48 hr after the infusion. Systematic variation of the volume of dorsal and ventral hippocampal lesions showed that successful retrieval required the integrity of the entire dorsal 70% of the hippocampus. Our data suggest that although spatial tasks can be acquired with local ensembles of hippocampal neurons when other parts of the hippocampus are inactivated, spatial memory is normally both encoded and retrieved by a widely distributed hippocampal network.

Key words: spatial learning; memory; hippocampus; water maze; rat; distributed network; septotemporal; dorsal hippocampus; muscimol

Considerable evidence points to the hippocampus as a brain structure of importance for encoding and retrieval of explicit memory (Squire, 1992; Schacter and Tulving, 1994; Eichenbaum, 1997). The mnemonic role of the hippocampus is particularly clear in tasks in which subjects are required to remember location (O'Keefe and Nadel, 1978). Hippocampal lesions disrupt acquisition and retrieval of spatial maze tasks in rats (Jarrard, 1978; Morris et al., 1982, 1990), and principal cells in the rat hippocampus fire in a location-specific manner during exploration of spatial environments (O'Keefe and Nadel, 1978; Muller, 1996). In humans, the right hippocampus is activated in navigation tasks in which retrieval of spatial relations is essential (Maguire et al., 1997).

A currently debated issue is whether the ensemble of neurons responsible for encoding and retrieval of a spatial learning episode is localized or distributed within the hippocampus. Based on the internal connectivity of the hippocampus, a range of theories have proposed that the hippocampus, acting as an autoassociative matrix (Kohonen, 1984; Rolls and Treves, 1997), encodes episodes and locations in a distributed manner (Marr, 1971; McNaughton and Morris, 1987; Buzsáki et al., 1990; Treves and Rolls, 1994; Hasselmo et al., 1995). However, although the hippocampus has many of the anatomical and physiological features expected of a distributed associative system, there is a lack of behavioral evidence for distributed function. The strongest piece of evidence is perhaps the nontopographic representation of location by hippocampal principal cells, with cells coding for the same location being distributed over the entire hippocampus (O'Keefe and Nadel, 1978; Jung et al., 1994; Poucet et al., 1994). However, location can nevertheless be predicted at high accuracy from the spatial correlates of a local cluster of hippocampal neurons (Wilson and McNaughton, 1993), suggesting that the neuronal elements required for representing an environment may exist within quite circumscribed regions of the hippocampus.

The size of the network involved in a hippocampus-dependent learning task can be determined by partial lesions or partial inactivation of the hippocampus. It has been shown previously that small blocks of the hippocampus ($\leq$25%) are sufficient for learning a reference memory task in a water maze, provided that the block is located within the dorsal two-thirds of the hippocampus (Moser et al., 1993, 1995, 1997). This suggests that spatial learning may take place with a fairly concentrated cluster of neurons. However, it is conceivable that the cells participating in encoding of memory for location are more distributed when the entire hippocampus is available, as it is in the normal rat. To explore this possibility, we first trained rats in a water maze task and then lesioned or temporarily inactivated parts of the hippocampus. We found that retrieval, but not new learning, was disrupted by restricted bilateral lesions at either side of the dorsal two-thirds of the hippocampus. The results suggest that although spatial tasks can be acquired with a small hippocampal remnant, a widely distributed hippocampal network is used for encoding and retrieval in normal rats.

MATERIALS AND METHODS

Subjects. One hundred forty-three naïve male Long–Evans rats (300–450 gm) were housed in groups of four to six in large transparent polycarbonate cages (59 × 38 × 20 cm) with food and water available *ad libitum*. They were kept on a 12 hr light/dark schedule and tested in the light phase. The animals received either a bilateral partial lesion of the hippocampus or bilateral intrahippocampal injection of the $GABA_A$ receptor agonist 5-aminomethyl-3-hydroxyisoxazole (muscimol). Control animals received sham lesions or intrahippocampal saline infusions, respectively.

Behavioral training. All rats were trained in a water maze (Morris,

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1984): a white circular polyvinylchloride tank (198 cm in diameter, 50 cm deep) filled to a depth of 40 cm with water at 25 ± 2°C. Latex liquid was added to make the water opaque. A pneumatically controlled escape platform (11 cm in diameter) was located in the center of the southwest quadrant and could be moved by remote control between an available level (1.5 cm below the water surface) and an unavailable level (22 cm below the surface). The pool was located in a room (4 × 7 m) with multiple cues on all sides. A wall separated the pool from the experimenter during the trials.

Unless otherwise indicated, the rats were trained to asymptotic performance before surgery. Training consisted of 11 sessions of four consecutive trials each. One session was conducted in the morning and one in the evening over a period of 6 d, except for the first day when the rats received only one session. In each trial, the rats were released from one of eight equally spaced start positions along the perimeter of the pool in a predetermined and pseudorandom order. The position of the hooded rat during swimming was identified and stored at 10 Hz by a tracking system (HVS Image, Hampton, UK; Watermaze Software, Edinburgh, UK). If the rats had not entered the platform after 120 sec, they were guided onto it. The rats were left for 30 sec on the platform. On the last day of pretraining, only a spatial probe test was conducted. The platform was kept in its lower position for the first 60 sec of the test and the search pattern was recorded. The platform was subsequently raised to its upper accessible position, after which the rat usually found the platform. The rats were released from the quadrant opposite to the platform on the probe test. They were ranked, matched, and assigned to surgery or drug groups according to the proportion of time they spent around the platform.

Seven days after completion of pretraining and surgery, a retention test was conducted. Again, the platform was kept in its lower position for the first 60 sec, and the swim pattern was recorded. Immediately afterward, new walls and landmarks were placed around the pool and the rats were retrained with the platform in a new location. Training in the new environment consisted of four blocks of two trials each. The blocks were separated by 1 hr intervals in lesioned animals and by 10 min intervals in drug-infused animals. After the last block, another spatial probe test was conducted.

Surgery. Within 36 hr after completion of pretraining, the rats were anesthetized with equithesin (pentobarbital and chloral hydrate; 1.0 ml/250 gm body weight). Hippocampal lesions were made by bilateral injection of ibotenic acid (Biosearch Technologies, San Rafael, CA) at ≤28 sites (Jarrard, 1989). Ibotenic acid was dissolved in 10 mg/ml PBS, pH 7.4, and injected with a 1 μl Hamilton syringe mounted to the stereotaxic frame. Injections of 0.05–0.10 μl were made over 10–20 sec at each site. The syringe was retracted 2 min after injection. In sham-operated rats, the syringe was lowered through the neocortex, but no drug was infused. In three separate rats, EEGs (0.2 sec epochs at 30 sec intervals) were recorded from the dorsal hippocampus after ventral ibotenic acid infusions for 5–9 hr until anesthesia had worn off. After-discharges (which could lead to damage outside the injected area) were not observed.

Temporary inactivation. A subset of the rats (n = 32) did not receive lesions but were implanted with two 26 ga guide cannulas (C315G; Plastics One), one above the dorsal pole of each hippocampus (3.0 mm behind bregma, 2.2 mm lateral, 1.0 mm below dura). Cannulas and anchor screws were encased in dental acrylic. Seven days after surgery, the $GABA_A$ agonist muscimol (Sigma, St. Louis, MO) dissolved in PBS, pH 7.4, was infused via a 33 ga internal cannula (C315I; Plastics One) with the tip protruding 1.0 mm beyond the guide cannulas. A total of 0.07 μg of muscimol in 0.14 μl was injected into each hippocampus at 0.15 μl/min (controlled by a syringe pump). Control rats (n = 11) received saline (same volume and same rate). The internal cannula was retracted 4 min after each infusion. Retention was tested 20 min later. A separate group (n = 8) received the muscimol 48 hr before the retention test.

Histology. The rats were killed with an overdose of equithesin and perfused intracardially with saline and 4% formaldehyde. The brains were stored in formaldehyde for ≥1 week. Frozen sections were cut coronally (30 μm) and stained with cresyl violet. To determine the volume of residual hippocampal tissue in the lesioned animals, the sections were placed under a microscope attached to a video camera and a personal computer. Images were grabbed by GrabIt (AIMS Lab) and taken into Canvas (Deneba Systems), where an outline of remaining hippocampal tissue was traced. The area of the outlined region was determined by Canvas. Volume of hippocampal tissue was calculated by treating the hippocampal remnant as a series of truncated cones with parallel surfaces, in which each surface corresponded to one section and the area of the surface was equal to the area inside the outline. The volume of the hippocampal remnant was expressed as percentage of the mean volume of hippocampal tissue in the sham-operated group. Such volume estimates have been shown previously to be highly reliable, with interobserver reliabilities >0.99 (Moser et al., 1995).

RESULTS

Effects of partial hippocampal lesions on retrieval and new learning

The effect of small lesions in the hippocampus on encoding and retrieval was investigated in 42 rats that had been pretrained in a Morris water maze. During pretraining, all of these rats learned to swim directly to the hidden platform well before training was completed (Fig. 1*A*). On the last day of pretraining, a spatial probe test with the platform unavailable at the bottom of the pool was conducted. The animals spent most of the trial searching within a small zone around the platform (Fig. 1*B*). They were ranked according to time spent in the platform zone, matched, and assigned to three groups, which received (1) partial hippocampal lesions starting from the ventral pole, (2) complete hippocampal lesions, or (3) sham surgery. Surgery started 1 hr after the probe test.

The partial hippocampal lesions encompassed nearly all hippocampal tissue from the ventral pole of the structure up to the level of the lateral geniculate nucleus (Fig. 2). The lesions spared 60.7 ± 2.5% (mean ± SEM) of the total hippocampal volume. The border between damaged and healthy tissue was sharp, implying that the density of intact neurons within the remaining dorsal hippocampus was within the normal range. There was partial damage to the ventral subiculum in most of the animals. Four animals were excluded because of patchy hippocampal lesions, neocortical damage, or unintended hippocampal damage (sham animal).

Retrieval was measured on a second probe test 7 d after pretraining and surgery (Fig. 1*C*). Only the sham-operated group clearly searched more within a circular zone (35 cm radius) around the platform than in corresponding zones of the other quadrants. Although each zone covered 12.5% of the pool, these animals spent 35.0 ± 3.8% (mean ± SEM) of the search period in the platform zone. Rats with partial or complete hippocampal lesions failed to show any preference for the platform zone (15.1 ± 2.2% and 5.8 ± 1.8%, respectively). The short time spent around the platform in the complete lesion group was attributable to swimming near the start position opposite to the platform quadrant at the beginning of the trial. A repeated measures ANOVA of time spent in the four quadrant zones showed a significant Groups × Zones effect ($F_{(6,87)} = 9.2$; $p < 0.001$) with significant group differences in the target zone ($F_{(2,31)} = 19.7$; $p < 0.001$). Subsequent planned orthogonal comparisons showed that the rats with partial or complete lesions had lower target zone times than the sham-operated controls ($F_{(1,31)} = 39.2$; $p < 0.001$), whereas the lesioned groups themselves did not differ ($F_{(1,31)} = 3.0$; $p > 0.05$)

The ability of the above rats to learn a new task was tested on the same day by training them in a new environment. The same partially lesioned animals now performed as well as the sham group. At the end of four blocks of training, both groups had escape latencies <15 sec (Fig. 1*A*), and on a subsequent probe test, both groups searched primarily in the platform zone (Fig. 1*D*). Rats with complete hippocampal lesions still failed to show any preference for the target area. There was a significant Groups effect on the escape latencies ($F_{(6,87)} = 3.7$; $p < 0.005$) and a

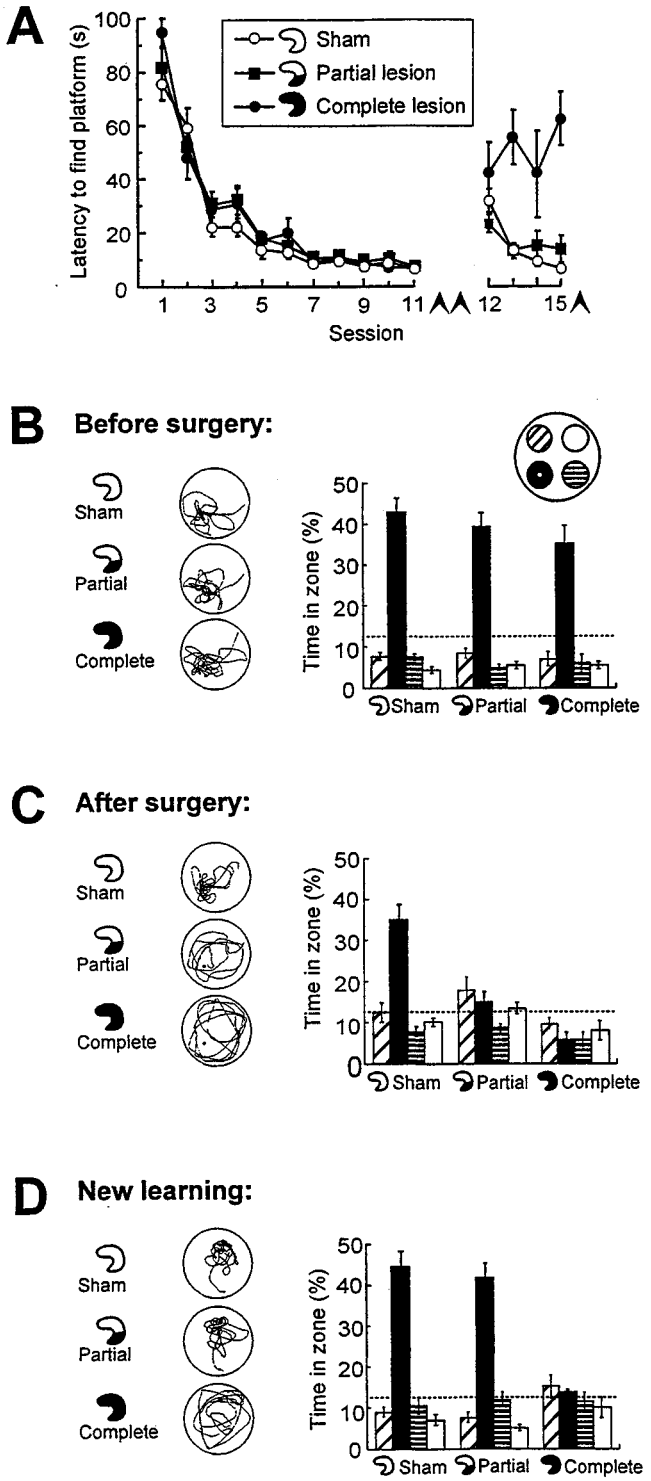

Figure 1. Retrieval of spatial memory in a water maze after a small hippocampal lesion. Control groups received sham surgery or complete hippocampal lesions. Lesions were induced after 11 sessions of pretraining. Seven days later, retrieval was tested, and the animals were trained in a new task. *A*, Latency to locate the hidden platform before surgery (sessions 1–11) and after surgery (sessions 12–15, conducted in a new environment). *Arrowheads* indicate spatial probe tests. *B–D*, Retrieval on probe tests at the end of pretraining (*B*), 7 d after pretraining and surgery (*C*), and after postoperative training in the new environment (*D*). *Left column*, Typical swim paths. *Right column*, Time spent in a circular zone around the platform position (*filled bars*) and in corresponding zones of the three other quadrants (mean ± SEM). Chance level is 12.5% (*dashed lines*).

significant Groups × Zones effect on time distribution on the probe test ($F_{(6,87)} = 10.0$; $p < 0.001$). Both effects reflected the difference between the complete lesion group and the two other groups. The preservation of spatial learning in rats with the dorsal 60% of the hippocampus intact is consistent with previous observations (Moser et al., 1995) and argues for adequate function of the spared hippocampal tissue.

To further rule out nonspecific factors related to incomplete recovery from surgery on the retention test on day 7, separate groups with similar lesions were tested for retention 16 d after surgery ($n = 23$). In the partial lesion group, 56.6 ± 1.6% of hippocampal volume was spared, with the lesion starting at the ventral end of the hippocampus. The same differential behavior was observed as after 7 d. The rats spent 31.0 ± 3.7% (sham surgery), 17.2 ± 2.1% (partial lesions), and 9.1 ± 2.9% (complete lesions) of the search time in the platform zone (Groups × Zones effect, $F_{(6,60)} = 3.2$; $p < 0.01$).

Relationship between volume of hippocampal tissue and success of retrieval

The fact that retrieval of a spatial task was impaired after a lesion confined to the ventral half of the hippocampus was surprising, because ventral lesions have no effects on encoding (Moser et al., 1993, 1995). The ventral hippocampus might be a central part of the substrate for retrieval but not for encoding. Alternatively, both encoding and retrieval rely primarily on the dorsal hippocampus, but the area used for retrieval might be larger and thus stretch well into the ventral half of the hippocampus (and into the damaged region of rats with ventral lesions). To dissociate effects of location from effects of volume, we pretrained rats to asymptotic performance (as above) and made additional lesions sparing a broader range of hippocampal volume. Lesions were now made from either the ventral end of the hippocampus (as above) or from the dorsal end. In total, 100 rats received surgery (including the rats of the first experiment). Sixty-one of these received dorsal ($n = 39$) or ventral ($n = 22$) hippocampal lesions.

The partial lesions were confined to either the dorsal or the ventral hippocampus. The volume of the dorsal remnants ranged from 41 to 91% of total hippocampal tissue, whereas the ventral remnants ranged from 27 to 92%. Ventral hippocampal lesions were associated with some ventral subicular damage. Seven animals with dorsal remnants, two with ventral remnants, three with complete lesions, and three sham-operated animals were excluded because of unintended neocortical or thalamic damage or because of patchy hippocampal lesions.

Successful retrieval 7 d after pretraining and surgery was observed only with the largest volumes of spared dorsal hippocampal tissue (Fig. 3). In the group with dorsal remnants, those with >60% of the hippocampus intact preferred the platform zone to the other zones, but only those with >70% were comparable to the sham rats (sham group vs 60–70% group, $t_{(31)} = 3.4$; $p < 0.005$; sham group versus >70% group, $t_{(26)} = 1.5$; $p > 0.05$). In the group with ventral remnants, rats with <70% intact performed at random, whereas rats with >70% showed some retention but still significantly less than the sham group ($t_{(29)} = 3.1$; $p < 0.005$). There were no group differences in behavior during the pretraining. Thus, rats need at least 70% of the hippocampus to retrieve a spatial task that is encoded preoperatively. These 70% must be at the dorsal side of the structure.

Reversibility of the retrieval deficit

We investigated whether the retrieval deficit was reversible by inactivating a small portion of the dorsal hippocampus with the

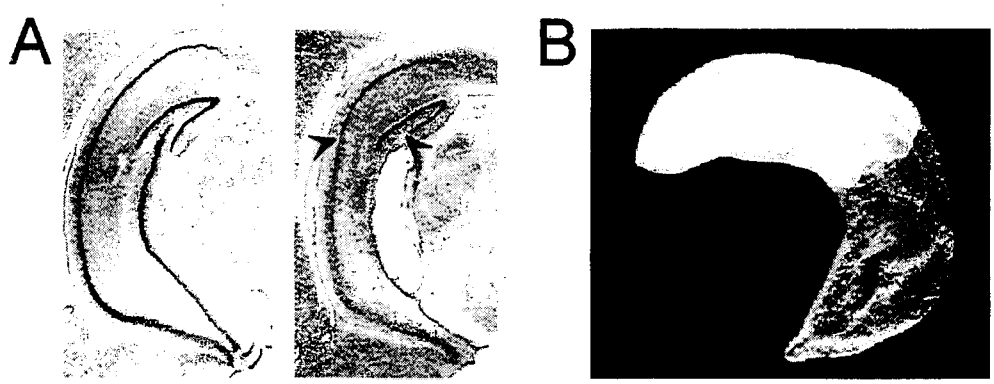

Figure 2. Location of remaining hippocampal tissue in a rat with a partial hippocampal lesion that impaired retrieval but not new learning (Fig. 1). *A*, Coronal section showing cresyl violet stains of neuronal cell bodies in the intermediate to ventral portion of the hippocampus of a sham-operated rat (*left*) and a rat with a lesion that spared 58.5% of hippocampal volume dorsally (*right*). *Arrowheads* indicate border between lesioned and healthy tissue. *B*, Three-dimensional reconstruction of the remaining dorsal hippocampal tissue (*white*) of the lesioned rat in *A* superimposed on a reconstruction of the entire hippocampus (*gray*).

