Correction

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The authors note that Fig. 5 appeared incorrectly. The corrected figure and its legend appear below.

The authors also note that on page 19464, right column, lines 1–5, “We found that this paradigm abolished the already small degree of decorrelation that we had observed over long time periods in CA3 [all mean population vector (PV) correlations >0.96; all post hoc comparisons between time intervals ≥0.5 h, n.s.]” should instead appear as “We found that this paradigm did not change the low degree of decorrelation that we had observed over long time periods in CA3 [all mean population vector (PV) correlations >0.82; F(5) = 10.1, P < 0.001; all post hoc comparisons between time intervals ≥0.5 h are n.s. except P < 0.001 for 6 h compared to 24 h].”

The authors also note that on page 19464, right column, line 5, the sentence starting with “At the same time” should end with the added statement “and the decrease over time remained larger in CA1 than in CA3 (at 30 h, t = –9.0, P < 0.001).”

Fig. 5. When testing with a single enclosure shape, firing patterns of the CA3 network remained highly consistent for repetitions of the same environment over extended time intervals, whereas activity patterns in the CA1 network changed. (A) An experimental design with a single enclosure shape was used to test whether the decorrelation of hippocampal activity patterns could have been an effect of intervening experiences in a different context (Fig. 3). The mean PV correlation between pairs of recordings in the same enclosure shape (B) and the corresponding cumulative distribution function for the PVs (C) are shown as described in Fig. 3. Highly consistent firing patterns in the CA3 population were observed over time intervals of 30 min to 30 h. In contrast, the CA1 network continued to show a pronounced monotonic decrease in firing similarity with time (see text for statistics).

www.pnas.org/cgi/doi/10.1073/pnas.1502758112
Neuronal code for extended time in the hippocampus

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Edited by Roger A. Nicoll, University of California, San Francisco, CA, and approved October 12, 2012 (received for review August 15, 2012)

The time when an event occurs can become part of autobiographical memories. In brain structures that support such memories, a neural code should exist that represents when or how long ago events occurred. Here we describe a neuronal coding mechanism in hippocampus that can be used to represent the recency of an experience over intervals of hours to days. When the same event is repeated after such time periods, the activity patterns of hippocampal CA1 cell populations progressively differ with increasing temporal distances. Coding for space and context is nonetheless preserved. Compared with CA1, the firing patterns of hippocampal CA3 cell populations are highly reproducible, irrespective of the time interval, and thus provide a stable memory code over time. Therefore, the neuronal activity patterns in CA1 but not CA3 include a code that can be used to distinguish between time intervals on an extended scale, consistent with behavioral studies showing that the CA1 area is selectively required for temporal coding over such periods.

Results

We trained six rats to randomly forage in a square and in a circular enclosure in the morning (AM) and, after an interval of 6 h, in the afternoon (PM) of each day. The training at each time of day consisted of four 10-min sessions, two in each enclosure shape, presented in random order (Fig. 1 A). The training schedule remained consistent over many days. Rats were therefore highly familiar with the behavioral paradigm when recordings began such that any observed differences in firing patterns could not be attributed to novelty-related effects.

Because it was critical for all aspects of the data analysis that each recorded cell could be identified accurately throughout the entire sequence of sessions, we used a technique for electrode placement that was optimized for recording stability across time, and we applied stringent standards to the acceptance of cells for further analysis (Materials and Methods and Fig. S1). In particular, we ranked the recordings in behavior with recording periods during rest. Most hippocampal cells fire bursts of action potentials during rest periods (30, 31), and these recordings can thus be used to confirm that the signals of each cell could be reliably recorded before, after, and throughout the entire experimental paradigm, even if the cell was not active during all periods of random foraging.

In the dataset with confirmed cell identity within an entire recording day (n = 99 CA1 cells and n = 126 CA3 cells), we found in both the hippocampal CA1 and CA3 subregions that repetitions of the same enclosure shape resulted in highly reproducible firing of hippocampal cells at well-defined spatial locations, as reported consistently since hippocampal place fields were first described (32–35). However, when considering the network activity pattern of the CA1 cell population, we found that the similarity in


The authors declare no conflict of interest.