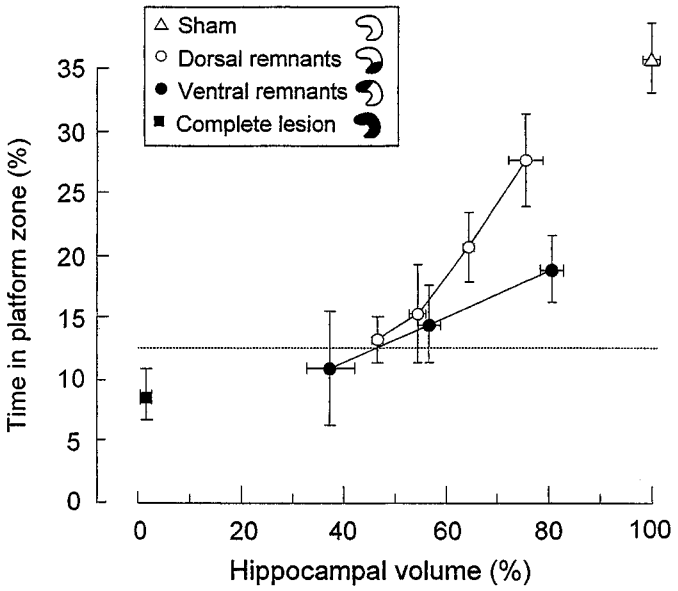

Figure 3. Retrieval on a spatial probe test 7 d after pretraining and surgery as a function of remaining hippocampal volume in animals with dorsal remnants (10% bins) or ventral remnants (20% bins). Retrieval is expressed as the proportion of time spent in a circular zone around the platform position (mean ± SEM). *Dashed line* indicates chance level.

$GABA_A$ agonist muscimol in a separate set of animals (n = 32), all pretrained to asymptotic performance in the water maze (Fig. 4*A*). All rats showed a preference for the zone around the platform on the probe test at the end of training (Fig. 4*B*). The infusion cannulas were placed within the dorsal one-third of the hippocampus on both sides of the brain (Fig. 5). The amount of hippocampal damage was <0.2%. In two animals, the cannulas did not hit the hippocampus; these animals were excluded from further analysis. Three animals were excluded because of cortical lesions or infection.

Seven days after pretraining and implantation, the animals were tested for retrieval of the spatial task. Twenty minutes before testing, they received an intrahippocampal infusion of either saline or a low dose of muscimol. In a separate group, muscimol was infused 48 hr before the retrieval test; thus, little or no drug was left in the brain at the time of testing. Rats tested with muscimol at the 20 min interval swam no more in the platform zone than in the corresponding zones of the other quadrants (Fig. 4*C*). In contrast, rats tested at 48 hr clearly preferred the platform zone, as did the saline-infused animals, suggesting that the retrieval impairment was reversible. ANOVA of the probe test times showed a significant Groups × Zones effect ($F_{(6,72)} = 4.2; p < 0.001$) and a significant Groups effect on time in the platform zone ($F_{(2,26)} = 7.7; p < 0.005$). Planned orthogonal comparisons showed that the muscimol 20 min group spent less time in the target zone than the saline and muscimol 48 hr groups ($F_{(1,26)} = 15.4; p < 0.001$), whereas the two latter groups did not differ ($F < 1$).

All three groups were able to acquire a navigation task in a new environment during the subsequent hour. In all groups, the rats learned to swim to the hidden platform within ~15 sec (Fig. 4*A*). On the final probe test in the new environment, all three groups showed a preference for the platform zone (Fig. 4*D*). There was no significant Groups effect on escape latencies, nor was there a Groups × Zones effect on the probe test (F values < 1.3). Thus, temporary inactivation of a small part of the hippocampus disrupted retrieval but not new learning.

Retrieval after postoperative training

Our data suggest that retrieval relies on a widespread hippocampal network. The reason could be that a widely distributed network was engaged during encoding and that the same distributed network must be activated for retrieval of the stored information. Alternatively, the need for large portions of the hippocampus may be inherent to the retrieval process itself, i.e., retrieval may require processes that were not involved in original encoding. If the latter is true, small lesions may disrupt retrieval regardless of the amount of hippocampus used for acquisition. Thus, we tested 20 rats that had acquired the water maze task after partial hippocampal lesions were made. The lesions of these rats were similar to those of the rats learning the task before surgery, with 62.3 ± 1.9% of total hippocampal volume being spared at the dorsal end.

As expected, both the rats with small hippocampal lesions and the sham-operated rats learned to find the hidden platform rapidly and precisely (Fig. 6*A*). These groups achieved escape latencies <10 sec and clearly searched in the area around the platform on the probe test at the end of the training (Fig. 6*B*). In contrast, a group of rats with complete hippocampal lesions failed to learn where the platform was located. There was a significant effect of Groups on the escape latencies ($F_{(2,17)} = 47.3; p < 0.001$), as well as a Groups × Zones effect on time spent in the four zones on the probe test ($F_{(6,51)} = 5.7; p < 0.001$). Both effects reflected the difference between the group with complete lesions and the two other groups.

When tested for retention 7 d later, both the sham-operated and the partially lesioned animals searched in the target zone,

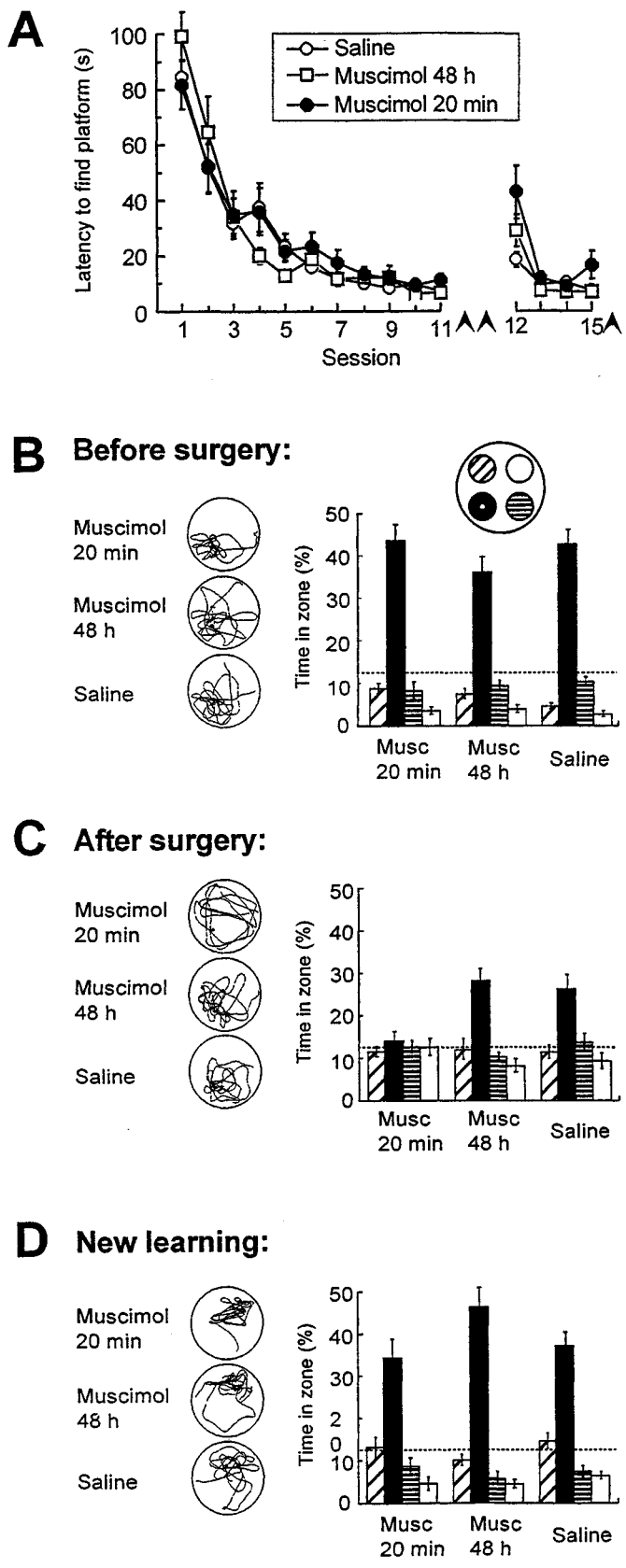

Figure 4. Distribution of swim time in pretrained rats after partial inactivation of the dorsal hippocampus by microinfusion of the $GABA_A$ receptor agonist muscimol. The interval between pretraining and drug infusion was 7 d. *A*, Latency to locate the hidden platform during pretraining (sessions 1–11) and after drug infusion (sessions 12–15, conducted in a new environment). *Arrowheads* indicate spatial probe tests. *B–D*, Retrieval on probe tests at the end of preoperative training (*B*), 7 d after pretraining and surgery (*C*), and after new learning in a different environment 1.2 hr subsequent to the test in *C* (*D*). Muscimol was infused 20 min or 48 hr before the test in *C*. A separate group received saline 20 min before testing. No drug was given in *B*. *Left column*, Typical swim paths. *Right column*, Time spent in the central zones of each quadrant (Fig. 1). *Dashed lines* indicate chance level.

whereas rats with complete hippocampal lesions still failed on both tests (Fig. 4*B*). Again, there was a significant Groups × Zones effect ($F_{(6,51)} = 5.7$; $p < 0.001$) and a significant Groups effect on time in the platform zone ($F_{(2,19)} = 11.3$; $p < 0.001$). Orthogonal comparisons showed a difference between rats with total hippocampal damage and rats with either partial lesions or no lesions at all ($F_{(1,19)} = 22.3$; $p < 0.001$). There was no difference between the partial lesion group and the sham group ($F < 1$). Thus, less hippocampal tissue was needed for retrieval if the task was acquired after the partial hippocampal lesion.

DISCUSSION

The present study shows that retrieval of spatial memory is disrupted by small lesions within the dorsal 70% of the hippocampus in rats trained before surgery. Retrieval was not impaired if acquisition occurred subsequent to the partial lesions, although the interval between training and retrieval was similar. The lesions had no effect on new learning. The results suggest that a widespread hippocampal network is used in normal rats during encoding and retrieval of spatial memory, that this network is located within the dorsal 70% of the hippocampus, and that smaller networks within this region can be used for encoding in rats with partial hippocampal lesions.

Dissociation between retrieval and new learning

Small hippocampal lesions impaired retrieval, suggesting that retrieval involves a widespread hippocampal network. This interpretation rests on the assumption that the remaining hippocampal tissue functioned normally. It is conceivable that ibotenic acid disrupted information processing also in the noninjected parts of the hippocampus, e.g., by triggering seizures. However, the tissue remnants seem to be normal in several important respects. Both synaptic activation and short- and long-term plasticity in the main excitatory synapses of such blocks have been shown to be within the normal range (Moser et al., 1995), and the animals seem to be capable of normal spatial learning in a water maze. Moreover, temporary inactivation of a part of the hippocampus impaired retrieval shortly after the infusion but not 2 d later when the drug presumably had disappeared from the synapses. Thus, access to spatial memory seems to reappear in the muscimol-infused animals, and it is unlikely that the selective disruption of retrieval after partial inactivation or lesions of the hippocampus reflects damage to the hippocampal network. Rather, we believe pretrained animals were impaired, because a substantial volume of the hippocampus was required for retrieval of spatial memory.

Although retrieval was disrupted, new learning was unimpaired in the partially lesioned rats. Rats with partial damage acquired a new spatial task at the same rate as sham-operated animals. Together with previous observations (Moser et al., 1993, 1995, 1997), this suggests that relatively small pieces of dorsal hippocampal tissue (a quarter or less of total hippocampal volume) are sufficient to encode a representation that successfully guides the rat toward a hidden goal. If less than a quarter of the

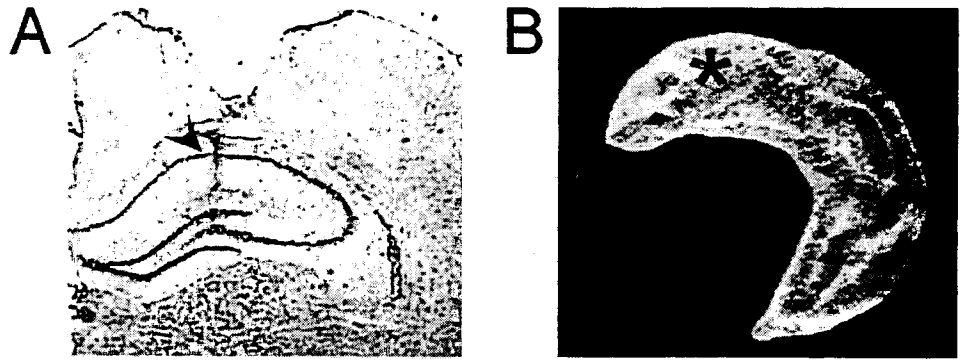

Figure 5. Location of the muscimol infusion within the dorsal hippocampus. *A*, Cresyl violet stain showing position of internal cannula (*arrowhead*) in the hippocampus of a representative muscimol-infused rat. The neocortical damage was attributable to the implanted guide cannula. *B*, Three-dimensional reconstruction showing the position of the cannula (*asterisk*) in one hippocampus of the same rat.

hippocampus (half of the dorsal hippocampus) is left intact, new learning is also affected (Moser et al., 1995; Duva et al., 1997).

In animals familiar with the procedural rules of the water maze, knowledge of a new platform position can be acquired within a single trial (Morris et al., 1986). The present data demonstrate such fast learning also in rats with partial hippocampal lesions, even when the rats do not remember the spatial layout of the original training environment. This is consistent with previous work showing that relearning in radial mazes after large neurotoxic lesions of the hippocampus is only mildly disrupted (Handelmann and Olton, 1981; Jarrard, 1986) and suggests that procedural knowledge about the water maze task is stored and accessed outside the hippocampus (Morris et al., 1982, 1986; Squire, 1992).

The observation that larger volumes of hippocampal tissue are required for retrieval than for new learning may be surprising, because in many regions of the cortex, repetition and practice are accompanied by reduced neuronal activation in specific areas. Well practiced normal subjects show such reductions during perceptual and conceptual priming (Squire et al., 1992; Raichle et al., 1994; Buckner et al., 1998), during recall of familiar stories and word lists (Andreasen et al., 1995a,b), and during motor behavior (Petersen et al., 1998). In primates, single neurons in the inferior temporal and rhinal cortices respond less frequently to the second than to the first presentation of a stimulus (Miller et al., 1991; Riches et al., 1991), although this does not necessarily imply that the activated area becomes smaller. In the hippocampus, discharge probabilities were not reduced by repeated presentations (Riches et al., 1991), suggesting that many hippocampal neurons that were active during encoding may be required also for retrieval.

The hippocampal volume required for retrieval mirrors the volume used for original encoding

Encoding and retrieval of episodic memory may be associated with differential patterns of activity. There is only partial overlap between the systems activated during these processes in the neocortex (Tulving and Markowitsch, 1997; Fletcher et al., 1997). A similar differentiation may exist for spatial memory within the hippocampus, in which case the need for large volumes of hippocampal tissue during retrieval could be a property of retrieval per se rather than a consequence of the way memory was encoded.

Our data suggest that the amount of hippocampal tissue required for retrieval of a spatial task is not fixed but rather reflects the volume of hippocampal tissue with which the task was acquired. If only a block of the dorsal hippocampus was present during training, that small block also appeared to be sufficient for subsequent retrieval (Fig. 6), probably because the necessary cellular modifications took place within that block. If the rats learned the spatial task with an intact brain, however, successful retrieval required ≥70% of the hippocampus (Figs. 1, 3), suggesting that a widely distributed network was engaged during encoding. Thus, in normal rats, a widespread neuronal ensemble may be engaged both during encoding and again during retrieval of the stored information. The overlapping nature of the two ensembles would be consistent with models, suggesting that the same hippocampal principal neurons may perform both encoding and retrieval functions (Paulsen and Moser, 1998).

Spatial memory functions may take place in the entire dorsal 70% of the hippocampus

At least 70% of the hippocampus was required to retrieve a spatial task encoded with an intact brain. However, retrieval was successful only if the 70% remnants were at the dorsal side of the hippocampus. Probably because the critical substrate was so large, impairment was seen also after large ventral lesions (affecting ~40% of the hippocampus) (Fig. 2) as these encroached on the dorsal to intermediate area.

Encoding is possible with a minimum of preserved tissue in either the dorsal or the intermediate portion of the hippocampus but not in the ventral portion (Moser et al., 1997). Thus, the same sectors of the dorsoventral axis of the hippocampus seem to be involved in encoding and retrieval of spatial memory. Recent tracing studies have shown that sensory input likely to be important for spatial learning is distributed to the dorsal and the intermediate hippocampus but not to the ventral pole of the structure (Deacon et al., 1983; Witter et al., 1989; Dolorfo and Amaral, 1998a,b). Thus, behavioral and anatomical data converge and suggest that the hippocampus may consist of a large dorsal region involved in spatial learning and a smaller ventral region not essential for navigation.

The location of the border between the putative functional divisions of the hippocampus may have implications for the interpretation of studies investigating whether pyramidal cells have place fields not only in the dorsal hippocampus but also in the ventral hippocampus. Place cells have been reported in the ventral half of the hippocampus, although such cells may be fewer, discharge at lower rates, and have larger place fields (Jung et al., 1994; Poucet et al., 1994). However, if more than half of the hippocampus is used for navigation learning, the ventral cells with place fields may still have been located at the dorsal side of the putative border.

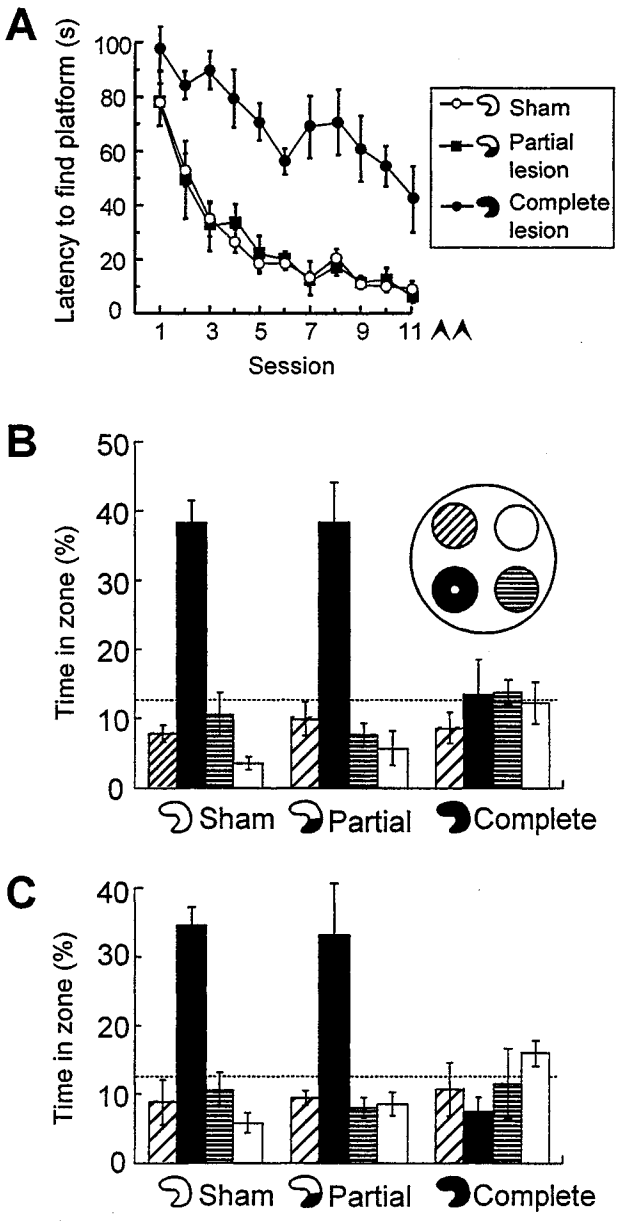

Figure 6. Retrieval in animals that received training after the partial hippocampal lesion. The rats had lesions sparing the dorsal hippocampus (as in Fig. 2), complete hippocampal lesions, or sham lesions. *A*, Latency to locate the hidden platform during postoperative training. *Arrowheads* indicate spatial probe tests. *B*, *C*, Retrieval at the end of training (*B*) and 7 d later (*C*). *Symbols* are the same as in Figure 1.

Longitudinal integration of hippocampal activity during encoding and retrieval

The representation of location by hippocampal place cells is nontopographic (O'Keefe and Nadel, 1978; Muller, 1996). A local cluster of place cells in the dorsal hippocampus usually covers most parts of a spatial environment, and the location of the animal in this environment can be predicted from the discharge pattern of these cells (Wilson and McNaughton, 1993). However, we found that retrieval was disrupted by small hippocampal lesions, which suggests that a local cluster of neurons may not be sufficient for retrieving a useful place representation. Place learning may involve associations between events that each give rise to activity at separate levels of the hippocampus. With sensory information entering the hippocampus along as much as three-quarters of the longitudinal axis of the structure (Dolorfo and Amaral, 1998a,b), it is conceivable that widely separated inputs are associated during spatial learning.

Integration of signals from different levels of the hippocampus may depend on the extensive longitudinal connections of the structure (Amaral and Witter, 1989). Although the average direction of the excitatory pathways of the hippocampus is in the transverse plane (Andersen et al., 1971), the major excitatory and inhibitory connections are highly collateralized and divergent (Amaral and Witter, 1989; Tamamaki and Nojyo, 1990, 1993; Sik et al., 1995), and both dentate mossy cells and CA3 pyramidal cells have extensive associational fibers projecting widely in the longitudinal direction (Swanson et al., 1978; Ishizuka et al., 1990; Li et al., 1994). These recurrent collaterals provide a substrate by which input entering at multiple and distributed sites along the dorsoventral axis of the hippocampal formation could become associated during spatial learning (Marr, 1971; McNaughton and Morris, 1987; Treves and Rolls, 1994; Hasselmo et al., 1995).

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SCIENCE

Association Between Brain Temperature and Dentate Field Potentials in Exploring and Swimming Rats

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Association Between Brain Temperature and Dentate Field Potentials in Exploring and Swimming Rats

Edvard Moser, Iacob Mathiesen, Per Andersen*

Attempts to correlate behavioral learning with cellular changes, such as increased synaptic efficacy, have often relied on increased extracellular potentials as an index of enhanced synaptic strength. A recent example is the enlarged excitatory field potentials in the dentate gyrus of rats that are learning spatial relations by exploration. The altered hippocampal field potentials do not reflect learning-specific cellular changes but result from a concomitant rise in brain temperature that is caused by the associated muscular effort. Enhanced dentate field excitatory potentials followed both passive and active heating and were linearly related to the brain temperature. These temperature-related effects may mask any learning-induced changes in field potential.

The hippocampal formation is a phylogenetically old part of the cerebral cortex. Although there is strong evidence for its involvement in learning the spatial relation between objects (spatial learning) (*1*), neurophysiological correlates to learning such as synaptic weight changes have been difficult to find in freely moving animals (*2*). However, it was reported that exploration of an unfamiliar environment was associated with an increased dentate field excitatory postsynaptic potential (f-EPSP) after perforant path stimulation but with decreased population spike amplitude and latency. The effect was interpreted to reflect a changed synaptic weight due to the learning experience (*3*). We now examine an alternative possibility: These changes may be due to a temperature effect (*4*). This possibility would also explain why the enlarged f-EPSP was associated with a decreased population spike (*5*). Both exercise and feeding elevate the brain temperature in rats, whereas inactivity and sleep result in lower temperatures (*6*). The major factor that controls brain temperature is muscular heat production, which warms the cerebral arterial blood (*7*). In rats that were swimming in a Morris maze, we observed field potential changes that are exactly opposite to those reported above, namely, f-EPSP reduction with spike increase. Similar field potential changes are seen during brain cooling (*4*).

For these reasons we have recorded the hippocampal temperature in freely moving rats and correlated it with dentate field potentials during exploration and swimming (*8*). In both situations there was a strong, linear correlation between the behaviorally induced potential changes and the brain temperature.

When rats explored items on a platform the slope of the f-EPSP was increased (Fig. 1A), which confirms earlier reports (*3*). However, this enhancement was paralleled by an increase in brain temperature. In addition, the f-EPSP latency, the population spike amplitude, and latency all decreased. The brain temperature rose during 10 to 20 min of exploration from 37.0° ± 0.1°C (mean ± SEM) with a mean rate of 0.11° ± 0.01°C per minute (n = 20); the largest increase was 3.2°C. After the exploration the brain temperature and the f-EPSPs declined along the same exponential time course, with both the f-EPSP and spike reaching base-line levels after 20 to 80 min. The temperature and f-EPSP curves were always parallel (43 sessions in nine of nine rats). The correlation factor (r) between the brain temperature and the f-EPSP slope during exploration was never <0.5 and in most runs was >0.75. For the exploring rat in Fig. 1, the r values between the brain temperature and the following signal elements were: f-EPSP slope, 0.76 ($P < 0.001$); population spike latency, −0.78 ($P < 0.001$); and population spike amplitude, −0.34 ($P < 0.01$). In rats with two thermistors, implanted at the same depth in the same or opposite hemispheres, the bilateral activity-induced temperature changes were nearly identical (difference <0.15°C). Therefore, the temperature at the contralateral homotopic point could be used as a reference value. Warming the brain by radiant heating (Fig. 1B) gave a similar parallel increase in brain temperature and f-EPSP slope (n = 19). Increasing the temperature of the animal by letting it run on a treadmill yielded comparable effects (n = 15), with larger changes seen after the faster running speed (Fig. 1C). During treadmill runs of animals that were not fully habituated, there was an initial reduction of the f-EPSP slope, similar to the results of Green, McNaughton, and Barnes (*3*). Similar parallel changes of brain temperature and f-EPSP were seen in the responses of the olfactory bulb to lateral olfactory tract stimulation in exploring rats (n = 5; Fig. 1D).

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Parallel changes in the brain temperature and f-EPSPs also occurred during spontaneous behavior in an opaque test cage similar to the rat's home cage (six rats followed for 8 hours). Changes of up to 2°C in the brain temperature, closely linked to the f-EPSP increase, were observed whenever the rat moved from rest to activity. Conversely, both types of signal declined during inactivity and sleep.

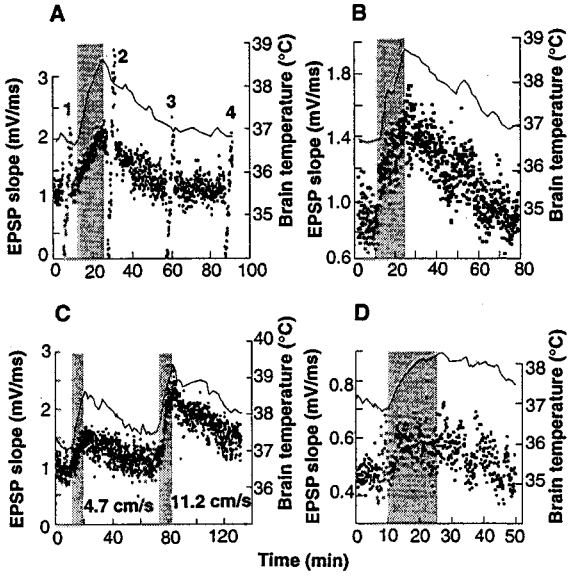

Fig. 1. Changes in f-EPSPs and brain temperature by exploration and treadmill running. (**A**) Brain temperature (continuous line, contralateral hippocampus) and dentate f-EPSP slope (dots) during and after 15 min of exploration (shaded column) of a platform (120 cm by 60 cm) with six to ten objects. Records that are marked 1 through 4 are input-output tests. Before and after exploration, the rat was left undisturbed for 10 and 60 min, respectively, in an opaque test cage (38 cm by 25 cm), to which the rat had habituated before the test. (**B**) Brain temperature and dentate f-EPSP slope in response to radiant heating (shaded column). The rat rested in the opaque test cage throughout the session. (**C**) Similar to (A) but dentate f-EPSP slope in response to 10 min of running on a treadmill (shaded columns) at speeds of 4.7 and 11.2 cm/s, respectively. (**D**) Brain temperature and f-EPSP in the olfactory bulb after stimulation of the olfactory tract during exploration (shaded column).

Because exploration was accompanied by relatively large changes in brain temperature, we tested whether swimming in a water maze produces brain cooling and, if so, whether any changes in f-EPSPs could be observed. Therefore, we measured the brain temperature and dentate f-EPSPs in rats that were swimming at different temperatures in a Morris water maze without a platform (9). In water of 18°C, the rat brain temperature rapidly decreased about 5°C (Fig. 2B). In parallel, the f-EPSP slope diminished and its onset was delayed (Fig. 2, A and C), whereas the population spike paradoxically increased both in size and latency (Fig. 2, D and E); these effects are the exact opposite of those observed during exploration. Similar but smaller changes were seen in water of 26°C. Hardly any change occurred at 33°C, and all changes reversed direction in water of 40°C. The observations were robust, appearing in all 55 trials in 15 animals. There was a strong correlation between brain temperature on the one hand and the f-EPSP slope (for the rat in Fig. 2, $r = 0.97$ and $P < 0.001$), population spike latency ($r = -0.99$ and $P < 0.001$), and population spike amplitude ($r = -0.58$ and $P < 0.001$) on the other. Equivalent results were observed for responses in the molecular layer of the dentate gyrus, the intrahippocampal synapses between the Schaffer collaterals and CA1 pyramidal cells (three rats), and the olfac-

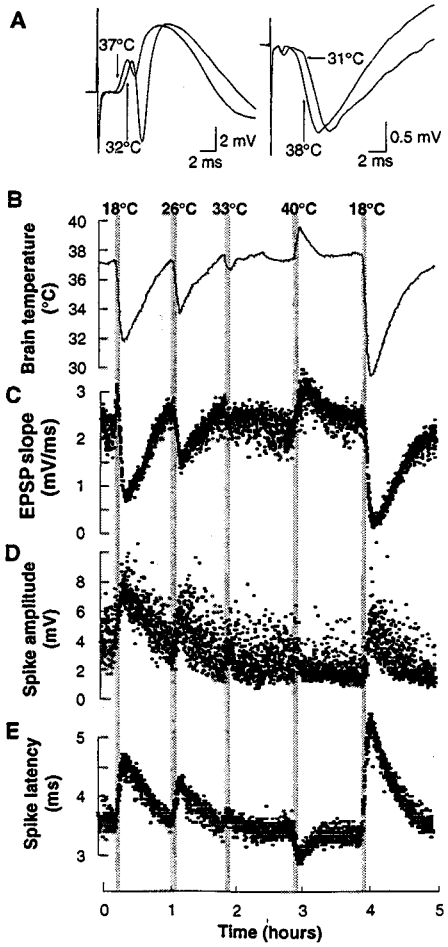

Fig. 2 (left). Changes in f-EPSP and brain temperature in a rat as it swam in a water maze 198 cm in diameter without a submerged platform at four different water temperatures. (**A**) Superimposed f-EPSPs of the dentate gyrus (left) and the olfactory bulb (right) in response to stimulation of the perforant path and the lateral olfactory tract, respectively, taken at the indicated brain temperatures before and after swimming in water of 18°C. (**B**) Brain temperature of the right hippocampal formation in a rat as it swam for five 5-min periods (shaded columns) with water at the indicated temperatures. (**C** to **E**) The EPSP slope and the population-spike amplitude and latency, respectively, of simultaneously recorded perforant path–dentate field potentials.

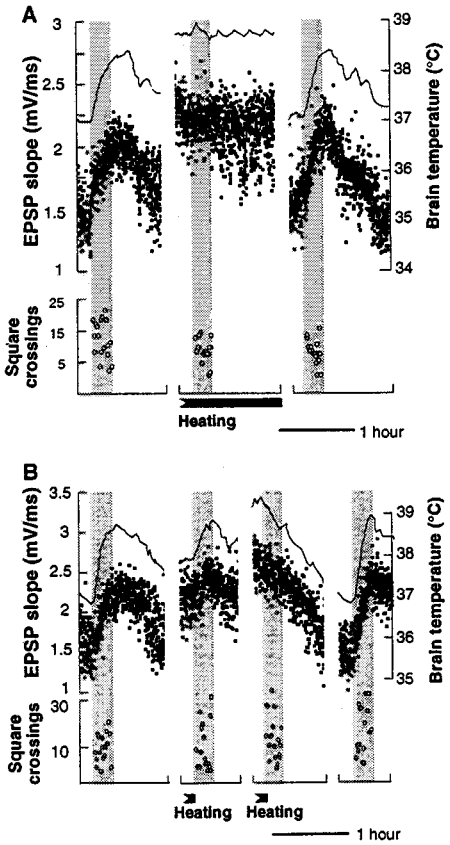

Fig. 3 (right). Dissociation of dentate f-EPSP changes from the exploratory behavior. (**A**) Records of brain temperature (continuous line, contralateral hippocampus), dentate f-EPSP slope (dots), and motor activity (open circles) during two 15-min control explorations that started at normal brain temperature (shaded columns, left and right panels) and during a similar period in which the rat had been preheated (horizontal thick bar) with an infrared lamp (middle panel). The exploratory activity was recorded by an observer and plotted as the number of border crossings of squares (8 cm by 8 cm) in the exploration area per 50 s. (**B**) Similar to (A) but the explorations (shaded columns) were started at various brain temperatures produced by preheating. The heating lamp was turned off at the start of exploration.

tory bulb (five rats) (Fig. 2A).

Finally, attempting to dissociate the f-EPSP changes from the exploratory behavior, we used radiant heat to bring the brain temperature to the maximum value that was obtained during exploration on the previous day. By intermittent infrared heating, the brain temperature was kept just above this temperature (Fig. 3A, middle). In five of five rats, exploratory activity had normal intensity under these conditions, but no further change in the f-EPSP was observed (Fig. 3A). Input-output tests (as in Fig. 1B) showed that the lack of additional changes in the f-EPSP was not due to a ceiling effect. In another series of experiments, the brain was warmed before the exploration. The magnitude of the exploration-induced potential changes depended on the increment in brain temperature and disappeared altogether at a sufficiently high starting temperature (Fig. 3B). Again, the exploratory intensity was unchanged from that of the control sessions. The dissociation of the exploratory behavior from the f-EPSP changes argues against a causal relation between the two processes.

Our results show a consistent relation between the field potential parameters and brain temperature, whether the latter is changed by heat produced by muscle activity or by artificial warming. In essence, the observed f-EPSP changes during exploration appear to be caused primarily by an increased brain temperature due to muscular heat production rather than by a learning-induced change in synaptic strength. The results represent a caveat for the interpretation that in freely moving rats changed f-EPSPs are signs of altered synaptic efficiency. They do not rule out the possibility that f-EPSP changes are produced by learning but they do indicate that such changes must be evoked independently of changes in brain temperature that are induced by activity, environment, or drugs.

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5. During exploration there was an unexpected relation between the f-EPSP and the population spike. Normally, when the f-EPSP increases the population spike grows and its latency diminishes. The paradoxical spike amplitude reduction with reduced latency during exploration is probably an effect of increased brain temperature. The larger f-EPSP and its shorter latency are both probably caused by a temperature-induced increased speed of transmitter release [B. Katz and R. Miledi, *J. Physiol. (London)* **181**, 656 (1965)], an effect of physiological significance. The reduced spike latency results from the f-EPSP increase. The effects of warming and of cooling on the population-spike amplitude are exactly opposite. Cooling gives three effects: (i) a small depolarization lowers the threshold for cell discharges, which causes more cells to fire; (ii) each discharging cell contributes a larger signal [A. L. Hodgkin and B. Katz, *J. Physiol. (London)* **109**, 240 (1949)]. and (iii) because each action potential in the cooled state is broader than it is in the warm condition [G. M. Schoepfle and J. Erlanger, *Am. J. Physiol.* **134**, 694 (1941)], the algebraic summation of the individual units to a compound potential results in a larger sum, in spite of the fact that the onset times are more spread out (less synchronous) than they are in the warm condition.
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8. Male Long Evans rats (250 to 500 g) were anesthetized with a mixture of chloral hydrate and pentobarbital (Equithesin, 1.0 ml per 250 g of body weight). Bipolar stimulation electrodes (SNEX 100; Rhodes Medical, Woodland Hills, CA) were implanted in the right angular bundle (7.5 to 8.0 mm posterior and 4.3 mm lateral to bregma), and a tungsten recording electrode was placed in the dentate hilus or granule cell layer (4.0 mm posterior and 2.6 mm lateral to bregma). Responses in the CA1 pyramidal layer to stimulation of Schaffer collaterals or olfactory bulb responses to lateral olfactory tract stimulation were recorded in some animals. A thermistor (0.5-mm diameter; 111-802 EAJ-B01, Fenwal Electronics, Milford, MA) was implanted contralaterally at the homotopic point of the recording electrode. Electrode and thermistor leads were connected to a socket fastened to the skull with dental acrylic. Before implantation, each thermistor was calibrated in a water bath against a precision thermometer. The thermistors allowed temperatures to be measured at a precision of 0.05° to 0.1°C. The rats were allowed 1 week of recovery before testing started. Behavioral testing was performed at 23°C (air temperature) and began 1 to 2 hours after the rat was connected to the recording equipment, when both the electrical and temperature records had reached stable values. Test f-EPSPs were elicited by a constant stimulus (100 to 500 μA, 50 μs) at 0.2 or 0.07 Hz. The slope of the f-EPSP was measured near its maximum as the amplitude difference at two fixed latencies. The population spike was taken as the vertical distance between the peak and a joint tangent to the preceding and succeeding positivities. The spike latency was defined as the time from stimulus onset to the spike peak.
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Impaired Spatial Learning after Saturation of Long-Term Potentiation

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Impaired Spatial Learning after Saturation of Long-Term Potentiation

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If information is stored as activity-driven increases in synaptic weights in the hippocampal formation, saturation of hippocampal long-term potentiation (LTP) should impair learning. Here, rats in which one hippocampus had been lesioned were implanted with a multielectrode stimulating array across and into the angular bundle afferent to the other hippocampus. Repeated cross-bundle tetanization caused cumulative potentiation. Residual synaptic plasticity was assessed by tetanizing a naïve test electrode in the center of the bundle. Spatial learning was disrupted in animals with no residual LTP (<10 percent) but not in animals that were capable of further potentiation. Thus, saturation of hippocampal LTP impairs spatial learning.