This article is a PNAS Direct Submission. Freely available online through the PNAS open access option.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1214107109/-/DCSupplemental.
neuronal activity decreased for the 6-h interval compared with intervals of less than 1 h \((t = -7.71, P < 0.001)\), indicating that events that are farther separated in time have more distinct neuronal representations (Fig. 1 B and C). In contrast, the similarity of firing patterns in the CA3 cell population decreased to a much smaller extent between the <1-h interval and the 6-h interval \((t = -2.95, P < 0.05\) for the comparison between time intervals; \(t = -9.92, P < 0.001\) for the comparison between CA1 and CA3). Differences between hippocampal subregions were also observed for the firing rates of single cells such that subsets of CA1 but not CA3 cells showed a high variability between repetitions of the same enclosure shape in the morning and afternoon. Thus, we find that CA3 maintains highly similar representations for repeated events, irrespective of the elapsed time between them, whereas CA1 representations vary to a larger extent between repeated events that are separated over intervals of several hours \([F(103, 49) = 1.98, P < 0.01]\) (Fig. 2). Next, we considered whether differences in hippocampal firing patterns were circadian. Pronounced fluctuations in hippocampal gene expression patterns, neurotransmitter release, and in hippocampal synaptic excitability occur with the circadian clock (36–40). Any differences in the population code for an identical environment after a time interval of 6 h might therefore reflect a circadian or ultradian modulation of activity patterns. If hippocampal neural network activity were controlled by internal states associated with circadian or other endogenous clocks, one would expect to find a cyclical pattern in neuronal firing such that the similarity at matching times of the day is higher than at nonmatching times within a day. To examine this possibility, we identified the hippocampal place cells that could be reliably tracked through the morning and afternoon of two consecutive recording days \((n = 50\) CA1 cells and \(n = 71\) CA3 cells). We first looked for circadian fluctuations in normalized firing rates. Consistent with a previous study (41), we found no differences across time in the average normalized firing rate \([F(15) = 0.90\) for CA1 and 0.32 for CA3; not significant (n.s.)] (Fig. 3 A and B). We then asked whether the differences in neuronal activity patterns in the hippocampal CA1 area, when comparing sessions recorded 24 h apart and thus matched for time of day, showed any increase in similarity compared with those at 6-h time intervals, as would be expected for a circadian cycle. Instead, the recordings after 24 h exhibited a further decrease in similarity compared with the 6-h time point, such that the similarity in hippocampal firing in CA1 monotonically decreased for time intervals between 30 min up to at least 30 h \([F(4) = 46.3, P < 0.001]\) (Fig. 3 C and D; Fig. S2). The network activity in CA3 also showed a gradual decrease in similarity with time \([F(4) = 50.5, P < 0.001]\), although to a much smaller extent than the decrease in CA1. The correlation at 30 h was 0.80 ± 0.003 in CA3 compared with 0.57 ± 0.006 in CA1. Thus, in CA1, we do not find a distinct coding scheme in the CA1 or CA3 network for representing the exact time of day at which an event occurred, but rather find that the degree of similarity between two representations differs depending on the temporal distance between them, in particular within the CA1 cell population. If the decrease in similarity over time reflected merely an unconstrained random drift in firing patterns, such continuous change could eventually result in a decrease in the accuracy with which hippocampal neuronal firing patterns code for a repetition of the same event after a long time interval and, consequently, in a change of the ability to represent the relative similarity between contexts (42, 43). We observed that, at any time point, there were many CA1 cells that discriminated between the two different enclosure shapes, but that individual CA1 cells showed inconsistency across extended time periods in their strength of preference for firing in one of the enclosure shapes (Fig. 4 A and B; see figure legend for statistics). In contrast, individual CA3 cells showed a striking consistency in enclosure shape coding by firing rate over extended time periods. Although individual CA1 cells showed high variability in preferentially firing in one of the enclosure shapes, the average degree of shape preference for the CA1 cell population did not change over time periods of 6 h or between days [absolute shape preference: CA1, \(F(3) = 0.28, n.s.,\) CA3, \(F(3) = 0.33, n.s.\); spatial correlation: CA1, \(F(3) = 0.30, n.s.,\) CA3, \(F(3) = 2.59, n.s.\)] (Fig. 4 C and D). This pattern of coding in CA1 can result in consistent representations of contextual differences, even though the population vector correlations for repetitions of identical shapes were as low as 0.57 between days (see above and Fig. 3). Did the dissimilarity in the CA1 hippocampal population code emerge as a function of elapsed time, or could differences in neuronal firing patterns be the outcome of cumulative rate changes during the behavioral sessions such that larger differences were reached with increased random foraging experience? If experience in the recording enclosure were primarily responsible for the reorganization of the network, then the changes in network activity would be expected to occur predominantly during running in the enclosure, the period during which place cells are active and when activity-dependent network plasticity occurs (44). To examine this possibility, we established several control conditions. First, we tested whether the amount of random foraging experience between two time points had an effect on the similarity of CA1 population coding. At the 24-h and at the 30-h interval, we found the same degree of decorrelation irrespective of the number of intervening four-session blocks \((t = -0.64, n.s. for 24-h intervals; t = -1.82, n.s. for 30-h intervals)
Fig. 2. CA1 place fields show variability in firing rate between morning and afternoon sessions. (A) Firing rates for two representative CA1 place fields and (B) for two CA3 place fields. Fields 2, 3, and 4 were recorded simultaneously. Each 10-min recording session throughout the AM and PM is shown. Symbols above each graph indicate the order of enclosure shapes. Each bar (green for the square shape and purple for the circular shape) represents the firing rate of the cell during a pass of the animal through the place field (see Fig. S6 for methods and Fig. S7 for additional examples). For each pass, the corresponding running speed of the animal is plotted downward below the x-axis (cm/s, in blue). The variability in firing rates cannot be explained by movement velocity or by the proximity of the path to the field center (Fig. S8). For each firing field the corresponding color-coded rate maps (averaged across each 10-min recording session) are shown below the line graph. The color scale for rate maps is from 0 Hz (blue) to the peak rate of the day (red). Coding differences emerged, in part, from CA1 place fields that changed or became silent at a subset of time points. In all cases in which cells became silent, it was verified in preceding or subsequent rest sessions that spikes from these cells could be detected (Fig. S1). (C) For each place field, mean peak rates within each enclosure shape were calculated in the morning and in the afternoon. These rates were compared within each hippocampal subregion. Place fields that were not active (mean peak rate <2 Hz) at either time point were excluded. For active cells, the firing rates between AM and PM were more variable in CA1 compared with CA3 (see text for statistics; SI Materials and Methods).

intervals; Fig. S3A). Second, we observed decorrelation with time even when limiting our analysis to time intervals without any intervening AM or PM blocks \( F(3) = 41.6, P \text{ values for all post hoc comparisons} < 0.001 \) except that the comparisons between the 18- and 24-h interval is n.s. (Fig. S3B). Third, we asked whether the continued switching between two different enclosure shapes might explain why CA1 firing patterns accumulated firing differences across a series of recording sessions. To examine this possibility, we performed a 2-d series of AM and PM recordings in only one enclosure shape (Fig. S4). We found that this paradigm abolished the already small degree of decorrelation that we had observed over long time periods in CA3 [all mean population vector (PV) correlations \( > 0.96 \); all post hoc comparisons between time intervals \( \geq 0.5 \) h, n.s.] (Fig. S5 B and C). At the same time, a monotonic decrease in population similarity with extended time was still observed in hippocampal CA1 cell populations \( F(5) = 54.0, P < 0.001; P < 0.001 \) for all post hoc comparisons, except \( P < 0.01 \) for \( < 1 \) h compared with 6 h, and