An important prediction of the hypothesis that activity-dependent synaptic plasticity in the hippocampus (such as LTP) plays a critical role in certain kinds of learning (*1*, *2*) is that physiological saturation of synaptic weights should disrupt new memory encoding. Saturation of an intrinsic pathway can be viewed as a neural state in which no further potentiation is feasible, at least for a period of time, at any site in the pathway (*3*). Repeated tetanization at a single site in the perforant path has been reported to block spatial learning when leading to cumulative LTP in the dentate gyrus (*4*), but this result has not been

replicated successfully (*5–7*), and some studies have even found enhanced learning (*8*). The reasons for this failure clearly include the possibility that the hypothesis is wrong but also that tetanization at a single site in the perforant path produces saturation only at selected synaptic loci and only along part of the longitudinal axis of the hippocampal formation (*9*).

Saturation is most likely to be achieved by an electrode array that straddles an afferent pathway and by a stimulation protocol that consists of multiple tetanization episodes with cathodal stimulation at different cross-sectional sites. The variable success of such an arrangement must be assayed by a separate test-stimulation electrode that selectively (but randomly) samples fibers within that pathway. If LTP can still be induced by tetanization of the test electrode, saturation cannot be claimed to have occurred. Thus, to reinvestigate the relation between saturation and spatial learning, we induced LTP through a multielectrode array across the angular bundle of the perforant path fibers in rats.

To increase the sensitivity of animals to the saturation of plasticity at synapses that might be used for learning, we first decided to decrease the volume of hippocampal tissue by making unilateral ibotenic acid lesions of the hippocampus and dentate gyrus (*10*). Two weeks later (day 14), the specially designed array of three bipolar stimulating electrodes and one recording electrode was implanted into the nonlesioned side of the brain. Two electrodes were implanted so that they straddled the angular bundle of the perforant path at the point passed by a high proportion of cortical afferents destined for the dorsal hippocampus (Fig. 1A) (*11*). The vertical placement of each electrode was adjusted so that the use of either the tip or the shaft of one of these concentric electrodes as a cathode and the use of either the tip or the shaft of the other electrode as an anode resulted in high-amplitude dentate field potentials (Fig. 1B). The field potentials were recorded by means of an electrode in the hilar zone of the ipsilateral dentate gyrus. Acute mapping experiments that were conducted under urethane anesthesia in nonlesioned animals revealed that cross-bundle stimulation was able to induce >10-mV amplitude field potentials at sites extending from the septal pole and along the dorsal 60% of the longitudinal axis of the dentate gyrus. We did not record signals in the temporal part of the hippocampus, which is unable to support spatial learning with the present training protocol (*12*).

The third stimulating electrode was positioned between the other two and aimed at the center of the perforant path (Fig. 1A). This served as a low-frequency (LF) test electrode during both baseline recording and induction of cumulative LTP. It also served as the tetanization test electrode to check whether the cumulative LTP that was induced from the other electrodes was saturated. An animal could be said to have saturated LTP if the cumulative LTP had reached an asymptote and if the later attempt to induce LTP from this separate electrode was unsuccessful. Once positioned, the electrodes were cemented in place, and the animals were allowed to recover from the acute effects of surgery for 2 weeks.

High-frequency (HF) tetanization was then conducted on a single day (day 28) with a cathode on one side of the bundle and an anode on the other side. All possible combi-

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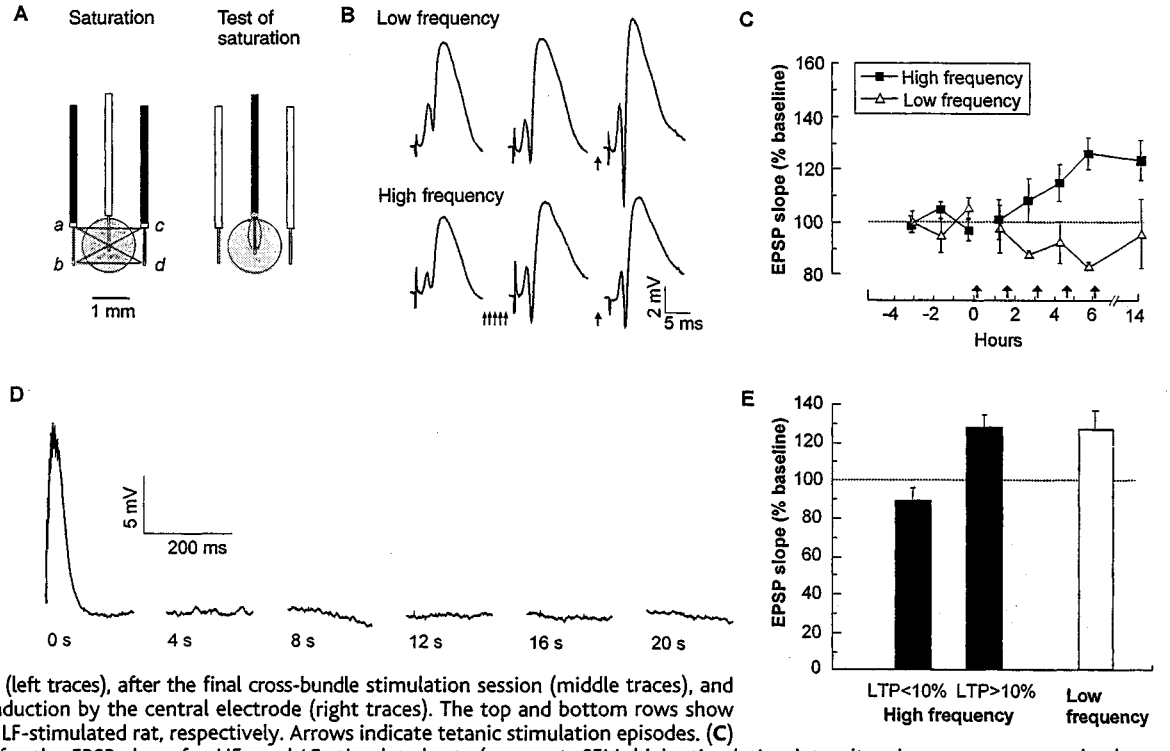

Fig. 1. Saturation of LTP in perforant path synapses of the dentate gyrus. (**A**) The placement of two bipolar electrodes on each side of the medial and lateral parts of the angular bundle (black bars) and one bipolar electrode in the middle (white bar). (Left) Induction of LTP by cross-bundle tetanic stimulation (horizontal and diagonal lines), with anode and cathode sites at different sides of the bundle (a, b, c, and d). (Right) A test of LTP saturation by tetanic stimulation through the naïve central electrode at the end of the experiment. (**B**) Representative evoked potentials shown before cross-bundle stimulation (left traces), after the final cross-bundle stimulation session (middle traces), and after the residual LTP induction by the central electrode (right traces). The top and bottom rows show traces from a HF- and a LF-stimulated rat, respectively. Arrows indicate tetanic stimulation episodes. (**C**) The normalized values for the EPSP slope for HF- and LF-stimulated rats (means ± SEM, high stimulation intensity, six responses per animal per session). The recording was conducted at 1.5-hour intervals, except for the last session, which occurred 7 hours after the end of the last tetanic stimulation. The cross-bundle stimulation (arrows) was delivered immediately after the third through seventh recording sessions at 0, 1.5, 3, 4.5, and 6 hours. The HF stimulation gradually increased the fEPSP slope values. Error bars indicate SEM. The dotted line indicates the mean EPSP slope during baseline recording. (**D**) Representative traces taken during tetanic stimulation at 0 s and at 4-s intervals during the minute after tetanization. There is an absence of afterdischarges. (**E**) The change in the EPSP slope at the highest stimulation intensity for 1 hour after tetanic stimulation at the central stimulation electrode at the end of the experiment. The animals were considered to have residual LTP if the slope of the fEPSP was enhanced by >10% at this pulse intensity. Error bars indicate SEM. The dotted line indicates the mean EPSP slope during baseline (2 min before tetanization).

nations of cathode (tip or shaft) and anode (tip or shaft) were used (Fig. 1A) (*13*). The animals were placed in dark, enclosed chambers in which, to reduce the attenuation of LTP by stress (*14*), they had been familiarized on the preceding 3 days. After baseline responses had been sampled, five series of cross-bundle tetanization episodes were given, starting at 0, 1.5, 3, 4.5, and 6 hours after the last baseline recording. The fifth episode was an anode and cathode arrangement that was identical to the first episode in order to check whether there would be further cumulative potentiation. Low-frequency control animals received the same stimulation sequence of cathode and anode locations, but only single pulses were given at each location. Nonstimulated (NS) controls, with electrodes implanted, were handled and placed in the recording chambers.

The stimulation resulted in cumulative LTP with waveforms showing a gradual increase in the early rising portion of the extracellular field potential (Fig. 1B, middle trace) over the course of the recording period (Fig. 1C). Little change was seen immediately after the first tetanization episode, possibly because the test electrode used for the measurement of the degree of potentiation was in the center of the angular bundle. The field potential slope at 7 hours after the last tetanization session was significantly elevated above the pre-tetanization baseline in the HF group and significantly elevated above the LF group [groups: $F(1,13) = 6.7$, $P < 0.05$; groups × session: $F(4,52) = 3.3$, $P < 0.05$] (F is the variance ratio and P is the probability). The level of LTP after the fifth episode of tetanization was comparable to the level after the fourth episode, with the mean LTP level of fibers in the center of the perforant path being comparable to the level obtained in studies where the tetanization electrodes were placed in the center of the bundle (*4–7*). Thus, cross-bundle stimulation did not induce a greater magnitude of LTP than previous studies did, but the cross-bundle stimulation may have induced LTP on a higher proportion of fibers afferent to the hippocampal formation. The trend toward a slight decline in slope in the LF group may be a temperature effect (*15*), as these animals became less active across the recording sessions; however, no direct recordings of hippocampal temperature with implanted thermistors were made in this study. High-frequency cross-bundle tetanization did not result in seizures in traces recorded at 4-s intervals for 1 min after each tetanization in a subset of eight animals (Fig. 1D) (*16*).

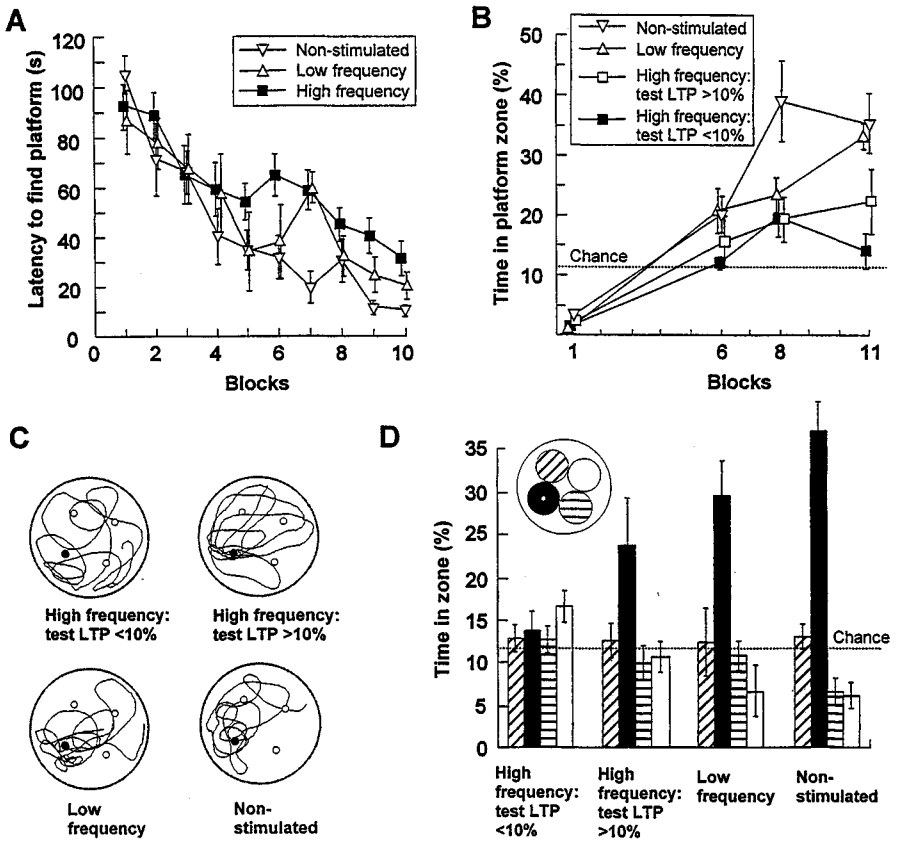

Fig. 2. The effect of LTP saturation on performance in a water maze learning task (means ± SEM). **(A)** The latency to enter the platform of rats receiving HF stimulation, LF stimulation, or no stimulation at all. Error bars indicate SEM. **(B)** The development of spatial behavior across trial blocks in tetanized rats (with and without residual LTP at the central stimulation electrode) and LF and NS control rats. The search time in a circular area (radius of 35 cm) around the platform zone was measured during the first 40 s of four trials with the platform submerged to the bottom of the pool. The dotted line indicates the chance level. Error bars indicate SEM. **(C)** Records of the search pattern of a representative animal from each group during the final spatial probe test (60 s). **(D)** Time spent inside a circle (radius of 35 cm) around the platform position (black bar) and in corresponding, equally large zones in the three other pool quadrants (diagonally striped, horizontally striped, and white bars) during the final spatial probe test (60 s). The dotted line indicates the chance level. Error bars indicate SEM.

After the last recording session, all animals were trained in an open-field water maze to find a platform that was hidden at a single location in the pool (*17*). All animals showed a decline in escape latency across the 10 trial blocks of training (Fig. 2A). Analysis revealed significant effects of groups [$F(2,24) = 5.4$, $P < 0.01$] and groups × block [$F(18,216) = 2.4$, $P < 0.001$] that reflect the higher mean escape latency of the tetanized group toward the end of training. Probe tests, in which the pneumatic platform was kept submerged for the first 40 s of the trial before raising, showed a gradual increase in time spent in a platform zone of 35-cm radius around the center of the platform in blocks 1, 6, 8, and 11 (the final probe test) (Fig. 2B). Low-frequency and NS test animals showed the most focused searching in the correct zone, with representative swim paths shown in Fig. 2C. High-frequency test animals showed a distribution, with some animals doing quite well but with most animals swimming all over the pool with no spatial bias toward the target area. Statistical analysis revealed significant groups × quadrants [$F(6,72) = 8.5$, $P < 0.001$] and groups × quadrants × probe test [$F(18,216) = 2.2$, $P < 0.005$] interactions.

The reason for the distribution of the search pattern by individual animals in the HF group became apparent when we returned the animals to the recording chamber to examine the extent to which the cumulative LTP that was previously observed reflected a true saturation of synaptic plasticity (*18*). The critical test involved the use of the stimulating electrode located at midbundle as a new site at which to induce LTP (Fig. 1E). Up to this point, the midbundle electrode had only been used for LF test pulses. Midbundle tetanization gave LTP [defined as a >10% enhancement of the slope of the field excitatory postsynaptic potential (fEPSP)] in all LF test animals. The HF group was divided into animals that showed <10% LTP on the test pathway (the saturated subgroup; $n = 7$) and animals that showed >10% LTP (the nonsaturated subgroup; $n = 6$). Analysis of the potentiation induced on the test pathway showed a significant effect [groups $F(2,15)$

= 10.3, $P < 0.005$] (*19*). The hypothesis that saturation of LTP will result in a learning deficit predicts that the saturated subgroup should have learned less about the location of the hidden platform than the nonsaturated subgroup. This prediction was upheld (Fig. 2, C and D). An analysis of variance (ANOVA) of the proportion of time spent in the target zone during the final transfer test revealed an overall difference between groups [$F(3,26) = 7.5$, $P < 0.001$]. Subsequent planned orthogonal comparisons revealed that the animals with >10% residual LTP did not differ from the LF group ($F = 1.1$, not significant), but these two groups performed better than the animals with <10% residual LTP ($F = 7.7$, $P < 0.025$). These three groups, all of which had electrodes implanted and were stimulated, also performed more poorly than the NS controls. Thus, successful saturation of LTP did impair spatial learning in the water maze.

These results uphold a key prediction of the "LTP and learning" hypothesis and can explain previous failures to see the effects of cumulative LTP on spatial learning (*5–7*). First, previous studies used only single bipolar tetanization electrodes in the angular bundle of the perforant path and may have activated only a small proportion of the entorhinal afferents. Thus, some studies would succeed in seeing a behavioral effect of tetanization and others would not. Second, our use of animals with a unilateral hippocampus may have increased the sensitivity of the behavioral task to a disturbance of synaptic plasticity in the dorsal hippocampus, which is the region of hippocampal formation whose integrity is essential for this form of spatial learning (*12*). Third, previous studies did not check whether the cumulative LTP was, in practice, saturated. Assuming that our test electrode sampled a representative subset of fibers traveling in the angular bundle, its use constitutes an independent identification of animals that show saturated LTP from those that merely show cumulative LTP. Although it was not possible to induce further LTP by means of the test electrode in the subset of animals that failed to learn where the platform was located, we do not know the proportion of maximally potentiated synapses in these animals (*3*). However, the effects of saturation of LTP on subsequent learning are likely to follow a sigmoidal function where deleterious effects will be observed well before a saturation maximum is achieved (*7*).

The fact that impaired and nonimpaired animals in the tetanized group received identical stimulation suggests that a blockade of learning after saturation of LTP is unlikely to be caused by nonspecific side effects of the HF stimulation of large populations of fibers (*20*). Although such side effects remain a theoretical possibility, their deleterious effects on behavior would have had to covary with the capacity to induce residual LTP on the terminals of the perforant path. The induction of seizures could be such a factor (*6*), but afterdischarges were not seen with our stimulation paradigm.

The procedure of cross-bundle tetanization of the perforant path demonstrably induced LTP in the dentate gyrus, but the procedure may also have induced LTP in the terminal zone of the perforant path in area CA3 or may have affected synaptic transmission at synapses at the outer dendritic portion of area CA1, where fibers emanating from layer III entorhinal cells terminate. Some LTP may also have been induced transsynaptically (*21*). Thus, this tetanization procedure does not speak directly to the issue of whether a blockade of dentate LTP alone is sufficient to impair spatial learning. The possibility that dentate LTP is unimportant has recently been raised by studies of mice harboring mutations of genes that affect dentate but not CA1 LTP (*22*). Further analyses of this mutant have revealed, however, that some residual LTP is present when studied in freely moving mice (*23*).

Several current models of hippocampal function emphasize its role as a distributed associative memory system that is responsible for capturing event-related information online with an LTP-like synaptic mechanism (*2, 24*). In these models, the distributed nature of information representation within the hippocampus and dentate gyrus provides opportunities for pattern completion in response to partial cues. Also, these models predict that artificial saturation of synaptic weights across a substantial proportion of cortical afferents should disrupt the representational capacity of the system and hence disrupt learning. Our results support those models in indicating that saturation of LTP can disrupt one form of hippocampal-dependent learning.

The link between LTP and learning rests on three pillars: blockade, saturation, and erasure. The disruption of spatial learning associated with a blockade of hippocampal LTP is well established (*25*). The present findings reestablish the predicted impairment of learning after saturation of LTP. However, it remains to be shown that an erasure of LTP causes forgetting.

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3. We view saturation of an intrinsic pathway as a neural state in which the pathway cannot be further potentiated in the intact and awake animal. It is unlikely that any method of physiological stimulation will potentiate all synapses of the perforant path to their maximum values. Many synapses are thought to be silent [D. Liao, N. A. Hessler, R. Malinow, *Nature* **375**, 400 (1995); J. T. Isaac, R. A. Nicoll, R. C. Malenka, *Neuron* **15**, 427 (1995)]. Their whole-scale potentiation would render a brain region susceptible to hyperexcitability or even seizures, a state that may ordinarily be prevented by intrinsic inhibitory activity or rapid depotentiation.
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10. Forty-three naïve male Long-Evans rats (300 to 450 g), which were housed in pairs, were on two occasions anesthetized with Equithesin, a mixture containing chloral hydrate and pentobarbital (1.0 ml per 250 g of body weight). During the first surgical session, all rats received complete unilateral hippocampal lesions by the injection of ibotenic acid (Biosearch Technologies, San Rafael, CA) at 14 sites [modified from L. E. Jarrard, *J. Neurosci. Methods* **29**, 251 (1989)]. Ibotenic acid was dissolved in phosphate-buffered saline (pH 7.4) at 10 mg/ml and injected with a 1-μl Hamilton syringe that was mounted to the stereotaxic frame.
11. Two weeks after the induction of the lesions, three bipolar stimulation electrodes (SNEX 100; Rhodes Medical, Woodland Hills, CA) were implanted in the angular bundle of the intact hemisphere 7.0 mm behind and 3.0, 4.0, and 5.0 mm, respectively, lateral to the bregma. A stainless steel recording electrode was placed in the dentate hilus or granule cell layer (3.8 mm behind and 2.4 mm lateral to the bregma). Electrode leads and contacts were encased in dental acrylic, and the animal was allowed 2 weeks for recovery.
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13. Two weeks after implantation, evoked waveforms were recorded in the dentate gyrus at 1.5-hour intersession intervals in response to perforant path stimulation. Recording started 5 min after the rat had been placed in a dark, enclosed recording chamber. Waveforms were sampled in the dentate gyrus in response to constant square-wave pulses (100 μs, 0.1 Hz) delivered to the perforant path at three intensities that were adjusted to give population spikes of 0, 1, and 3 mV, respectively (80 to 1000 μA). The slope of the fEPSP was measured as the amplitude difference at two fixed latencies in the middle of the rising phase of the potential. After the third through seventh recording sessions, the rats received either HF stimulation ($n = 17$), LF stimulation ($n = 12$), or no stimulation at all ($n = 14$). Pilot experiments failed to show more saturation (less residual LTP) in rats receiving LTP across several days than in rats receiving a single day of massed stimulation, possibly because of slowly developing homeostatic changes in synaptic weights in populations undergoing substantial up- or down-regulation of synaptic transmission [G. G. Turrigiano, K. R. Leslie, N. S. Desai, L. C. Rutherford, S. B. Nelson, *Nature* **391**, 892 (1998)]. Thus, a massed stimulation protocol was adopted, with the HF-stimulated rats receiving a total of five episodes of tetanic stimulation at 1.5-hour intervals between the four stimulation sites of the cross-bundle stimulation electrodes. This 1.5-hour interval was used to obtain an optimal saturation of LTP induction in the stimulated pathways, taking into account the fact that LTP does not preclude the further induction of a potentiation during a late

phase of previously induced LTP [U. Frey, K. Schollmeier, K. G. Reymann, T. Seidenbecher, *Neuroscience* **67**, 799 (1995); U. Frey and R. G. M. Morris, *Nature* **385**, 533 (1997)]. The tetanic current was passed between pairs of the four poles of stimulation sites (a, b, c, and d) of the two cross-bundle stimulation electrodes (Fig. 1A, left). The choice of anode and cathode was systematically altered between tetanization episodes, subject to the constraint that anode and cathode were always on opposite sides of the angular bundle. In each episode, eight pulse trains (each consisting of eight stimuli at 400 Hz) were first passed at 2-s intervals between two of the poles (for example, a and c); 1 min later, a similar train was given at the opposite polarity. Then, after another 1-min interval, the whole sequence was repeated with the two other poles (for example, b and d). The choice of the anode and cathode pairs was as follows: ac and bd (episode 1), ad and bc (episode 2), bd and ac (episode 3), and bc and ad (episode 4). The fifth episode was a repetition of the first (ac and bd). Electroencephalogram epochs were recorded at 4-s intervals for 1 min after each tetanization (all combinations of stimulation across the bundle) in a subset of eight tetanized animals, and the samples were screened carefully for afterdischarges. Control rats (also lesioned and implanted) also received eight pulses at 2-s intervals, which were repeated twice within each stimulation episode. In both groups, the intensity was adjusted to evoke fEPSPs at 80 to 90% of the maximum obtained with these electrodes (500 to 2000 μA and 100-μs pulse width). Nonstimulated rats also received a unilateral hippocampal lesion, and 9 rats out of 14 were implanted.

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16. Four of these animals showed <10% on the test for residual LTP (*18*).
17. Behavioral testing was conducted in a water maze, a 198-cm-diameter pool with a featureless white surface, filled to a depth of 40 cm with water at 25° ± 2°C [R. G. M. Morris, *J. Neurosci. Methods* **11**, 47 (1984)]. Latex liquid was added to make the water opaque. A pneumatic escape platform (11 cm in diameter) was located at a fixed position midway between the center and the periphery of the pool. The platform could be moved vertically between an upper available position (1.5 cm below the water surface) and a lower unavailable position (22 cm below the water level) by remote control. Behavioral training started 7 hours after tetanic stimulation was completed. The rats were trained hourly in blocks of two trials, which were separated by 15 s (a total of 10 blocks, corresponding to trials 1 through 20), and were released from one out of eight equally spaced start positions along the perimeter of the pool in a pseudorandom predetermined order. If the rat failed to find the platform within 120 s, the rat was guided onto it. The rat was always left on the platform for 30 s. The position of the black head of the swimming rat was identified and stored at 10 Hz by a video tracking system (VP200, HVS Image, Hampton, UK; Watermaze Software, Edinburgh, UK). Probe tests (with the platform initially unavailable) were conducted on the first trial of blocks 1, 6, and 8 to assess the spatial precision of the search behavior. The platform was kept on the bottom of the pool for the first 40 s and then raised. A final transfer test with the platform submerged for 60 s was conducted at the end of training (called block 11, although consisting of only one trial). On probe trials during training, the latency to cross the platform location was substituted for the actual latency to climb the platform.
18. The extent of saturation at perforant-path/granule-cell synapses was estimated after the completion of the water maze training by tetanizing the fibers activated by the central electrode. The tetanization consisted of two blocks of eight 400-Hz pulses repeated eight times at 2-s intervals and at the same polarity. There was a 1-min interval between the blocks. The tetanization intensity was adjusted to evoke fEPSPs at 80 to 90% of maximum, as above. After the completion of these tests, the rats were killed with an overdose of Equithesin and perfused intracardially with saline and 4% formaldehyde. The brains were stored in formaldehyde for >1 week. Frozen sections were cut coronally (25 μm) and stained with cresyl violet, and the sections were examined for hippocampal and extrahippocampal damage. Sixteen animals (4 HF, 6 LF, and 6 NS) were excluded because of neocortical or thalamic lesions or because of incomplete hippocampal lesions. The exclusion of these animals did not change the pattern of results. Analyses conducted on the entire data set ($n = 43$) gave group [$F(2,40) = 4.3$, $P = 0.02$] and groups × block [$F(18,360) = 2.0$, $P < 0.005$] effects on escape latency and gave a groups × quadrants effect on the probe tests [$F(6,120) = 3.3$, $P < 0.005$].
19. The population spike increased 0.61 ± 0.30 mV (HF group) and 1.34 ± 0.68 mV (LF group). The increase in the HF group was not related to fEPSP enhancement.
20. This conclusion is corroborated by a pilot experiment suggesting that the disruption of spatial learning after LTP saturation is reversible. Six animals receiving HF stimulation were impaired when tested subsequently in a delayed-matching task in the water maze [R. G. M. Morris, J. J. Hagan, J. N. P. Rawlins, *Q. J. Exp. Psychol.* **38B**, 365 (1986)]. These animals showed no improvement in escape latency from the first to the second trial (trial 2 latencies were, on average, 6.5 s longer; there was an intertrial interval of 2 hours). One month later, when LTP had decayed, the animals showed clear evidence of learning from trial 1 to trial 2 on the same test. Latencies were 20.9 s shorter in trial 2 than in trial 1. In NS control rats, the difference was 34.0 s.
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An increase in dendritic spine density on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses

(hippocampus/plasticity/confocal microscopy/complex environment)

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ABSTRACT **The search for cellular correlates of learning is a major challenge in neurobiology. The hippocampal formation is important for learning spatial relations. A possible long-lasting consequence of such spatial learning is alteration of the size, shape, or number of excitatory synapses. The dendritic spine density is a good index for the number of hippocampal excitatory synapses. By using laser-scanning confocal microscopy, we observed a significantly increased spine density in CA1 basal dendrites of spatially trained rats when compared to nontrained controls. With unchanged dendritic length, the higher spine density reflects an increased number of excitatory synapses per neuron associated with spatial learning.**

The hippocampal formation is closely related to spatial learning. This conclusion is based upon the presence of cells signaling the position of the animal in space and the interference with the ability to learn a spatial environment following mechanical or chemical inactivation of the hippocampus and neighboring cortex (1–4). Many of the hippocampal synapses have plastic properties, which may play a role in the learning process (5–7). Since learning effects are long-lasting, structural changes of hippocampal synapses are possible correlates to spatial learning. Among possible changes, the alteration of the size, shape, or number of excitatory synapses is among the most likely ones. Because virtually all excitatory synapses on hippocampal pyramidal cells contact dendritic spines (8), the number and distribution of these structures may be taken as an index of synaptic changes. Training in a complex environment causes spatial learning (9–12). Whereas exposure to an enriched environment gives various structural changes in the visual cortex, there are few reports on such effects in the hippocampus (13). Using two-dimensional electron microscopy, Altschuler (14) found an increased number of synapses on CA3 cells in young rats after training in an enriched environment. In the absence of information on dendritic length, which is environmentally modifiable (15), it is uncertain how such changes translate to spine density. Further, the large increase in number of boutons (82%), observed during a developmental period with rapid changes of spine density (16), need not be related to learning, but to normal development. Wenzel *et al.* (17) reported increased CA1 spine density after training, but the selected brightness discrimination learning probably does not depend upon the hippocampal formation (18). Further, the spine density for the control and experimental material reported by Wenzel *et al.* (17) were both within the normal spine density range given by Andersen *et al.* for guinea pigs (19).

On this background, we chose a hippocampus-dependent task. We tested whether or not spatial training of rats can give changes in dendritic spine density in the CA1 field of the hippocampus. To avoid interference with developmental processes, we used adult animals.

MATERIALS AND METHODS

Environment. Adult male rats (250–460 g) were kept together in a large (2.5 × 2.0 × 1.7 m) cage with up to five floors mounted at various positions and connected with narrow ladders (Fig. 1*A*). Items expected to generate exploration (wooden blocks, freshly cut wood chips, branches, fresh leaves, plastic containers, paper bags) were distributed on the floors. Water and food bowls were hidden. All floors, ladders, and the position of water and food were changed between sessions. The rats were exposed to this environment for 4 hr/day for 18 days (behavioral study, $n = 7$) or 14–30 days (morphological study, $n = 13$). Between the exposures, the rats were housed in groups of 6 or less in transparent cages (59 × 39 × 20 cm). The activity of the animals (number of floors visited, area covered, latency to new floors, latency to all floors) was monitored by two independent observers during the first hour of each training session. Control rats were either housed in pairs ($n = 8$) in transparent cages (37 × 22 × 18 cm) or individually ($n = 7$) in opaque cages (33 × 20 × 18 cm). All rats were food-deprived for 19 of the preceding 24 hr.

Water Maze. In a subset of rats ($n = 7$, trained; $n = 10$, paired; $n = 9$, isolated) spatial learning was tested in a water maze (20), a circular pool (diameter 198 cm), filled to a depth of 40 cm with water to which milk was added. A platform was positioned in one of four possible places, halfway between the center and periphery. Each rat was assigned a platform position that was maintained throughout the experiment. The rats were trained in two daily sessions 4 hr apart. Each session consisted of four trials 40 s apart. The rat swam from randomized start positions. If the rat failed to find the platform, it was guided to it after 120 s. All rats were left on the platform for 30 s. The position of the rats was monitored by a vertically mounted camera and stored at 10 Hz. When all rats had learned to swim directly toward the platform (after session 9), the environment was restructured, curtains were drawn to form a square around the pool at a distance of at least 1 m, and cues were attached. After the environmental change, the platform was located in a new, fixed position on all trials.

Staining and Preparation. Animals assigned to the morphological study ($n = 26$) were exposed to one of three environments ($n = 11$, trained rats; $n = 8$, paired rats; $n = 7$, isolated rats) but were not tested in the water maze. The animals were deeply anesthetized with halothane and decapitated by a colleague in the laboratory, so that the investigators remained ignorant of the behavioral history of the animals. The brain was

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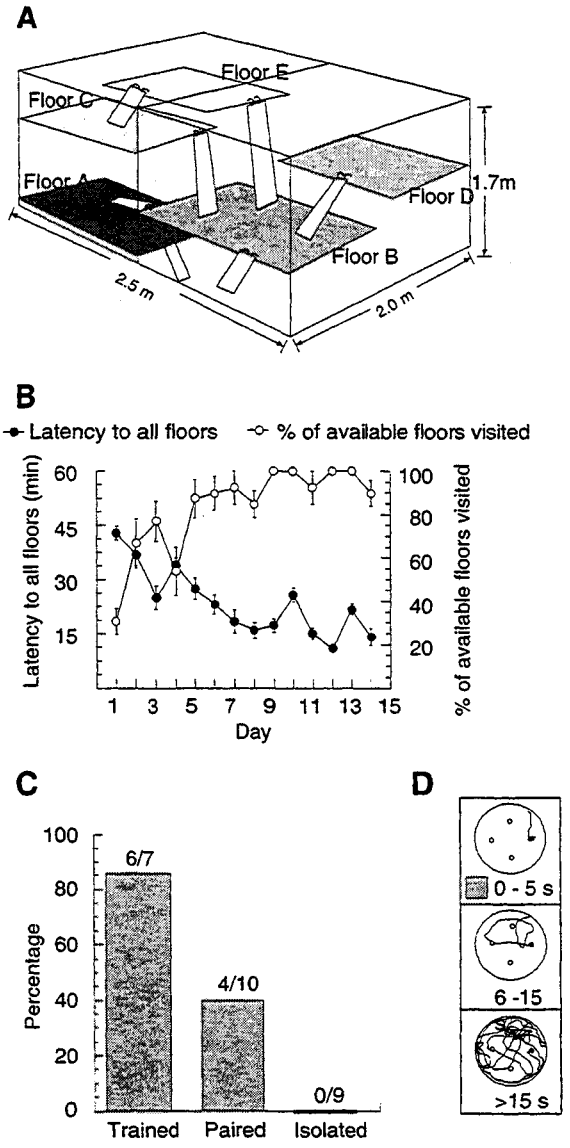

FIG. 1. Spatial training in the complex environment and testing of the acquired learning in a water maze. (*A*) Diagram of the complex environment. (*B*) Exploration intensity during the first hour of the total 4-hr exposure in the complex environment, expressed as the mean latency for all animals ($n = 11$) to reach floors (●) and as the percentage of available floors visited during the first hour (○). (*C*) Percentage of rats belonging to the best category (0–5 s, in *D*) on trial 2 in a new water maze environment. (*D*) Swim paths of three rats, in the good (0–5 s), medium (6–15 s), and poor learning categories (>15 s).

taken out and the hippocampus was dissected free in cold (1–3°C) artificial cerebrospinal fluid (in millimolar: NaCl 124, $NaHPO_4$ 1.25, KCl 3, $MgSO_4$ 2, $CaCl_2$ 2, $NaHCO_3$ 26, glucose 10), and 400-μm-thick slices were cut with a vibratome. The slices were placed in the interface between artificial cerebrospinal fluid from below and a humidified gas mixture (95% O_2/5% CO_2) above. The inner part of the gas chamber was kept at 32 ± 0.5°C. Fiber-containing electrodes were filled with Lucifer yellow (4% in 0.2 M LiCl; resistance, 75–200 MΩ). After 1 hr in the bath, CA1 cells were penetrated and the dye was injected with hyperpolarizing pulses (1.5 nA). Adequate spine staining required the cells to show action potentials above 80 mV and a duration of <2 ms. After filling, the slices were fixed in paraformaldehyde (4%), dehydrated in an alcohol series (75%, 85%, 95%, 96%, and 100%), cleared in methyl salicylate, and coverslipped in DPX (BDH).

Morphological Analysis. Only cells with an extensively filled dendritic tree without obvious truncations were analyzed in a laser-scanning confocal microscope (Phoibos 1000 Sarastro, equipped with the software package Image Space, Molecular Dynamics). The data were stored on optical disks for later analysis. A 40× objective (numerical aperture, 1.3) was used for an overview of cells by making projections from section series (image size, 512 × 512 pixels; pixel size, 0.5 μm; step size, 3.0 μm; 40–80 sections). Dendritic length and branching pattern were calculated with the programs PDENLEN and NEUREC (unpublished) provided by T. Blackstad (Department of Anatomy, University of Oslo). For the spine counts, four or five dendritic segments were picked from the middle portion of the basal dendrites. For detailed analysis, sections were scanned with a 100× objective (numerical aperture, 1.3) (image size, 256 × 256 pixels; pixel size, 0.1 μm; step size, 0.1 μm; 20–70 sections). The criteria for spine detection followed those given in ref. 21. The data analysis was made blind with a subset of spines studied by all three investigators. Student's *t* test for comparison gave no significant difference between either pair of the three investigators' analyses.

RESULTS AND DISCUSSION

Spatial Learning. We first wanted to ascertain that the training caused an increased spatial learning ability. During the spatial training period in the complex environment, the animals gradually increased their activity both with regard to the numbers of visited floors and the total area covered (Fig. 1*B*).

We tested the effect of the spatial training by recording the animals' performance in a Morris water maze. The spatially trained animals acquired the standard water maze task faster than both control groups [$F(2, 23) = 4.16$, $P < 0.03$]. However, after six or seven training sessions (four trials each), animals from all groups found the hidden platform equally fast and showed similar precision in a test without the platform. At the end of the acquisition period, the animals were tested for their ability to solve a new task of the same nature. A new environment was made by fastening cues to curtains forming a square around the water maze at a distance of at least 1 m. The animals trained in the complex environment acquired the new platform position faster and swam more directly to the new position on the second trial than the nontrained animals (Fig. 1 *C* and *D*, $P < 0.05$, one-sided Wilcoxon test). The latency to find the platform on the second trial (mean ± SEM) was 4.7 ± 0.6 s in the trained group against 8.9 ± 1.4 s in the paired and 25.2 ± 9.3 s in the isolated groups. The mean number of pool quadrants entered before reaching the platform was 1.0 ± 0, 2.6 ± 0.6, and 5.3 ± 1.6 for the same groups, respectively. Thus, the spatial training in the complex environment gave an improved acquisition rate of the water maze tasks, even though the three groups reached similar performance levels with repeated training.