Fig. 3. The decorrelation in CA1 network activity over extended time periods does not repeat cyclically across days. (A) To determine whether differences in CA1 network activity patterns can be explained as a circadian effect, we extended the hippocampal recordings across 2 d. \( B \) The mean ± SEM normalized firing rate for all active CA1 (Left) and CA3 (Right) principal neurons is shown for each 10-min recording session across the 2-d experiment. For each cell, the normalized firing rate was calculated by dividing the average firing rate for each session by that cell’s maximum average firing rate in any of the sessions. A circadian variation in firing rate was not observed (see text for statistics). (C) PV correlations between pairs of recordings in the same enclosure shape are shown as dots. Every pair-wise comparison is aligned to its time interval, so that, for example, the AMPM comparisons on day 1 and the AMPM comparisons on day 2 are all aligned to the 6-h interval (see Fig. S9 for the complete pair-wise correlation matrix). The black error bars report the mean ± SEM for pair-wise comparisons at each time interval. The correlation coefficients for the CA1 population activity (red) decreased monotonically as a function of elapsed time between recording sessions up to at least 30 h (see text for statistics and Fig. S2 for comparisons of up to 60 h). Repeated CA1 recordings at matching times of day on two consecutive days (24-h interval) show a smaller correlation than recordings at shorter intervals but at different times of the day \( (P < 0.001 \) for the post hoc comparison). Thus, the effect we observed is not due to circadian fluctuations. (D) Cumulative distribution functions for PV correlations between pairs of recordings in the same enclosure shape at different time intervals.
n.s. for 6 h compared with 18 h, 18 h compared with 24 h, and 24 h compared with 30 h]. Finally, we examined whether a repetitive increase or a repetitive decrease in the firing rate of place fields within each 10-min session might contribute to progressive changes over long time periods. We observed that a large fraction of CA3 place fields (57.9%) exhibited either a consistent decrease or a consistent increase in firing rate within each 10-min behavioral session. However, consistent rate changes were uncommon in CA1 cells (11.1%; Fig. S4). Systematic changes in firing patterns within recording sessions—as expected for activity-dependent plasticity mechanisms—were thus primarily observed in the CA3 hippocampal subregion, which showed only minor differences in activity patterns over long time periods, and not in the CA1 subregion, where marked changes occurred across longer timescales.

**Discussion**

Theories of long-term memory coding require that stored firing patterns are accurately reinstated during later retrieval (26–29). In contrast, theoretical considerations for representing temporal aspects in long-term memory require fluctuations of activity patterns in neuronal networks such that relative temporal distances or temporal order can be represented even when the events are otherwise identical (21–25). We provide evidence for both of these neuronal coding schemes within distinct subregions of hippocampus on a timescale of hours and days. In the CA1 cell population, the degree of similarity of neuronal responses for identical locations in the same context decreases monotonically as a function of the time between experiences for at least 30 h. These changes in neuronal network activity were measured in highly familiar environments and thus appear unrelated to changes that have been reported during new learning (41, 45–47). The fluctuations also seem not to correspond merely to noise, as it would be unlikely for a cell to stop firing for a time and then resume its prior firing field, firing rate, and shape preference by chance. Finally, we could not detect a circadian component in the coding difference within the hippocampal CA1.

**Fig. 4.** Differences in firing patterns over extended time periods did not preclude the encoding of spatial information or of contextual differences. (A) For each place field a shape preference score was calculated as a measure of the difference in firing rates between the circular and the square enclosure (scores of −1 or +1 indicate that the cell fired only in the circle or only in the square, respectively). This score was compared between recording blocks (Left, CA1; Right, CA3) in the AM and PM (Upper) and between days (Lower). For calculating each day’s score, all AM and PM recording sessions within a day are used. Individual CA1 fields show more variable shape coding over 6-h intervals and over 1-d intervals than individual CA3 fields (P(82, 43) = 2.51 and P(50, 31) = 2.53, P < 0.01 for comparisons of shape preference scores at both time intervals). (B) Firing rates within two representative CA1 place fields from the same cell over 2 d (data presented as described in Fig. 2; see Fig. S10 for additional examples). The CA1 place fields showed a change in the degree of discrimination between the square and circular enclosure between time points (Fig. 2A). Field 1 normally fired at its highest rate in the circular shape, but became silent in the AM session on day 2. The field resumed its firing in the circular enclosure during the PM session on day 2. It was therefore observed that shape preferences could be lost and regained between blocks of sessions at different time points. (C and D) Even though individual CA1 cells show coding differences between time points, the average degree of context and place coding is consistent within CA1 and within CA3 cell populations (see text for statistics).

**Fig. 5.** When testing with a single enclosure shape, firing patterns of the CA3 network remained highly consistent for repetitions of the same environment over extended time intervals, whereas activity patterns in the CA1 network changed. (A) An experimental design with a single enclosure shape was used to test whether the decorrelation of hippocampal activity patterns could have been an effect of intervening experiences in a different context (Fig. 3). The mean PV correlation between pairs of recordings in the same enclosure shape (B) and the corresponding cumulative distribution function for the PVs (C) are shown as described in Fig. 3. Highly consistent firing patterns in the CA3 population were observed over time intervals of 30 min to 30 h. In contrast, the CA1 network continued to show a pronounced monotonic decrease in firing similarity with time (see text for statistics).
but not

as a cue for locating a food reward (15, 19). At the time of surgery, the rats were anesthetized with fl

Subjects. Six male Long Evans rats with a preoperative weight of 400–485 g were housed individually and maintained on a 12-h light/12-h dark schedule. One rat was tested at 6:00 AM and the other in the circle enclosure. For each animal, we selected the shape

Surgical Procedures. At the time of surgery, the rats were anesthetized with isoﬂurane gas [2–2.5% in O2 (20 mL/ l per minute)] and an electrode assembly that consisted of 14 independently movable tetrodes was implanted above the right hippocampus (3.8–4.0 mm posterior and 3.0 mm lateral to bregma).

Behavioral Procedures. After 1 wk of recovery from surgery, rats were partially food-deprived and trained to forage for randomly scattered cereal crumbs in an enclosure with walls that could be shaped either as a square (80 × 80 cm) or as a 16-sided polygon (50-cm radius; referred to as a circle) (43). A polarizing white cue card (20 cm wide) was placed on an inside wall of the enclosure. The center of the enclosure was always located at the same place in the room, and the angle of the cue card compared with external room cues was kept constant. Training was performed in two daily blocks. For all rats, the first block started between 8:30 and 9:30 AM, and the second block between 2:30 and 3:30 PM. For each individual rat, the daily start time of each block varied by less than 30 min. Rats were returned to the animal housing room between the morning and afternoon training sessions.

Rats were trained to run for four 10-min sessions during each block, with two sessions in the square enclosure and two sessions in the circle enclosure. The order of the shapes varied randomly within each training block. The rats were allowed to rest for 5 min between sessions, and training blocks were flanked by sleep sessions (10–20 min before and after each block). The floor of the enclosure was cleaned with water between each session. Following the sleep session at the end of the afternoon training block, rats were screened for single-unit activity. Electrophysiological recordings throughout the morning and afternoon sessions were initiated when multiple well-isolated cells (>300 μV) were observed on most tetrodes. The recording phase of the experiment began after 14–26 d of behavioral training, except in one rat in which the recordings commenced after 9 d.