Spine Density. Another group of animals was similarly trained in the complex environment for 14–30 days. After training, slices were taken from these rats and from two groups of control animals as before. With our technique based on laser-scanning confocal microscopy of cells injected with Lucifer yellow (21), a large number of spines were counted from various portions of identified neurons. Satisfactory large and representative samples, coupled to a high rate of spine detection, make the technique useful for measurement of spine density changes following experimental interference. Fig. 2 shows examples of basal dendritic segments taken from two different CA1 cells to illustrate the appearance of spines and the variability of the spine density. Cells taken from rats after intense spatial training had a spine

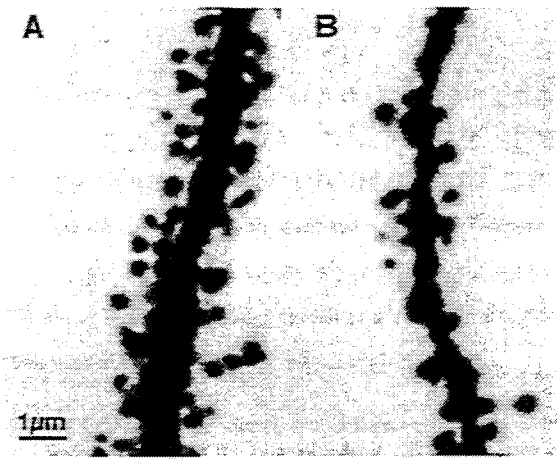

FIG. 2. Examples of parts of two basal dendritic segments to illustrate the variation of spine density. The spine densities were 2.71 spines per μm (trained rat, *A*) and 1.75 spines per μm (isolated rat, *B*).

density of 1.96 ± 0.09 spines per μm (mean ± SEM; n = 11 rats, 23 cells, 137 dendritic segments, and 7460 spines). The spine density of the paired control group was 1.77 ± 0.09 (n = 8 rats, 13 cells, 74 segments, and 3720 spines) and measured 1.78 ± 0.06 spines per μm in the isolated control group (n = 7 rats, 10 cells, 68 segments, and 3460 spines). The distribution of spine densities is shown in Fig. 3. Because the values from the two control groups were similar, these were combined for the statistical analysis. The difference in mean spine density between the spatially trained and the pooled control groups was statistically significant [Wilcoxon, one-sided test, $W(11, 15) = 181$, $P < 0.05$]. The total dendritic length and the degree of dendritic branching were similar in the three animal groups. Thus, the enhanced spine density in the trained group is not due to an altered size of the sampled neurons.

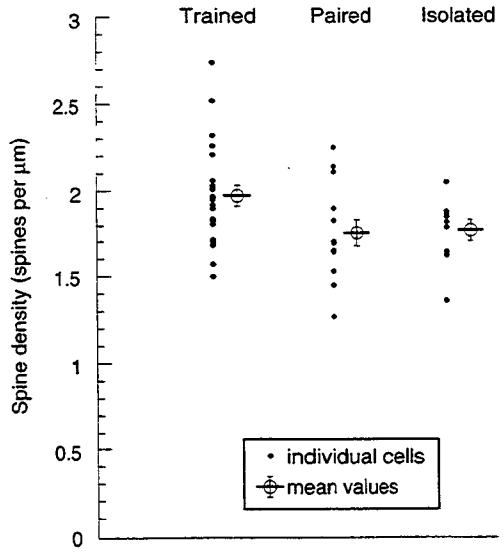

FIG. 3. Distribution of spine densities (spines per μm; filled dots) in the trained, paired, and isolated groups. Each dot represents the mean of all segments from one cell. Circles and horizontal lines give the means and the vertical lines give the SEM of the spine density in these cells in the three groups.

There was an appreciable variability between different dendritic segments from the same cell and from different cells and rats, with figures ranging from 0.90 to 2.99 spines per μm (cell means ranging from 1.26 to 2.73 spines per μm). This fact underlines the need to obtain a large sample of segments to get a representative sample for the analysis. The density of spines was considerably higher than found with Golgi impregnated material (22).

In conclusion, spatial training of adult rats by exposing them to a spatially challenging environment is associated with an increased spine density on CA1 pyramidal cells. The trained animals also showed increased learning ability as signaled by a faster acquisition in a water maze task. Because the total dendritic length was unchanged, the increased spine density means a higher number of synapses per cell, and thus an altered connectivity, as a result of spatial learning. These findings demonstrate that behavioral training can induce structural change in the hippocampal cortex of adult rats.

We thank L. Scholte and T. Eriksen for technical assistance, S. Ringvold for help in observing the rats in the complex environment, T. Blackstad for valuable discussions and providing programs to calculate dendritic features, and E. Moser, O. Paulsen, and T. Egeland for valuable discussions. This research was supported by the Norwegian Medical Research Council, Grants 103582/310 and 101416/320.

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Spatial Training in a Complex Environment and Isolation Alter the Spine Distribution Differently in Rat CA1 Pyramidal Cells

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ABSTRACT

The hippocampus is critically involved in spatial learning. Spatial training in adult rats, which improved their spatial learning ability, increased the number of excitatory hippocampal CA1 spine synapses on basal dendrites as compared with either isolated or standardly housed animals (Moser et al. [1994] Proc. Natl. Acad. Sci. USA 91:12673–12675). In this article, we report that spine synapses on oblique apical dendritic branches do not increase in density or number after the same type of training. When examining the variability of the spine density on basal CA1 dendrites by using variance component analysis, the variance associated with the cells was twice as large in all three groups as that coupled to the rats. Analysis of the spine density plots shows that the enhanced spine density after spatial training is found in most cells recorded from the trained group but that a small subset of CA1 neurones are particularly well supplied with spines. The trained group had a significant right-skewed tail of the spine distribution, i.e., training caused high spine density to occur in a small subset of dendritic segments. Conversely, the isolated group had a significant left-skewed spine distribution, indicating that some of the dendritic segments were undersupplied with spines, whereas the paired group displayed no asymmetry. J. Comp. Neurol. 380:373–381, 1997.

Indexing terms: hippocampus; dendritic spines; synapse formation; confocal microscopy

The hippocampus is both involved in and necessary for learning and memory of spatial relations (O'Keefe and Dostrovsky, 1971; O'Keefe and Nadel, 1978; Morris et al., 1982). This cortical region also contains excitatory synapses with considerable capacity for plastic change. Because tetanic stimulation may produce both long-term potentiation (LTP; Bliss and Lømo, 1973; McNaugthon et al., 1978) and synaptic structural changes (Fifkova and van Harreveld, 1977; Lee et al., 1980; Fifkova and Anderson, 1981; Desmond and Levy, 1983, 1986; Chang and Greenough, 1984; Trommald et al., 1990), spatial learning may also be associated with activity-dependent alterations in synaptic morphology in the hippocampus.

Although a series of experimental manipulations have been related to synaptic rearrangements in different animals (Bailey and Kandel, 1993), less is known about the type of morphological synaptic changes produced by purely behavioural learning situations. We have previously shown (Moser et al., 1994) that intense spatial training of adult rats is followed by an increased mean spine density on the basal dendrites of hippocampal CA1 pyramidal cells. The animals, which were trained in a spatially challenging environment for several hours over 3 weeks, showed enhanced rate of acquisition and improved precision learning in a water maze test. Because the total dendritic length did not differ between the trained and control rats, we concluded that the spatial training was associated with

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the creation of new spines in some dendrites, suggesting the formation of new synapses. Conversely, administration of excess glucocorticoids produces reduced spine density, which is localised to the apical CA1 dendrites only (Woolley et al., 1990).

From this background, we now ask whether the enhanced spine synapses due to spatial training are distributed over the entire dendritic tree of a CA1 target cell or are concentrated on certain dendritic segments. Furthermore, are the changes distributed evenly on a large number of cells or only concentrated on a subset of target cells? Can a reduction of spine synapses also occur, and if so, how is this reduction distributed over cells and dendrites in the CA1 region?

MATERIALS AND METHODS

Experimental material

We analysed the spine density on a sample of oblique secondary branches of the apical dendrites of CA1 pyramidal cells. This material was processed and analysed as described in Moser et al. (1994). In addition, we studied the distribution of new spines on basal dendrites by using the spine material as presented previously (Moser et al., 1994). The animal experiments were conducted according to the rules of the University of Oslo and accepted and controlled by the Norwegian Experimental Animal Board according to the rule of the European Convention for the Protection of Vertebrate Animals Used for Experimentation and Other Scientific Purposes.

Briefly, adult male rats (250–460 g) were randomly assigned to three different groups. One group of rats (trained, T) was spatially trained together in a complex and daily changing environment. To find food and water, the animals needed to search extensively in all parts of the five moveable floors that were interconnected by ladders of different configurations, thereby being trained on the spatial relations of the 5-m^3 cage. The food-deprived (19 hours per day) rats were exposed to this environment 4 hours per day for 14–18 days. For the rest of the day, they were housed in pairs in standard cages. The procedure differs from that used in complex or enhanced environments in the frequent drastic changes and the need to search for food and water, both of which gave the animals training in the spatial aspects of the cage. Two groups of control rats, also food-deprived, were housed either two by two (paired, P) in transparent cages or individually in opaque smaller cages in a separate quiet room (isolated, I). The spatial learning ability of all animals was subsequently tested in a water maze (Morris, 1984). The trained group showed statistically increased learning on three different tests (Moser et al., 1994).

Subsequently, the brain was removed from anaesthetised (halothane, 160 mg/liter inspired air) animals, and hippocampal slices were cut and mounted in a recording chamber. Individual CA1 pyramidal cells were recorded intracellularly, filled with Lucifer yellow, and later processed and analysed by confocal microscopy (Trommald et al., 1995). The code for the training history was not broken until all counting and measurements were finished.

This method allows the dendritic tree to be fully displayed so that a number of dendritic segments could be selected for analysis at a defined position with regard to cell landmarks (Fig. 1A). In a minority of cells, occasional dendritic branches were seen to be truncated at the edge of the preparation. By comparing such truncated branches with the length of fellow dendritic segments contained within the slice, we estimate that less than 3% of the total dendritic tree was lost. Spines in the stratum lacunosum-moleculare (lac-mol) were not analysed because these dendrites have a strikingly different appearance than the rest of the dendritic tree. The lac-mol dendritic branches have few side branches, run for a surprisingly great distance in the horizontal direction, and possess much fewer spines that the rest of the dendritic tree. In Figure 1, the boxes illustrate the standard position of the segments used for spine density measurement among the apical and basal CA1 dendrites. Figure 1B shows an excerpt (heavily lined box) from a single section with a number of spine heads, two of which are labelled by a circle and an asterisk. The criteria for accepting a spine was at least seven pixels (within an area of 0.10 μm^2) appearing at the same site through a minimum of three consecutive sections, 0.1 μm apart, and a distinct cleft between the spine head and the dendrite (Trommald et al., 1995). Figure 1C shows a projection of several sections. Although the problem with hidden spines (protruding at right angles to the plane of optical sectioning) also remains with the present method, a diligent use of the two display modes allowed an estimation of more than 70% of the total number of spines (Trommald et al., 1995).

Statistics

To compare the spine densities from trained and non-trained animals and to investigate skewed distributions, we used an unbalanced, nested, and mixed variance component model to deal with unbalanced designs (different numbers of units in each group). Standard analysis-of-variance tables were replaced by estimation of parameters based on restricted maximum likelihood estimation (Searle et al., 1990). Because the different dendritic segments statistically depend on both the cell and the rat they belong to, the variance component model nested the variables to the higher order variable. The model was mixed because there was a fixed group effect (the treatment groups were defined beforehand) and a random rat effect. The rats may be considered as sampled from the same distribution of animals.

The basic analysis of variance equation was:

$$y_{ijk} = \mu + \tau_i + \beta_{(j)i} + \epsilon_{(ij)k},$$

$$\text{where } i = 1, \ldots, a;\ j = 1, \ldots, b_i \text{ and } k = 1, \ldots, n_{ij}$$

In our case, $a = 3$ (the trained, paired, and isolated groups), b is the number of rats in each group, and k is the number of dendritic segments from a given rat, each providing one spine density measurement. The number of segments from each rat is denoted by n_{ij}. Therefore, y_{ijk} is the kth spine density (response) value for rat j in group i; μ represents the overall fixed effect (mean of the total population of spine densities); τ_i is a fixed effect depending on the treatment (spatial training); $\beta_{(j)i}$ is a random rat effect; and $\epsilon_{(ij)k}$ is a random error effect. We treated the $\beta_{(j)i}$ values as independent random variables with standard deviation σ_β and 0 expectation. In addition, there was in some cases a random cell effect, nested under rats, with

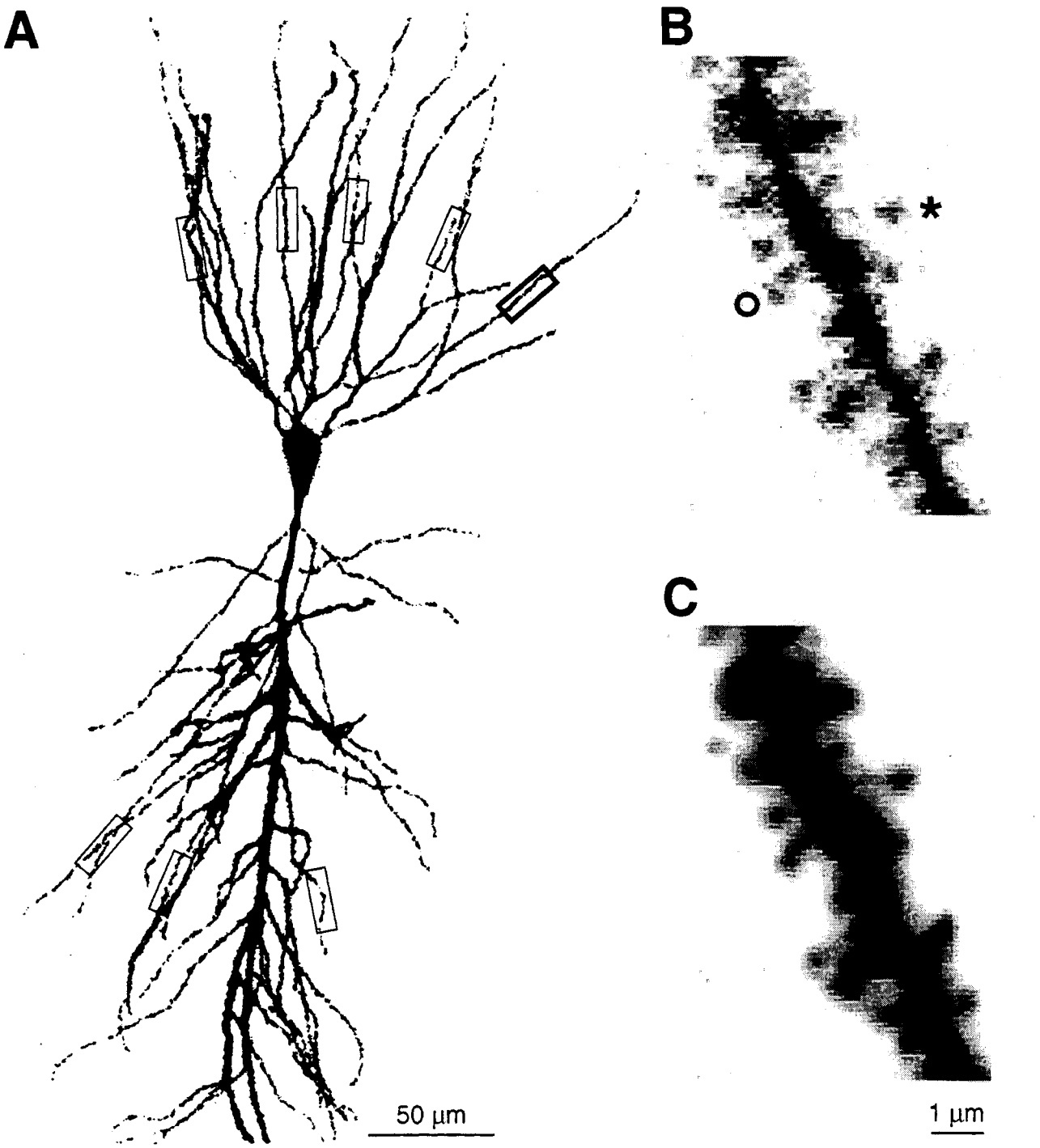

Fig. 1. Projection of a set of optical sections through the basal (top) and apical (bottom) dendritic tree of CA1 pyramidal cell filled with Lucifer yellow and analysed in a confocal microscope. **A:** Outline of the dendritic tree of the pyramidal cell. The boxes indicate areas that were scanned with high magnification for spine density measurements. **B:** Single section with two spines marked by an asterisk and a circle. **C:** Same basal dendritic segment but in a projection based upon 45 sections, taken with a z distance of 0.1 µm. The spine density of this segment was 2.23 spines/µm (trained rat). Scale bar in C also applies to B.

standard deviation σ_γ. The error terms were assumed to be independent with a standard deviation of σ.

The variance of the observations may be decomposed into contributions from rats, cells, and an error term. The P values were calculated, assuming that the variance components were normally distributed. In some cases, this assumption is of little importance considering the large data set. The spine densities from the same rat were

correlated; the correlation calculated from the basic model equation is:

$$\sigma_\beta^2/(\sigma_\beta^2 + \sigma^2)$$

In cases with an additional random cell effect, the correlation becomes:

$$(\sigma_\beta^2 + \sigma_\gamma^2)/(\sigma_\beta^2 + \sigma_\gamma^2 + \sigma^2)$$

for observations from the same cell and

$$\sigma_\beta^2/(\sigma_\beta^2 + \sigma_\gamma^2 + \sigma^2)$$

for observations from the same rat but based upon different cells. To test whether the trained group had increased spine density, i.e., if $\tau_1 > 0$, we used the standard statistic obtained by dividing the estimated value with the corresponding estimated standard deviation.

Statistical analysis of the skewness of the spine density distribution

We tested whether the tails of the spine density distributions of the different groups differed from a symmetric distribution generated from the same data by the same variance component model as described above, assuming that the distribution for the variance component did not deviate significantly from the normal. The skewness was analyzed by Monte Carlo tests because the estimates had to be simulated to get the corresponding P values. A brief intuitive description follows; see Ripley (1987) for a more formal justification. To evaluate the skewness, we employed the standard measure, i.e.:

$$\hat{\gamma} = \frac{1}{N}\sum_{i=1}^{N}\left(\frac{y_i - \bar{y}}{\hat{\sigma}}\right)^3$$

where N is the number of observations.