Recordings were first conducted for 2 d in the training paradigm (referred to as days 1 and 2 in the figure and in the text). Two behavioral sessions were performed on a third day. The third day was identical to the first two recording days, except that the start times of the blocks were shifted by 6 h, so that the first block occurred at 3:00 PM and the second at 9:00 PM. The second block was thus during the light phase of the light/dark cycle. Two animals returned for 1 d to the standard training conditions before two additional days of recordings were performed in which all four random foraging sessions in the morning and afternoon block were conducted in one enclosure shape (single shape, day 1; single shape, day 2). This paradigm was identical to the standard paradigm, except that only one of the two enclosure shapes was used in all behavioral sessions throughout both days. One rat was tested in the square and the other in the circle enclosure. For each animal, we selected the shape in which we identified the larger number of active cells during the recording on the preceding day.

Cell Tracking. Because our study depended on being able to follow the same set of principal cells over an extended time period, we developed a customized version of MClust (58) with added functions that allowed for the comparison of the cluster boundaries of each cell throughout a series of 10-min recording sessions. Clusters that persisted in the same region of parameter space throughout a day (or multiple days) were accepted for further analysis. Care was taken to accept only cells that could be precisely followed from the beginning to the end of the data analysis (Fig. 51), and for which all spikes were included in the cluster boundary such that observed rate changes could not be attributed to the definition of the cluster boundaries.

Materials and Methods

Rats were trained to run for four 10-min sessions during each block, with two sessions in the square enclosure and two sessions in the circle enclosure. The order of the shapes varied randomly within each training block. The rats were allowed to rest for 5 min between sessions, and training blocks were flanked by sleep sessions (10–20 min before and after each block). The floor of the enclosure was cleaned with water between each session. Following the sleep session at the end of the afternoon training block, rats were screened for single-unit activity. Electrophysiological recordings throughout the morning and afternoon sessions were initiated when multiple well-isolated cells (>300 μV) were observed on most tetrodes. The recording phase of the experiment began after 14–26 d of behavioral training, except in one rat in which the recordings commenced after 9 d.

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cell populations. The increasing decorrelation with longer time intervals in CA1, but not in CA3, is consistent with behavioral studies showing that the hippocampal CA1 area is selectively required for temporal coding over extended time periods (20), and that rats are able to use this information to locate a food reward (15, 19).

In contrast to CA1, CA3 cell populations showed highly reproducible firing patterns over extended time periods between repeated recordings. We therefore find a complementary neural code in CA3, which provides a highly stable representation of space and context without the possibility of contributing information about extended time. The striking stability of firing patterns in CA3 compared with the pronounced decorrelation in CA1 suggests that differences in neuronal activity over time are not due to the neuronal firing within highly plastic hippocampal networks. To the contrary, CA3 generates nearly identical firing patterns for repeated events over time intervals when changes in synaptic strengths are expected (36–39). This function is consistent with the proposed role of the recurrent network architecture in CA3 for pattern completion (27, 48–50). Although the typical definition of pattern completion proposes that a partial sensory input pattern is expanded to a pattern that is stored in memory, our finding of network stability for exact repetitions across long time intervals suggests that accurate neuronal firing patterns can be generated even from degraded sensory inputs, but also that fluctuation or degradation in synaptic strength may emerge within neural circuits over extended time intervals. Ongoing changes in synaptic strength could therefore not only be random variability, but also by network reorganization over long time periods, as predicted by theories of consolidation and reconsolidation (51). Our results indicate that hippocampal memory circuitry includes network mechanisms within CA3 that provide consistency of neuronal representations despite fluctuations or circuit reorganization over extended time intervals within CA1 and, possibly, within a wider cortical network.

Even within CA3, the firing patterns remained remarkably consistent, the input from CA3 to CA1 did not result in equally consistent firing patterns in CA1. This finding is perhaps not unexpected because CA1 also receives major direct input from entorhinal cortex (52) and is thus the site for convergence of firing patterns from layer III of entorhinal cortex—which may include temporal information (53, 54) and be critical for memory consolidation (55)—with the highly consistent firing patterns it receives from CA3. Even though the CA3 firing patterns are not completely transduced to CA1, they may nonetheless provide substantial firing stability to the CA1 population. This notion is supported by a finding that although each individual subregion became more decorrelated in the two-shape compared with the single shape condition, the difference in decorrelation over time between CA3 and CA1 remained consistent irrespective of the experimental condition (Figs. 3 and 5; Fig. S5). This consistency further supports the idea that a neuronal code for temporal distances might emerge in CA1 by adding fluctuations to a more stable representation of other aspects of context it receives from CA3.

Though temporal coding over periods of seconds and minutes can use delay-dependent and sequential coding (7–10), and though time-stamps over weeks might be explained by long-term structural reorganization (56), the mechanism that we describe corresponds to theoretical considerations (21–25) that predict that temporal information can be retrieved from the resulting variability in the population code over time. Our findings provide experimental evidence of a neuronal code that can be used for encoding temporal distances on a timescale of hours and days and that can co-occur with a precise neuronal code for other aspects of memory such that the “when, what, and where” aspects of memories can be simultaneously represented within the hippocampal CA1 cell population.
Data Analysis. For tracked cells, we calculated the spatial map of each 10-min session and the spatial correlations between maps. For a more detailed analysis of firing rates within the place field, we also analyzed the firing during individual passes through the place field (Figs. S6 and S11). From firing-rate measurements within the entire place field, we derived scores for differences between square and circular enclosures for different times throughout the experiment. For the purpose of population CA1 cells and for the population of CA3 cells, we calculated PV correlations between 10-min sessions that were recorded at different times, but within the same ensheathable scope.


Histology. Final tetrad locations were confirmed histologically. Tetrodes with tips in or near the border of the CA2 region were excluded from the analysis. Additional details on the cell tracking, data analysis, and histology can be found in SI Materials and Methods.

ACKNOWLEDGMENTS. This work was supported by the Ray Thomas Edwards Foundation, a Walter F. Heiligenberg Professorship, National Science Foundation/National Institutes of Health/Bundesministerium für Bildung und Forschung Grant 101046, and Alberta Innovates-Health Solutions.

PNAS | November 20, 2012 | vol. 109 | no. 47 | 19467
Supporting Information

Supporting Information Corrected February 20, 2015
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SI Materials and Methods

Electrode Turning. One tetrode remained in the cortex and was used as a reference for all recordings. Another tetrode was lowered to the stratum lacunoso-nummoleare to record hippocampal local field potentials. Of the remaining 12 tetrodes, approximately half were advanced toward the CA1 cell layer and the other half toward the CA3 cell layer over a period of at least 2 wk after surgery. The depth profile of the local field potential on different tetrodes (1) was used as a guide for electrode movement. When the tetrodes approached the cell layers, further movement of tetrodes was done in small increments over several days. Once the tetrodes were adjacent to the cell layer, as indicated by the presence of low-amplitude multiunit activity, tetrodes were not turned again and were allowed to settle into the cell layer for stable recordings over a period of many days.

Recording Procedures. For recording spikes and local field potentials, the electrode assembly was connected to a multichannel head-mounted preamplifier. The x-y position of light-emitting diodes on the preamplifier was tracked at 30 Hz by processing video images. Unit activity was amplified and band-pass filtered at 600 Hz to 6 kHz. Spike waveforms above a trigger threshold (40–60 μV) were time-stamped and recorded at 32 kHz for 1 ms.

Spike Sorting. Spike sorting was performed offline using the graphical cluster-cutting software MClust (2). Clustering was performed manually in 2D projections of the multidimensional parameter space (consisting of waveform amplitudes, the difference between peak and trough of the waveform, and waveform energies). Autocorrelation and cross-correlation functions were used as additional separation criteria. Putative excitatory cells were distinguished from putative interneurons by spike width and average rate. Only putative excitatory cells were included in analysis. Recording stability within a day and across days was confirmed as described in Fig. S1. In particular, sleep sessions before and after the behavioral sessions were used in addition to the recordings in behavior to assess recording stability, and a modified version of MClust was used that allows for the confirmation of the cluster boundaries of each cell in each 10-min recording session.

Cluster Quality Metrics. For each cluster, we calculated the L-ratio and isolation distance as described (3). When calculating the Mahalanobis distance, we included the features that we used for clustering (see above). We calculated the cluster quality for each experimental block by including all putative spikes that were detected during all behavioral sessions of the block. Full-day cluster quality was reported as the mean cluster quality between the two blocks of a given day. Cluster quality was compared across hippocampal subregions using a two-sample Kolmogorov–Smirnov (KS) test.

Spatial Maps. Spatial firing-rate distributions for each well-isolated neuron were constructed in the standard manner, by summing the total number of spikes that occurred in a given location bin (5 × 5 cm), dividing by the amount of time that the animal spent in that location, and smoothing with a Gaussian centered on each bin (4).

Spatial Correlations. Spatial firing patterns of individual cells were compared between sessions with a spatial correlation procedure (4, 5). The Pearson correlation coefficient for firing rates in corresponding pixels was calculated for pairs of spatial maps of each cell. Pixels visited less than 150 ms in either session were excluded to avoid artifacts in the correlation measure.

Place Field Boundaries. Standard practice has been to define boundaries by first finding the pixel with the maximum firing rate and then iteratively extending the field to any adjacent pixels that exceed a predefined threshold (4, 6–8). In our data, we found that a subset of fields had clearly separated peak values that were nonetheless joined by adjacent pixels and could thus not be separated by this method. We therefore developed a contour-based analytical method to separate fields (Fig. S11).

Individual Passes Through Place Fields. For each place field and each experimental session, the path segments inside of field boundaries were identified. Time periods when the path segments were continuously inside of the field boundary for at least 200 ms were considered a pass through the field if the path crossed inside of the contour at 60% of the field’s peak value. The average firing rate for a pass was calculated as the number of spikes during the pass divided by the duration of the pass. In addition, we calculated the average velocity during the pass and the minimum distance to the center of the field (Fig. S6). For any analyses reported by fields, we discarded fields through which the rat passed fewer than five times in any individual session.

Rate Changes Within Sessions. For each place field recorded on single shape, day 1, the rate change per session was calculated by fitting a regression line to the rates during individual passes through the field and by taking the difference of the y-values of the line between the end and start of the session. If rates fluctuated randomly in time, the average difference over repeated sessions should be close to zero. To measure whether a place field showed consistent rate changes, a Student t test was performed on the set of eight rate changes (one from each of the eight sessions within the day). If the mean rate change was found to be different from zero at the 0.05 level, that field was considered to change its rate consistently.

Shape Preference Score. To measure whether neuronal firing within a place field occurred preferentially in one enclosure shape, we calculated a shape preference score. For each field, the rates from the individual passes were combined to yield a distribution of firing rates for the square enclosure and a distribution of firing rates for the circular enclosure. The degree to which these distributions overlapped was calculated as the area under the receiver operating characteristic curve for the distribution in the square compared with the distribution in the circle. If all firing rates in the square enclosure were higher than in the circular enclosure, the area under the curve was 1. If all firing rates in the square enclosure were smaller than in the circle enclosure, the area was 0. If the distributions were identical, the area was 0.5. Then we did a linear transformation on this measure (subtracting 0.5 and multiplying by 2), so that the shape preference score would vary between −1 and 1, with 0 indicating no shape preference. The signed value of this score provides a nonparametric estimate of preferred firing in the square enclosure shape compared with the circle enclosure shape. The absolute value of the score gives an indication of the extent of firing preference for either shape.

Rate and Shape Preference Variability. To compare the variability in firing rate and in shape preference between CA1 and CA3 across time intervals, we calculated the average firing rate of each cell...
and the shape preference score of each place field at different time points. Morning firing rates were compared with afternoon firing rates, morning preference scores were compared with afternoon preference scores, and day 1 preference scores were compared with day 2 preference scores. The values for the two different time points were plotted against each other. If cells showed the same firing rate or the same degree of shape coding at the two time points, the data points would fall on the identity line. We used an F-test to determine whether the variance around the identity line differed between the CA1 and CA3 subregions. Because the average shape preference could systematically change over time, resulting in a best-fit line that is different from the identity line, we repeated our analysis with a standard linear regression line. The results with the regression line replicated the findings with the identity line. To evaluate whether the variability in shape preference could be related to cluster quality, we repeated F-tests within each brain region for place fields with clusters in the highest quartile of cluster quality compared with place fields with clusters in the lowest quartile of cluster quality.