The analysis now proceeded in two steps: first, a calculation of the skewness of the observed data and, second, the generation of a set of normal symmetrical distributions (n = 137, n = 74, and n = 68 for the trained, paired, and isolated groups, respectively) by using data from the appropriate variance component model and repeating it 999 times in each case. The mean and standard deviation of these drawn distributions and their skewness were reestimated. If the observed skewness factor is large compared with the simulated values, there are indications of skewness to the right. More specifically, "skewness to the right" is claimed with a P value approximately equal to the fraction of simulated values exceeding the observed value. Similarly, a small skewness factor would indicate skewness to the left. For an illustration, consider trained rats, in which case we may write the model equation:

$$y_{ik} = \beta_j + \epsilon_{(j)k};$$

$$j = 1, \ldots, 11; k = 1, \ldots, n_j, N = \Sigma_{nj} = 137$$

where β_j and $\epsilon_{(j)k}$ are independent and normally distributed, with standard deviations σ_β and σ under the null hypothesis of no skewness.

RESULTS

Dendritic branching and length

Exposure to the three different environmental conditions could change the dendritic dimension or branching pattern of the implicated nerve cells. To check for such effects we measured the total dendritic length and the proportion made up by the terminal segments. A change of the latter parameter would indicate that the dendritic tree had grown or retracted some branches. We also measured the number of branch points, which indicates how many times the dendrite branches before the terminal segments are formed.

Neither the dendritic branching pattern nor length changed following spatial training. None of the measures shown in Figure 2 showed any statistical difference between the trained and nontrained rats in either the apical or basal dendrites.

Differential spine density changes

Reliability of the spine counting method. A semirandom sample of 18 dendritic segments from the basal dendritic tree was selected by an independent colleague for reliability testing (6 from each group). The spines of these segments were counted by three observers. The analysis was performed blindly. There was a high correlation across observers. The Pearson correlation coefficient was above 78%, and paired t tests showed no significant differences between any pair of these observers (ts < 0.53, Ps > 0.17).

Apical dendritic spines. To determine the distribution of the spines, we counted a number of spines on different dendritic segments. In contrast to the findings for the basal dendrites, the mean spine density for the apical dendrites in the different groups did not differ significantly (T: 1.85 ± 0.10 spines/μm [mean ± S.E.M.] in 9 rats, 16 cells, 42 segments, and 2,182 spines; P: 1.87 ± 0.11 spines/μm in 7 rats, 11 cells, 30 segments, and 1,578 spines; I: 1.78 ± 0.11 spines/μm in 7 rats, 9 cells, 23 segments, and 1,076 spines; $P > 0.05$, same variance component model as above; Fig. 3A). Because of the smaller amount of apical versus basal dendritic data, we ran a test-power check, which showed the results from the apical dendrites to be statistically reliable ($P > 0.3$).

Basal dendritic spines. In contrast to the apical dendrites, there was an increased spine density on CA1 basal dendrites following spatial training when compared with paired and isolated animals (Fig. 3B; Moser et al., 1994). This conclusion remained valid for generous relaxations of the assumptions underlying the statistical tests due to the large data set. Because there was no change in the length of the dendrites, the increased mean spine density in trained animals was interpreted as an increased number of spines and therefore of excitatory synapses (Moser et al., 1994).

Analysis of the spine density distribution in basal dendrites

A significantly different mean spine density among basal dendrites does not identify the precise locus of change within the analysed population. The changed spine density could conceivably be evenly distributed to all dendritic segments. At the other extreme, the spatial training could affect only a small subset of dendrites or even specific parts of these dendrites. To characterise the changed spine

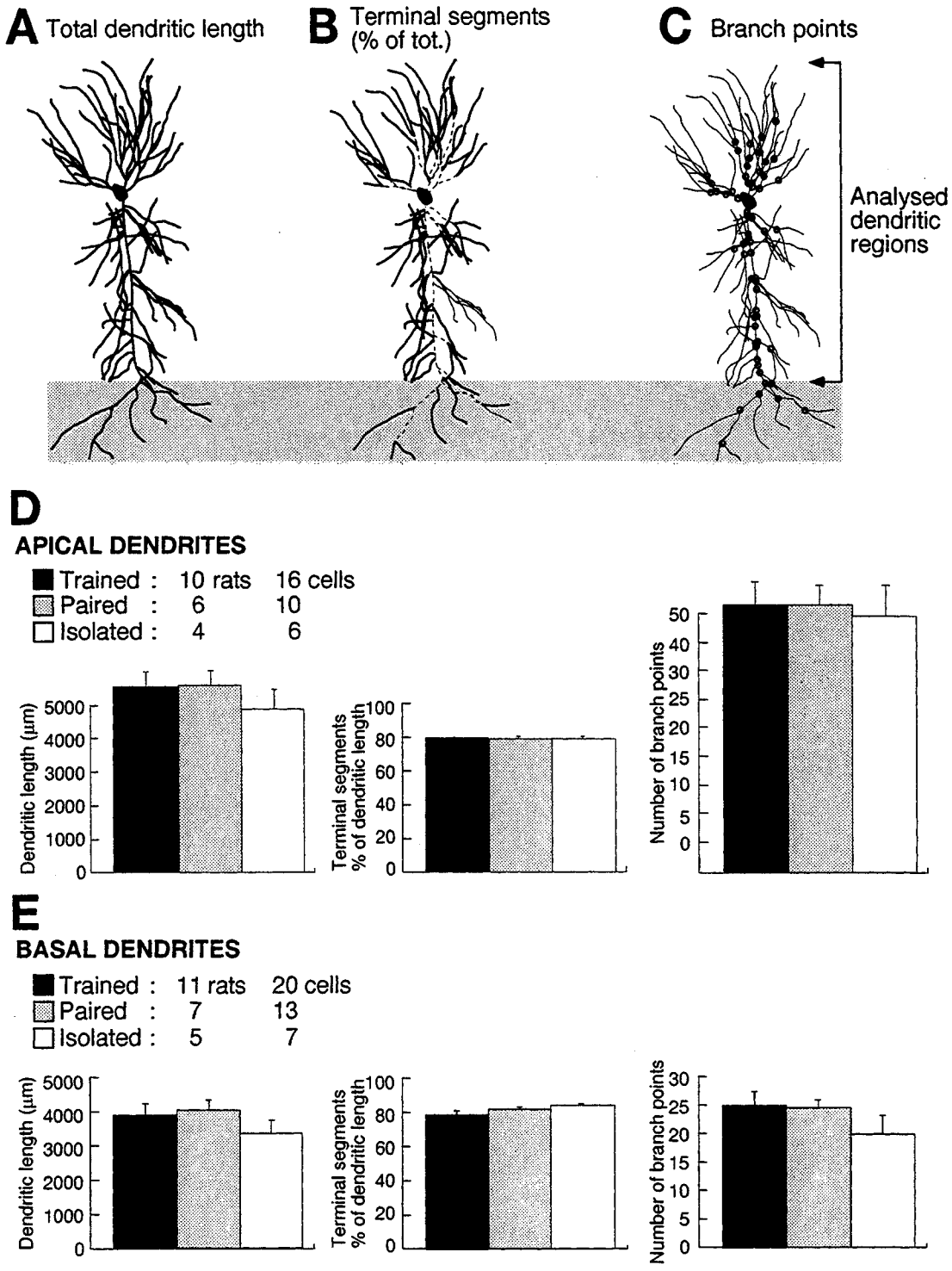

Fig. 2. Dendritic lengths and branching in trained, paired, and isolated rats. The analysis does not include branches in stratum lacunosum-moleculare. **A:** Reconstruction of a CA1 pyramidal cell. **B:** Same as A but with the terminal segments heavily outlined and earlier branches stippled. **C:** Same as A but with branch points indicated by circles. **D:** The left panel shows the mean and S.E.M. of the total dendritic length in stratum radiatum in trained (black), paired (grey), and isolated (white) animals. The middle panel shows the mean total length of terminal dendritic segments in stratum radiatum, and the right panel shows the mean number of branch points of the same dendrites for the three animal groups. **E:** Similar to D but with data from the basal dendrites.

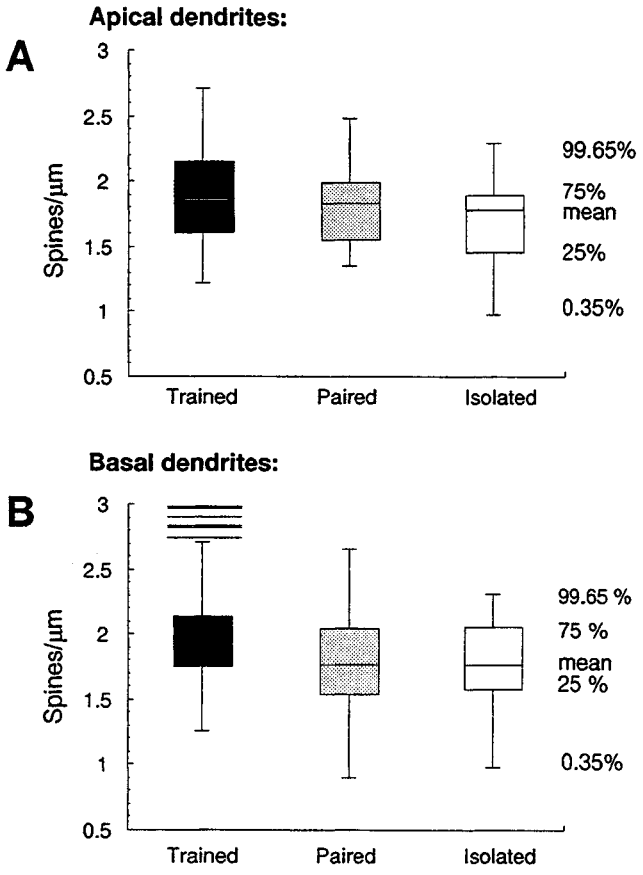

Fig. 3. Box plot showing the mean spine density and the indicated percentiles for each animal group. The boxes show the borders for the 25th and the 75th percentiles, and the ends of the vertical lines indicate the 0.35th and 99.65th percentiles. Dendritic segments with spine densities outside this range are indicated by individual horizontal lines. **A:** Apical dendrites. **B:** Basal dendrites.

pattern, we examined the distribution of spine densities in each animal group.

First, the median values of the basal dendritic spine density of the three groups, measuring 1.90, 1.85, and 1.82 spines/µm, were considerably closer than the mean values (T:1.96 ± 0.06 spines/µm in 11 rats, 23 cells, 137 segments, and 7,460 spines; P: 1.77 ± 0.09 in 8 rats, 13 cells, 74 segments, and 3,720 spines; and I: 1.79 ± 0.06 spines/µm in 7 rats, 10 cells, 68 segments, and 3,460 spines) for the trained, paired, and isolated rats respectively. Note also the higher number of outliers in the trained group. Although the distribution of spine densities in the trained group is not symmetrical when tested with the sign test ($P > 0.05$), these changes indicate that dendritic segments within the upper half of the spine density range show the highest spine increase due to spatial training. In other words, the spine density tends to increase most in those segments that already have a good spine coverage, assuming that the animals before the training had spine distributions similar to those in the paired group.

Second, a comparison of the distribution of basal dendritic spine densities in the three groups suggests an uneven change. The histograms in Figure 4 show the distribution of spine densities after they were sorted in bins of 0.1 spines/µm. Although the main form of the

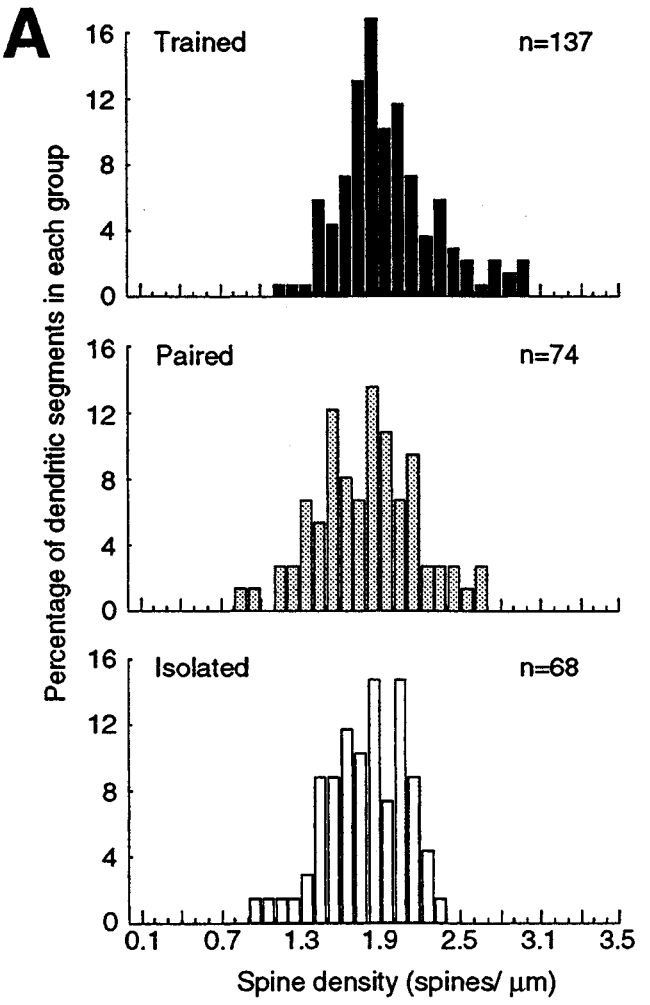

Fig. 4. The distribution of basal dendritic spine densities in the trained, paired, and isolated groups. **A:** The values from all segments were divided into bins of 0.1 spine/µm. The number of spine densities in each category is expressed as the percentage of the total spine density count. **B:** The probability density distribution of spine densities in the trained group (solid line) and the combination of the paired and isolated groups (dashed line).

histograms was similar, suggesting a wide dispersal of spine changes, the trained group showed a small increased incidence of segments with high spine densities but fewer segments with low spine densities. In contrast, there was a tendency to a left-skewed tail in the isolated group, that is, a higher incidence of segments with low spine densities and fewer segments with high spine densities. The spine density distribution in the paired group did not deviate from a normal distribution.

By combining the two control materials and converting the histograms to probability density plots (Fig. 4B), the increased spine density can be indicated by the right shift of the trained group data (solid line). If the changes were due to alteration of a restricted proportion of the dendritic segments, say only the highest spine densities, *only* the right-hand side of the distribution curve would have moved to the right, leaving the left flank unchanged, giving a broader profile and greater variance. However, because the two distribution curves have similar width, the changes are probably distributed over a large fraction of the dendritic segments.

To analyse the shape of the flanks in more detail, the distribution plots were subjected to Monte Carlo tests, where a skewness factor was calculated from the observed data of each group and compared with 999 distributions drawn from simulated symmetrical curves (see Materials and Methods). The probability densities of the simulated symmetrical curves (dotted lines) and of the observed data (solid lines) are shown in Figure 5.

The observed distribution of spine densities in the trained group was significantly skewed to the right. In fact, only 7 of 999 simulated values exceeded the observed skewness value, leading to a P value of 0.007 (Fig. 5A). The skewness is also illustrated by the relatively large number of outliers in this group (segments that fall outside the 99.65 percentile of the spine distribution; Fig. 3A). In contrast, the distribution of the spine densities in the isolated group was left skewed (Fig. 5C; $P < 0.05$). The paired group displayed no significant asymmetry (Fig. 5B). The data suggest that a small subpopulation of segments both in the trained and the isolated groups had changed spine density as an effect of the treatment but in opposite directions.

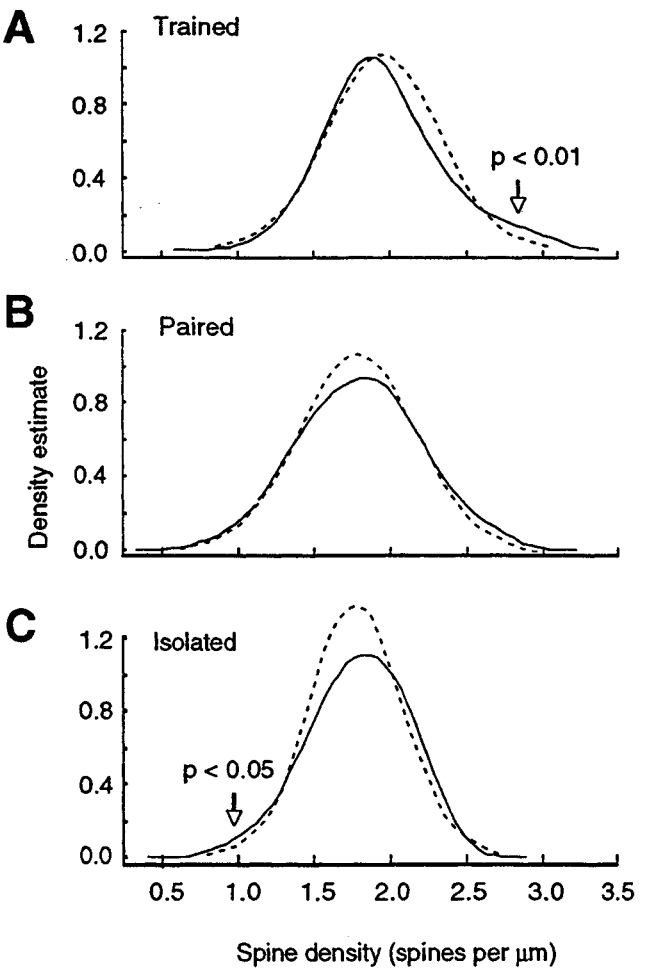

Fig. 5. Probability density distribution of spine densities from the trained (**A**), paired (**B**), and isolated (**C**) groups. Solid lines show the probability density distribution of the observed data, and the dotted lines show one of the Monte Carlo–simulated symmetrical distributions based on the nested variance component model. For further details, see Results.

Sources of the spine density variability

The spine density on individual dendritic segments varied considerably. Figure 6 displays the variability, which in turn is related to the correlations described in the Statistics section. The variance component analysis showed a relatively high correlation between dendritic segments within a given cell ($r = 0.297$). Thus, there is a dependence among dendritic segments from the same cell, implying that a dendritic segment to some extent can predict the spine density in another segment from the same cell. By counting spines on a sample of segments from a given cell, we may get a reasonable estimate of the total spine density of that cell. In contrast, the spine densities within individual rats were correlated by a smaller amount ($r = 0.148$).

DISCUSSION

Spatial training induces different effects on basal and apical CA1 dendrites

Our previously published results (Moser et al., 1994) showed that spatially trained animals in a frequently changing complex environment acquire the standard water maze task faster and with a greater precision than nontrained control rats. The trained animals also showed an increased spine density on basal CA1 dendrites. Because these spines carry asymmetrical synapses (Westrum and Blackstad, 1962) and the total dendritic length was unaltered, the results suggest that the training had generated new spine synapses on some CA1 basal dendrites.

The first new finding of the present investigation is that spatial training only affects basal and not apical dendrites in CA1. Although a larger number of dendritic segments were sampled from the basal versus the apical dendrites, a check on the test power showed that the lack of effect on the apical dendrites was statistically reliable. The increased plasticity in the basal dendrites is consistent with reports indicating that it is easier to induce LTP in basal than in apical dendrites (Kaibara and Leung, 1993; Arai et al., 1994). However, Chang and Greenough (1984) reported structural changes in the CA1 apical dendritic synapses after LTP induction. The basal dendrites were not tested.