Population Vector Correlations. For each behavioral session, rate vectors were constructed by arranging the spatial maps of all cells recorded from all animals in an x-y-z stack, where x and y represent the two spatial dimensions in 5 × 5 cm bins and z represents the cell-identity index (9, 10). CA1 and CA3 cells were analyzed in separate stacks. For multiple-day analysis, only cells that were tracked from the beginning of the first day through the end of the last day were included in the population vectors. The distribution of mean rates along the z axis for a given x-y location represents the composite population vector for that spatial bin and hippocampal subregion. For each pair of sessions, the Pearson correlation coefficient was calculated for spatial bins at corresponding locations. Cells with firing below 1 Hz in all bins of the two sessions were excluded from the analysis. To allow for comparisons between the square and circle enclosure shape, the analysis was restricted to the 16 × 16 cm bins that were common to both shapes, yielding 256 correlation estimates for a pair of sessions. The correlation coefficients of all spatial bins were averaged to estimate the average population vector correlation for a pair of sessions. All average correlations for a particular time interval (e.g., <1 h, 6 h) were compared using Student t tests or, for experiments with more than two time points, using ANOVA with time interval and enclosure shape as factors or, in Fig. S5, with time interval and number of enclosure shapes as factors. Bonferroni corrections were applied to correct t tests and post hoc analysis for multiple comparisons. To create the cumulative density plots, the population vectors of all spatial bins from pairs of maps with the same time lag were combined, and the functions were plotted for each time lag. The complete matrix of population vector correlations between all 10-min sessions in which cells were recorded over 2 d are shown in Fig. S9.

Histology and Three-Dimensional Reconstruction of the Tetrode Array in Serial Sections. Rats received an overdose of sodium pentobarbital and were perfused intracardially with saline and 4 % formaldehyde. The brains were extracted and stored in formaldehyde. Frozen coronal sections (40 μm) were cut and stained with cresyl violet. Each section through the segments of the hippocampus with electrode tracks was collected for analysis. All tetrodes of the 14-tetrode bundle were identified by finding their electrode tracks across sections. A small angular deviation between the plane of sectioning and the electrode tracks resulted in an apparent downward shift of the tissue damage. The electrode tip was considered to be located in the section where the tissue damage became negligible (Fig. S7E). Recordings from a tetrode were included in the data analysis if the tetrode’s deepest position was in the CA1 or CA3 pyramidal cell layer.

Clusters of spikes from single cells were tracked across multiple days. (A) The behavioral design and the experimental timeline are shown for the 2-d, single-shape experiment including rest and behavioral sessions. The sessions that are shown in B and D are highlighted in gray. (B) Each panel shows the projection of the peak amplitude recorded on one channel of a tetrode located in the CA1 pyramidal layer compared with the peak amplitude recorded on another channel of the same tetrode. The same projection is shown for each cluster diagram, and different diagrams correspond to different time points in the experiment. Each dot represents one sampled spike. Colored dots of a single color represent spikes that were assigned to a single cell. After deidentification, clusters on a single day, the cluster boundaries were applied to data recorded from the same tetrode on subsequent days. If clusters persisted in the same region of parameter space where they had been identified on the previous day, boundaries were adjusted to assure that all spikes that were part of the cluster were included in the cluster. (C) The isolation distance for each cluster was calculated as the distance between the peak of the L-Ratio and the peak of the CA1 or CA3 peak. (D) The shape preference for each cluster was determined by calculating the cumulative probability of the L-Ratio for each time point. (E) The cumulative probability of the shape preference for each cluster was calculated as the percentage of spikes that were assigned to the same cell on multiple days. The shape preference for each cluster was determined by calculating the cumulative probability of the L-Ratio for each time point. (F) The cumulative probability of the shape preference for each cluster was calculated as the percentage of spikes that were assigned to the same cell on multiple days. The shape preference for each cluster was determined by calculating the cumulative probability of the L-Ratio for each time point.
included within the boundaries. If the spikes for a set of clusters on one tetrode could be included with minor adjustments of the boundaries from the day before, the clusters were considered to be the same set of cells as on the day before, and the cells were included in our analysis as trackable across days. Because we occasionally found cells that appeared to turn on or turn off during the day, it was imperative to confirm that these effects were not simply a consequence of a tetrode having moved into or out of the range where the cell could be recorded. We thus only included cells in the analysis that were active at the beginning of the day (in the first sleep session or the first behavioral session in either shape) and at the end of the day (during the last sleep session or the last behavioral session in either shape). The highlighted (blue) cluster depicts a cell that fired robustly during all behavioral sessions on the first day and in the morning of day 2; it then fired very few spikes during the second afternoon (PM3–4, day 2) before regaining robust activity during the PM Rest2 session, indicating that the small number of spikes recorded during the afternoon was not due to the cell having moved out of the assigned parameter range, but was rather due to a genuine decrease in firing rate. Note that the pattern of clustered spikes remained stable throughout the entire recording sequence, confirming to the criterion for assessing stability of the recording electrode as described above. (C) Average waveforms from four clusters shown in B; the color of the waveform corresponds to the color of the corresponding spike cluster. The average waveform ± SD is shown for the first behavioral session (Left) and the last behavioral session on the second day (Right). The average waveforms and the average amplitudes remained consistent throughout the experiment as demonstrated by the comparison between AM1, day 1 and PM4, day 2. (D) Spatial maps from the same four cells in C during the behavioral sessions in B. The average firing rate in each 5 × 5 cm pixel is color-coded with a color scale from 0 Hz (blue) to the maximum peak rate within the day (red). The peak firing rate for each cell on each day is indicated to the right of the PM4 map. Although spatial maps from tracked cells were not used to determine whether clusters were the same from one day to the next, we visually inspected spatial maps from tracked cells. The location of place fields was identified to be the same from one day to the next, confirming that our method for tracking cells was reliable. (E) For each cluster that had a place field, isolation distance was used as a metric of cluster quality. Higher isolation distance indicates better cluster quality. (Left) There was no difference in the distribution of cluster quality between CA1 and CA3 on either the single day (Upper, KS test statistic = 0.21, not significant (n.s.)) or the clusters that were tracked across 2 d (Lower, KS test statistic = 0.29, n.s.). (Middle) The variability in shape preference observed in CA1 was not related to cluster quality. The same data as presented in Fig. 4 are shown with dots color-coded by cluster-quality quartile. The clusters with highest quality are shown as blue circles, the clusters with lowest quality are shown as yellow squares, the middle 50% of clusters are in gray (clusters whose quality metric was undefined are omitted from the plot). The degree to which clusters vary with reference to the identity line is not different between the best and worst quartiles for either single day (Upper; F(20, 20) = 0.97, n.s.) or 2-d (Lower; F(11, 13) = 1.37, n.s.) data. (Right) The same measures are shown for CA3 data. Variability was not different between the best and worst quartiles for single day (Upper; F(9, 9) = 2.79, n.s.) or 2-d data (Lower; F(6, 6) = 2.43, n.s.). (F) Cluster quality was assessed using a second metric, L-ratio. For this metric, lower scores indicate better clusters. Panels are laid out as described in E. There is no difference in the distribution of cluster quality between CA1 and CA3 for single-day data (KS test statistic = 0.24, n.s.). For clusters tracked across 2 d there is a small but statistically significant difference in distributions with CA1 clusters having lower scores than CA3 (P < 0.05, KS test statistic = 0.38), indicating that cluster quality in CA1 is slightly better than in CA3. There is no difference in variability between the clusters in the best quartile and worst quartile in either brain region [CA1, single day: F(20, 20) = 1.21, n.s.; CA1, 2 d: F(12, 12) = 1.21, n.s.; CA3 single day: F(9, 9) = 0.63, n.s.; CA3 2 d: F(6, 6) = 0.38, n.s.].

Fig. S2. The similarity in the activity patterns of the CA1 neural network continued to decrease on a third recording day. (A) To assess differences in CA1 network activity patterns across time intervals of up to 60 h, we extended the hippocampal recordings across 3 d in three animals. On the third day, behavioral sessions were shifted by 6 h, providing additional comparisons for the population of cells that was tracked across all 3 d (n = 30 CA1 cells). (B) Population vector correlations between pairs of recordings in the same enclosure shape are shown as dots. The black error bars correspond to the mean ± SEM for recordings at a particular time interval. The correlation coefficients for the CA1 population activity decreased as a function of elapsed time between recording sessions (F(8) = 78.8, P < 0.001). At 24- and 48-h intervals, which represent recordings at matching times of day, are indicated in gray. Repeated CA1 recordings at the same time of day on two consecutive days show a smaller correlation than repeated recordings at shorter intervals, but at a different time of day (P < 0.001 for the post hoc comparison). The decrease in the population vector correlation therefore does not show a circadian pattern. (C) Cumulative distribution functions for population vector correlations between pairs of recordings in the same enclosure shape.
Fig. S3. The activation of hippocampal neurons during intervening behavioral testing is unrelated to the pattern of decorrelation with extended time. (A) Population vector (PV) correlations for comparisons between recordings in the same enclosure shape at intervals of 24 h (Left) or 30 h (Right). Each comparison between two sessions is shown as a dot, and the black bars are the mean ± SEM for a time interval. Differences in the number of intervening blocks did not result in differences in the degree of decorrelation (comparisons between 24-h intervals: \( t = -0.64 \), n.s.; comparisons between 30-h intervals: \( t = -1.82 \), n.s.). (B) PV correlations between pairs of recordings in the same enclosure shape without intervening blocks. The 24-h comparisons are from recordings without an intervening AM block (Fig. S2). Each comparison between two sessions is shown as a dot, and the black bars are the mean ± SEM for a time interval. A decorrelation of the CA1 population activity with time was observed \( F(3) = 41.6, P \) values for all post hoc comparisons <0.001 except that the comparison between the 18- and 24-h interval is n.s.]