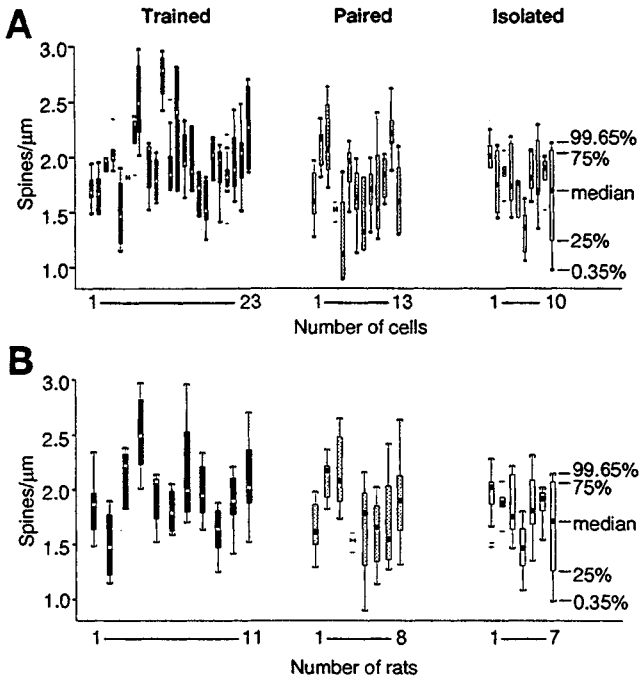

Fig. 6. Box plots of spine densities for individual cells in the trained, paired, and the isolated groups (**A**) and for individual rats in the same groups (**B**). Mean values are shown by the bars in the boxes. The boxes show the borders for the 25th and the 75th percentiles, and the ends of the vertical lines indicate the 0.35th and 99.65th percentiles.

Although learning-associated modifications take place in the basal dendrites (Moser et al., 1994), *estrogen*-induced synaptic density changes are expressed in apical dendritic branches (McEwen et al., 1993). Paradoxically, this increased CA1 apical spine density following high estrogen levels is associated with reduced performance in the water maze task (Galea et al., 1995). Similarly, exposure to glucocorticoids or to stress is associated with learning impairment (Luine et al., 1994) and even with atrophy of CA3 apical dendritic branches (Woolley et al., 1990).

In view of these hormonal effects, a critical question is whether our observations on spine changes relate to the spatial training or represent more general or unspecific *metabolic* responses to a new and challenging behavioural situation. The restriction of the spine density changes to a given part of the dendritic tree speaks to a specific effect. The same conclusion follows from the oppositely directed skewness in the trained and isolated groups (Fig. 5).

The increased CA1 *spine* density reported following discrimination learning in adult rats (Wenzel et al., 1980) may not be due to the training experience because this form of learning is hippocampus independent (Morris et al., 1986); in addition, Wenzel et al. (1980) did not control for dendritic length.

Distribution pattern of the basal dendritic synaptic changes

The second main finding of the present investigation deals with the particular distribution of the spines in the basal dendritic region after spatial training or isolation. The skewness analysis, based upon variance component analysis and Monte Carlo simulations, suggests that some of the changes are due to a redistribution of spine synapses with an overrepresentation on a subset of CA1 dendrites. The observation that the median value increased less than the mean value of spine densities in the trained group points in the same direction, indicating that a subset of basal dendritic segments contributes disproportionately to the spatial training effect. Thus, some new spines seem to be added to dendritic segments already well endowed with synapses. However, the skewed distribution can hardly explain all of the spine density increase. First, after removing the 9 highest density segments (outliers), the mean value shrank only from 1.96 to 1.91 spines/µm. The retention of these segments only explains a quarter of the spine increase. Second, although the number of cells in each group is too small to allow a precise estimate of the cell fraction that contributes the new spines, a rough estimate may be arrived at by considering a few scenarios. We may assume that the increased spine density (cell mean ± S.E.M.: 1.97 ± 0.06 spines/µm) relative to the controls (cell mean: 1.75 ± 0.05 spines/µm) could be caused by large increases in a small number of dendrites or by a more moderate increase in a larger dendritic population. Consider the extreme position where all new spines in the trained group are contributed by 1 of the 23 cells. This notion would require an average spine density in this neurone of 6.81 spines/µm, more than twice our highest value from individual cells, which is 2.74 spines/µm. In conclusion, the additional synapses are probably well distributed, but with an excess in some dendritic segments that had a reasonably high spine density at the outset.

The left-sided skewness of the spine distribution in the isolated versus control animals suggests that this condition also causes a redistribution of spine synapses. The rearrangement leads to some dendrites having less than normal spine coverage, contrasting the situation in the trained animals.

Not only the number but also the distribution of synapses may be under behavioural influence. Thus, the oppositely skewed spine distributions in the trained and in the isolated animals with an overrepresentation of high and low spine density segments, respectively, suggest that relevant learning may lead to an accumulation of spine synapses in some dendritic areas and that inactivity may be followed by regional thinning of synapses.

Usefulness of the confocal method

Confocal microscopy after intracellular marking represents a useful compromise between analysis of Golgi-stained material and reconstruction from serial electron micrographs. The resolution is better than for standard light microscopy and sufficient to detect a large fraction of the spines (Trommald et al., 1995). The high total dendritic length and spine density argue for an adequate representation of the neurones. This method allows sampling of many cells and several dendritic segments from the same cell, a condition necessary for reliable statistical analysis. However, for synaptic details and for a study of the presynaptic elements, the technique must be supplemented by electron microscopy.

Functional implications of the increased spine number

Two important factors determine the functional effect of the increased spine number in basal CA1 dendrites. Obviously, only spines in contact with boutons can play a functional role. The observation from serial electron micrographs that naked spines are hardly ever found (Harris and Stevens, 1989; Trommald et al., 1990) suggests that the vast majority of spines are, in fact, contacted by presynaptic endings and are probably functional. The second factor is the connectivity pattern formed by the new synapses. The largest effect would appear if a newly formed spine is contacted by an axon that already has one synapse on the target neurone. By causing release at two sites, an impulse along this axon will double its functional strength on this target cell. With a wide dispersal of the newly generated synapses, the functional effect of new synapses on a given cell will be determined by the type and degree of convergence of afferent axons on the target neurone at any given moment. Therefore, an assessment of the effect of a small addition of new synapses must await precise information on the presynaptic impulse patterns. Nevertheless, because the hippocampal network may operate as a distributed neuronal set (McNaughton and Morris, 1987; Eichenbaum, 1993), the statistically averaged effect is enhanced.

The enhanced spine density in some basal dendrites from trained animals and examples of reduced spine density in some dendritic segments from animals kept in isolation (Fig. 5C) suggest that the synaptic number in a dendritic region may be under dynamic behavioural control. Reminiscent of the developmental situation, spine synapses may be added or withdrawn, probably governed by the activity level of the cell. Whether such an alteration is a property of a subset of cells or spine synapses or a general property of cortical excitatory synapses remains a challenging problem.

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Pretraining and the Function of Hippocampal Long-Term Potentiation

Minireview

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Storage of information in the brain may rely on activity-dependent synaptic plasticity. It is well established that one form of long-lasting plasticity, long-term potentiation (LTP), can be induced in the major excitatory synapses of the hippocampal formation by afferent high-frequency stimulation reminiscent of the natural firing properties of hippocampal neurons. LTP satisfies many of the computational requirements of a memory-inducing cellular mechanism, and for this very reason there is now a huge body of literature dealing with the mechanisms of hippocampal LTP.

However, the relevance of this research hangs on the demonstration of a mnemonic role of LTP in behaving animals. Converging evidence does suggest that blockade of LTP interferes with some hippocampus-dependent memory processes, such as encoding of spatial memory (Martin et al., 2000). For example, an essential step in the induction of associative LTP is the activation of N-methyl-D-aspartate (NMDA) receptors. Intraventricular infusion of a competitive NMDA receptor antagonist disrupts LTP in the excitatory synapses of the dentate gyrus and impairs new spatial learning in a Morris water maze (Morris et al., 1986). Overexpression of an NMDA receptor subunit has been reported to enhance hippocampus-mediated learning (Tang et al., 1999). Similar parallel effects on LTP and learning have been noted following targeted mutations of other genes expressed in hippocampal excitatory synapses (Martin et al., 2000).

Although it appears from these studies that LTP-like activity may be necessary for hippocampus-mediated memory, there are several examples of intact learning in the absence of LTP, indicating that we have not yet grasped how LTP and memory are related. The most striking of these examples is perhaps the observation that NMDA receptor blockers fail to disrupt new spatial learning in the water maze in rats that have received pretraining in a different spatial environment (Bannerman et al., 1995; Saucier and Cain, 1995). We will argue that this observation, apparently inconsistent with the LTP memory hypothesis, may in fact provide a key to the understanding of the specific function of LTP-like activity in hippocampus-mediated memory formation.

Pretraining Prevents Spatial Learning Deficit following Blockade of LTP

When animals are trained to find a hidden platform in a water maze, they learn a lot more than the spatial configuration of the environment. A few years ago, Bannerman et al. (1995) and Saucier and Cain (1995) tried to isolate the encoding of spatial relations from other types of learning, such as learning an efficient swim strategy, learning that there is a platform, and learning to climb upon the platform. They pretrained the rats until the animals were familiar with the task in one pool and then tested the effects of NMDA receptor antagonists on new learning in a second pool with different external cues. Both groups reported that NMDA receptor antagonism failed to impair spatial learning. The benefit of pretraining was observed even in animals that received nonspatial pretraining behind curtains (Saucier and Cain, 1995; Hoh et al., 1999), suggesting that the effect did not reflect mere generalization between orientation cues around the two water mazes. Animals with hippocampal lesions did not profit from pretraining (Bannerman et al., 1995). Thus, spatial learning depends on the hippocampus but can, at least under some circumstances, occur in the absence of NMDA receptor–dependent LTP.

The simplest explanation of the pretraining effect would be that the NMDA receptor blockade was incomplete and that the few unblocked synapses were sufficient for synaptic plasticity and learning in animals already familiar with the general task requirements. However, tetanic stimulation of the perforant path failed to induce LTP in the dentate gyrus of the pretrained drug-treated animals (Bannerman et al., 1995; Saucier and Cain, 1995), and the intracerebral drug concentration was comparable to concentrations causing complete blockade of LTP in the excitatory synapses of the hippocampus in vitro (see Bannerman et al., 1995). Although the role of residual receptors cannot be determined until pretraining is studied in mice with inducible mutations of hippocampal NMDA receptors, the current data suggest that NMDA receptor–mediated LTP in the hippocampus is not essential for new spatial learning in pretrained animals.

Isolating Learning Impairment from Sensorimotor Impairment

There are several possible interpretations of the protective effects of pretraining in rats without capacity for NMDA receptor–dependent LTP. First, NMDA receptor antagonists may interfere with the acquisition of motor skills that are necessary for successful navigation in the water maze (Saucier and Cain, 1995; Saucier et al., 1996), and this, rather than the blockade of LTP, may be the cause of the performance impairment. Prior training has been shown to eliminate the disruptive sensorimotor effects of a variety of drugs, and animals that learn to swim, climb, and balance in advance may similarly be able to overcome the acute effects of an NMDA receptor antagonist (Saucier and Cain, 1995; Saucier et al., 1996). The "sensorimotor" interpretation predicts that even naive rats would learn if LTP were blocked without concomitant motor dysfunction.

Recent experiments shed light on the role of sensorimotor deficits. Otnaess et al. (1999) compared spatial learning in nave and pretrained rats after new LTP was prevented by a "saturation" procedure (Moser et al., 1998) that left the sensorimotor skills of the rat intact.

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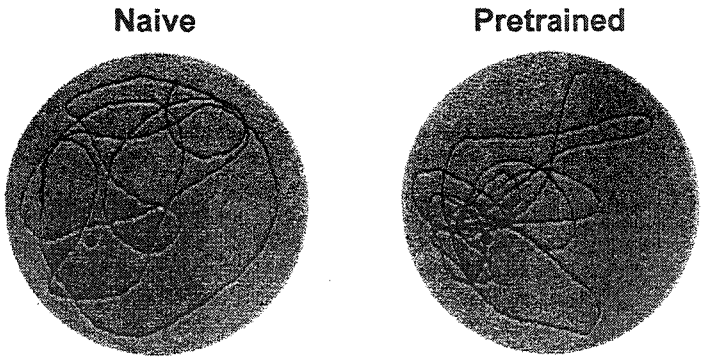

Figure 1. Spatial Learning in Naive and Pretrained Rats following "Saturation" of the Capacity for LTP in the Perforant Path Synapses of the Dentate Gyrus

Tetanic stimulation was delivered across the angular bundle until no further LTP could be induced through a stimulation electrode in the center of the bundle. Diagrams show swim paths of naive and pretrained animals at the end of training on a probe test with the platform unavailable for 60 s (Otnaess et al., 1999). The platform used to be in the southwest quadrant.

LTP was induced repeatedly across the perforant path fibers of the angular bundle until no further potentiation was obtained in the behaving animal. Stimulated animals were not different from control animals on a balance task or a climbing task in the water maze. Nonetheless, while saturation of the capacity for LTP interfered with spatial learning in naive animals (as in McNaughton et al., 1986; Moser et al., 1998), pretrained animals were unaffected and acquired the new task as rapidly and with the same precision as the rats in the control group (Figure 1). Thus, spatial learning was impaired in the absence of sensorimotor deficits, and pretraining eliminated this impairment.

So why doesn't NMDA receptor blockade disrupt spatial learning in a pretrained animal? We will consider two explanations: (1) LTP is essential for some aspect of learning other than the acquisition of a spatial map, and (2) LTP is indeed part of the normal mechanism for spatial learning, but there are parallel NMDA receptor–independent back-up mechanisms that are sufficient for new learning in a pretrained animal. These alternatives do not necessarily exclude one another.

Hippocampal LTP and Nonspatial Associations

It is possible that NMDA receptor–dependent LTP is indispensable only during a specific type of learning in the water maze and that only naive animals need this form of learning. Bannerman et al. (1995) suggested that NMDA receptors are critical for learning about the general structure of the water maze task, e.g., that there is an escape platform, that it is located some distance off the pool wall, and that entering the platform is followed by termination of the trial. This type of learning would be completed at the time training in the second pool is started. However, later work has shown that the beneficial effects of pretraining are not eliminated in rats that receive an NMDA receptor antagonist during the pretraining stage (Hoh et al., 1999). Antagonist-treated rats learned to make adaptive response sequences, such as swimming away from the periphery and climbing the platform, and subsequently acquired a new task at the same rate as did saline-treated control animals. Thus, paradoxically, both spatial and procedural learning can occur in the absence of NMDA receptors. What is then the function of NMDA receptor–mediated LTP?

Recent experiments have identified a condition during which NMDA receptor antagonism blocks spatial learning in the water maze even in pretrained animals. Steele and Morris (1999) trained rats in a delayed matching version of the water maze task, in which the platform position changed from day to day but stayed in the same place throughout the four trials of each day. Memory of the target position on trial 1 of a given day was characterized by reduced escape latencies on trial 2. The intertrial interval varied from 15 s to 2 hr. Steele and Morris found that animals treated with an NMDA receptor blocker exhibited poor retention at long test intervals, indicating that NMDA receptor–dependent LTP was necessary for long-term storage of spatial memory. Extensive pretraining did not prevent this retention impairment. So what LTP-dependent function may a pretrained animal need in the delayed matching condition but not in the standard reference memory task? In the matching condition, memory of location is not sufficient. The rat must be able to distinguish the most recent platform position from positions that were used on preceding days. It needs to remember both where the platform was positioned and when the platform occupied this position (recent or remote). This addition of a temporal tag to each spatial memory may be one of the LTP-dependent functions of the hippocampus. If it is, pretraining might not help if the platform position changed regularly, as in the delayed matching task. The reference memory test, in contrast, requires no new associations between location and time, at least not if the animal is already familiar with the course of events in a water maze session.

An involvement of hippocampal LTP in place–time associations (memory of "episodes") would be consistent with the suggested role of the hippocampus in memory of temporally sequenced information (reviewed by Eichenbaum et al., 1999; Lisman, 1999). Hippocampal lesions interfere with trace conditioning (see McEchron and Disterhoft, 1999) and disrupt the retention of which of two arms in a radial maze was visited most recently (Chiba et al., 1994). Although position is clearly the main determinant of firing rates in hippocampal pyramidal cells (O'Keefe and Nadel, 1978), some cells may also exhibit temporally specific activity, as shown after classical conditioning of the rabbit nictitating membrane response (Berger and Thompson, 1978). These observations suggest that hippocampal neurons may potentially encode a rather broad range of "episodic" memory attributes (Eichenbaum et al., 1999; Martin et al., 2000). Hippocampal associative LTP may contribute to the binding of spatial information with temporal or other nonspatial task elements.

Multiple Redundant Learning Mechanisms in the Hippocampus

However, it is not necessary to assume that hippocampal LTP has only nonspatial functions. Spatial memory

may normally be stored via NMDA receptor activation, but the hippocampus may contain additional NMDA receptor–independent plasticity mechanisms that are able to encode pure spatial relations, such as after advance training in a related environment. This view would explain why pretraining benefits learning in a reference memory task without discarding a role for NMDA receptor in spatial learning, as such.

New data suggest that spatial learning may indeed operate largely via the NMDA receptor in the naive rat. When a rat is exposed to a novel environment, the majority of the active pyramidal neurons in the hippocampus develop localized firing patterns (place fields) as the animal learns about the task (O'Keefe and Nadel, 1978). This place-related activity remains stable for weeks or more and may be necessary for spatial learning. Acute blockade of the NMDA receptor abolishes both LTP and overnight stability of place fields formed in a new environment (Kentros et al., 1998). These data suggest that NMDA receptor–dependent LTP does participate in the long-term storage of hippocampal spatial information.

However, despite the blockade of NMDA receptors, new place fields were maintained for at least 1.5 hr after the first exposure to the environment (Kentros et al., 1998). Similarly, in mice with a regionally specific mutation of the NMDA receptor, place fields remain stable across repeated tests on the same day, even though new LTP is abolished (McHugh et al., 1996). These observations suggest that the hippocampus may possess NMDA receptor–independent mechanisms that are sufficient to establish and maintain place-related activity in hippocampal neurons temporarily. Such residual encoding capacity, albeit not as precise and stable as in normal animals, may be sufficient to enable a pretrained rat to learn where a fixed platform is located in the water maze in the absence of NMDA receptor activity.

One way to test more directly whether a normal naive animal in fact uses NMDA receptor–dependent LTP to store spatial memory is to interfere with the pattern of potentiated and unpotentiated synapses in the hippocampus after the animal has learned the spatial configuration of the environment. Preliminary data show that long-term retention is disrupted when NMDA receptor–mediated LTP is induced randomly throughout the hippocampal network after training in the water maze (V. H. Brun et al., 1999, Soc. Neurosci., abstract). Thus, a naive rat may normally use NMDA receptors and LTP to store spatial memory in the hippocampus, but there may be additional plasticity mechanisms by which spatial relations may be encoded during antagonism of the NMDA receptor.

Conclusion

The pretraining studies show that rats can encode new spatial memory without NMDA receptor–mediated LTP. This suggested to many that LTP and memory were not related and that behavioral impairments in the naive condition reflected the disruptive sensorimotor effects of the drugs. New data suggest that (1) sensorimotor dysfunction does not explain the impaired performance after NMDA receptor blockade in naive rats, (2) the effectiveness of pretraining depends on the subsequent training protocol in the water maze, and (3) spatial representations in the hippocampus are normally maintained by NMDA receptor–mediated LTP. This does not preclude that there are additional NMDA receptor–independent memory mechanisms whose function may be sufficient to encode pure spatial relations under simplified conditions, such as after pretraining. When the complexity of the spatial memory task is increased, e.g., by requiring retention of temporal order, these back-up mechanisms may no longer help.

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