Fig. S4. Changes in network activity patterns at shorter timescales. (A) The change in firing rate within a session is shown for individual fields in CA3 and CA1, recorded across eight sessions in the same shape (four during the AM and four during the PM block). To obtain estimates for the firing rate at the beginning and at the end of each session, a regression line was fitted to the firing rates during single passes through the place field. For each field, the difference in firing rate between the beginning and the end of each of the eight 10-min sessions is shown as a dot. Colored dots depict rate differences when the mean rate change was significantly different (at the 0.05 level) from zero, which is the expected value in the case of random fluctuation in firing rate. The black bars show the mean ± SEM for each field. The fields of each hippocampal subregion are sorted in the order of their mean firing rate difference. Many CA3 cells consistently changed their firing rates within a series of 10-min recording sessions, often resetting toward the previous start-value. Together with the finding that CA3 changed to a small degree over extended time periods, these results show that changes in firing within a session do not result in corresponding changes in population activity over long time intervals. (B) Representative CA3 and CA1 place fields for the effects described in A. Place cells were repeatedly recorded within the same shape. The regression line for firing rate vs. time in each session is shown in black.
Fig. S5. The difference between population vector correlations in CA1 and CA3 is consistent across experimental paradigms. (A and B) For comparison, data from Fig. 3C (A) and Fig. 5B (B) are redrawn. In A, the PV correlation is plotted for the two-shape experiment, in which animals were run in two enclosure shapes during each recording block. In B, the same analysis is shown for the single-shape experiment, in which animals were exposed to only one shape for the entire 30-h experiment. (C) The difference between CA1 and CA3 was larger in the two-shape condition compared with the single-shape condition, but increased in parallel over time for the two conditions [main effects for time and paradigm: $F(4) = 22.3$, $P < 0.001$ and $F(1) = 97.4$, $P < 0.001$; no significant interactions]. This suggests that the decorrelation due to time may be added in CA1 to a small decorrelation that occurs in both subregions in response to other aspects of the experience, such as the ongoing changes in the shape of the recording enclosure.
Fig. S6. Method for calculating firing rates during individual passes through a place field. (A) The spatial maps from a representative cell are shown for the four behavioral sessions in the morning (AM sessions 1–4) and for the four sessions in the afternoon (PM sessions 1–4). The boundary of the place field is outlined in white (see Fig. S11 for the method that is used for defining boundaries). (B) For each 10-min behavioral session, the path of the rat is shown in gray. The spikes fired by the cell are superimposed on the path in red. As expected, most spikes occur within the place field boundary, shown in black. (C) Each of the four panels shows spikes of an individual pass through the place field during a 10-min session (AM3 on the Left, PM3 on the Right). The path of the rat from the entire 10-min session is shown, and single passes (from crossing the boundary into the field until exiting the field) are highlighted in color. Spikes that occurred during the pass through the field are superimposed on the path as dots. (D) For each pass through the field, we calculated the average firing rate as the number of spikes that occurred during the pass divided by the duration of the pass. We also calculated the average velocity at which the rat traveled. The average firing rate for each pass is shown as a colored bar (purple for foraging sessions in the circle), with firing rate on the y-axis and the time within the behavioral session on the x-axis. The average velocity of each pass is represented as a bar plotted downward from the time axis (in blue). The representative passes shown in C are marked with arrows. (E) Firing rates for all passes although the place field throughout the entire recording day.
Fig. S7. Firing rates of complete cell samples recorded simultaneously on the same tetrode as the representative examples shown in Fig. 2A and B. The examples that are also shown in Fig. 2 are highlighted in gray. (A) CA1 place fields recorded simultaneously on the same tetrode as field 1 in Fig. 2A. (B) CA1 place fields recorded simultaneously on the same tetrode as field 2 in Fig. 2A. (C) CA3 place fields recorded simultaneously on the same tetrode as field 3 in Fig. 2B. (D) CA3 place fields recorded simultaneously on the same tetrode as field 4 in Fig. 2B. For each place field in A–D, all 10-min recording sessions throughout the AM and PM are shown. The order of the enclosure shapes is indicated above the plot (Upper Left). Each bar represents the firing rate of the cell during a pass of the animal through the place field (Fig. S6). Time within the recording session is shown on the x-axis. The bars are colored green for square enclosures.
and purple for circular enclosures. For each pass, the corresponding running speed of the animal is plotted downward below the x-axis (blue bars). A pass during which the cell does not fire is indicated by the presence of a velocity bar for that pass without a corresponding rate bar. A regression line for firing rate vs. time is fitted to each session (shown in black). (E) Cresyl violet-stained section showing recording sites in the hippocampus. Final recording sites in CA3 are outlined in blue, for CA1 in red. (Scale bar, 500 μm.)
Fig. 58. Firing rates during individual passes through place fields were weakly correlated with the animal’s velocity and with the minimum distance to the center of the place field. (A) For each field, we calculated the correlation (Spearman’s rank correlation coefficient) between the animal’s average velocity and the cell’s firing rate on individual passes through the field. All passes in all behavioral sessions from a single enclosure shape (square or circle) were combined. Fields that were silent in a particular configuration were excluded from analysis, as were fields through which the rat passed fewer than five times in any 10-min session. The distribution of correlation values is shown for CA1 (Upper, median = 0.16) and CA3 (Lower, median = 0.23). Examples of representative fields in (B) CA1 or (C) CA3. Each panel shows a scatter plot of rate vs. velocity for all passes through the place field. Correlation coefficients are noted for each field, and coefficients that are significantly larger than zero are marked in blue. (D-F) Panels are laid out as in A–C, but here the correlation between firing rate and the minimum distance of the path to the center of the place field is reported [CA1, median = −0.18; CA3, median = −0.23; in E and F, fields with a correlation coefficient significantly less than zero (at the 0.05 level) are marked in pink]. We expect a negative correlation because the center of the place field is defined as the place at which the cell’s peak firing occurred. Traveling closer to the center should thus lead to increased spiking. Though there is a moderate correlation in firing rate with both velocity and distance to the center of the place field, this correlation is not sufficiently high to explain the variability in our data. Of particular relevance to our data, rats did not show a consistent trend over extended time in either their velocity profiles or in the average distance to the center of the place field. The difference in the mean velocity between morning and afternoon sessions never exceeded 2 cm/s and, on average, was less than 0.5 cm/s.
Fig. 59. Correlation matrices for repeated recordings in the same enclosure shape. (A) Each AM and PM recording block during two recording days (day 1, day 2) included two sessions in the square enclosure and two sessions in the circle enclosure (Fig. 3). Over 2 d, eight recording sessions were therefore performed in enclosures of the same shape. The correlation matrices depict all possible comparisons between repeated recordings in the same shape at different time points. The mean population vector correlation (i.e., the average across the spatial bins of the recording enclosure) of each pairwise comparison between identical enclosures is shown. The scale bar to the right of the matrices shows the color code for the correlation coefficients. All four matrices use the same color scale. Comparisons between the same sessions are shown along the diagonal, and their correlation coefficient is, by definition, 1. Comparisons at 24-h intervals are highlighted by black boxes. The matrices are symmetrical along the diagonal and the same values are thus shown in the Upper Right and Lower Left. In CA1, the highest correlation coefficients were measured at the time intervals closest to the diagonal and decreased correlation coefficients at longer time intervals. A small decorrelation was also observed in CA3, but the effects are minor compared with CA1 (see Fig. 3 for statistics). (B) For the single-shape control days, each AM and PM block during two consecutive recording days included four recording sessions in the same shape. The correlation matrices therefore include comparisons between each of the 16 sessions. As observed in A, population vectors in CA1 (Left) became substantially decorrelated with increasing time intervals. In contrast, population vectors in CA3 remained highly correlated for intervals of up to 30 h (see Fig. 5 for statistics).
Fig. S10. CA1 place fields that were recorded simultaneously on the same tetrode as those in Fig. 4B. The firing fields that are identical to those in Fig. 4B are highlighted in gray. For each place field, firing correlates are shown for all 10-min recording sessions within the AM and PM for each of the two recording days, with time within each recording session on the x-axis. The order of the enclosure shapes throughout the sessions is shown above the plot (Top). Each bar represents the firing rate of the cell during a pass of the animal through the place field as described in Fig. S6.
Fig. S11. Illustration of the method for calculating boundaries of place fields. To illustrate that the analysis procedure separates adjacent fields, a cell with multiple fields is shown as an example. (A) Spatial maps are first calculated for each of the eight behavioral sessions in a single day. The firing rates within each map are color-coded according to the scale bar on the right, and the shape of the box is superimposed on each map. (B) A reference map was calculated as the average of the eight maps in A. Note that the place fields in the reference maps are overlapping, but that the maps of individual sessions suggest that the firing rates in different areas appear to be modulated independently. (C) Contours were calculated at 20 levels between zero and the peak rate. The contours are shown superimposed on the reference map. (D) Peak contours (shown in red, green, and blue in the three panels) were found. Surrounding contours are in gray. The three fields share many of the contours at lower levels. (E) Each shared contour is divided into segments at inflection points of the contour, and each segment is assigned to the nearest field. Each segment is illustrated in the color of the field to which it is assigned. (F) The end points of the segments and the outside contours of different colors in E are combined into outer boundaries of the fields. (G) The maps in A are redrawn with the boundaries of the three identified fields superimposed. (H) Two additional examples of cells with multiple, adjacent fields. The large panels (left) depict the reference map, and the smaller panels show the eight individual sessions with the field boundaries superimposed (arranged as in A). (I) The method also identifies the boundaries of single fields. Three examples of cells with single fields are shown. (J) Histograms of the number of fields of CA1 cells (upper) and CA3 cells (lower) in the single-shape condition. (K) Histograms of the number of fields of each cell in the two-shape condition. The shift toward a higher number of fields per cell is a result of recording in two box shapes, because fields occasionally appear in one box shape but not in the other.