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Long-term stability of the place-field activity of single units recorded from the dorsal hippocampus of freely behaving rats

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Over 90% of all spontaneously active hippocampal pyramidal cells in freely moving rats signal the animal's spatial position by reliably changing their firing rate each time the animal enters a given place within an environment. This place-field activity exhibits plasticity when specific environmental variables are manipulated. Indeed, the hippocampus is perhaps best known as a system that serves as a model of neuronal plasticity. Although place-field activity has previously been examined only over relatively short experimental sessions, this behavioral correlate of hippocampal functional activity has been assumed to exhibit stability rather than plasticity in the absence of environmental changes. The present study shows that hippocampal neurons have stable place-field correlates that persist over very long periods of time. Single-unit activity was chronically recorded from the dorsal hippocampus of rats foraging repeatedly in a stable spatial environment. The location of the place fields of all units were stable over all time periods tested, for intervals up to 153 days in duration. The consistency of the information conveyed by this single-unit activity in a fixed spatial environment indicates that stability of neuronal activity may be as important as plasticity in the integrated processing of information that occurs in the hippocampus and throughout the nervous system.

INTRODUCTION

A critical assumption about the organization of nervous systems is that the elements are stable. That is, they show a continuity of function over time. This assumption is so fundamental that it is implicit in virtually every theory of brain function. It is assumed in theories of sensory processing, for example, that once the receptive field of a primary sensory neuron is established, it is stable for the life of the organism[29]. Similarly, the very concept of a 'motor unit' requires stability of established sensory and motor neuron responses[30]. Even recent interest in neuronal plasticity is properly examined in the context of such stability, since plasticity is a notable change from one stable functional state to a new stable state. It is somewhat surprising that there have been few rigorous experimental tests of this basic principle of long-term stability of neuronal activity. With the exception of studies of sensorimotor systems[12,26,34,52,54] and of the correlates of sleep–waking activity[57], the functional correlates of single-neuron activity are rarely examined over periods exceeding a single day of laboratory time. With the exception of the present report, no known studies have examined the more complex behavioral correlates (rather than simpler sensory evoked activity) of single neurons over periods of time exceeding one week.

One widely studied neuronal phenomenon, hippocampal *long-term potentiation*, is occasionally examined for periods exceeding 48 h in duration[51], but rarely if ever for more than a few weeks[9]. Over this so-called 'long-term' period, neuronal responses to changes in afferent activity are so dramatic that the hippocampus has come to serve as a testing ground for theories about neuronal plasticity. It is widely assumed that response plasticity is a ubiquitous property of hippocampal neurons. This emphasis on hippocampal plasticity has obscured the fact that a number of neuronal responses originating in this region are quite stable. In the kindling model of epilepsy, for example, induced changes in aggregate neuronal activity persist for long periods of time after withdrawal of the inductive stimulus[18,27]. Although most behavioral correlates of hippocampal single-unit activity are stable over short periods of time, up to 24 h[38], they are generally not described over significantly longer time intervals.

For example, individual hippocampal pyramidal units demonstrate reliable *place-field* correlates, increasing their firing rates in selected places within an environment and becoming virtually silent in other places[5,6,10,14,

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[20,24,32,40,46,56]. Changes in the sensory cues present in a spatial environment alter the place fields of hippocampal neurons in a systematic manner, but these changes in the neuronal spatial correlates are reversible when the spatial environment is returned to its former configuration[37, 41,42]. Limited deafferentation of the hippocampus disturbs both place-field activity and behavioral performance on tasks requiring cognitive processing of spatial information, while more complete deafferentation or hippocampectomy completely disrupts spatial behaviors[22,33,36,45]. Such results indicate that the hippocampus plays an important role in processing information about space, and indeed it has been theorized that one of its functions may be to serve as a spatial cognitive map[39, 41,43]. By definition, a *place field* requires short-term stability of the behavioral correlates of the unit activity under study for its identification. Such short-term stability, although generally assumed, has in fact been empirically demonstrated[38] over experimental sessions of 16 min duration, and in one less compelling instance for up to 6 days.

When studying the neuronal mechanisms underlying this or other behavioral correlates of neuronal activity, it is reasonable to ask if this correlate is influenced by events unrelated to the experimentally observed spatial behaviors. If the hippocampus and other limbic structures are particularly plastic brain regions, as some reports suggest, the effects of experiences in diverse environments might alter hippocampal activity in both specific and non-specific ways. Changes in functional activity undetectable over short time spans might become apparent over longer spans (as is the case in kindling). Consequently, we examined place-field activity over long periods of time in a fixed spatial environment to determine whether this specific behavioral correlate of neuronal activity exhibited long-term stability, irrespective of events occurring outside the fixed environment.

EXPERIMENTAL METHODS

Housing and training of experimental subjects

Young male Long–Evans rats were food deprived for approximately 22 h daily and maintained in individual home cages in one of two vivarium facilities on a consistent 12 h light/12 h dark cycle. Immediately following daily behavioral training, food was made available for 1 h in the home cages. Non-regulated variables in the experiences of individual rats in this environment included the density of the colony population within the room; the frequency of cage cleaning and changes; the frequency of entry and the duration of presence of human experimenters; and numerous non-cataloged variables relating to construction and maintenance within the building.

The rats were trained daily to run on an open 6-arm radial maze centered in a dimly lit, black-curtained sound-attenuated Faraday cage (2 × 3 × 2.3 m). A spatially fixed white noise source in the west ceiling was provided by a small ventilation fan. The location of all identified spatial cues (the start box used for entry to the maze; the doorway to the chamber; light sources; etc.) were maintained constant over the entire course of testing. Similar environments have been used extensively for testing the effects of various environmental manipulations upon hippocampally dependent spatial behaviors[45]. The radial arm maze (1.45 m in diameter, with arms 10 cm wide, elevated 0.7 m from the floor, divided into grids 50 mm on a side) provided a limited number of paths for an animal attempting to maximize its foraging effort, thus optimizing behavioral consistency at various places on the maze. Each arm of the maze was physically similar to all others, painted flat grey, and wiped clean with an ethanol solution after each animal's session to minimize odor cues. Food or fluid baits were placed in 10 ml capacity Plexiglas cups at the ends of the arm. Initial behavioral shaping in a win-shift paradigm was used to train the animals to explore the maze[46,47]. A behavioral criterion of 3 successive replicates of 6 correct choices per set of trials on 3 successive days of training was required for inclusion in the recording study. After an animal reached criterion, arms of the maze were baited with 0.15% sodium saccharin solution or Froot Loops cereal at random intervals on subsequent days of testing. Well-trained rats generally made 12 or more visits to each arm within an hour long session.

To test the effects of specific experiences external to the fixed spatial environment on place-field activity within the environment, 3 of the rats used in this study were also classically conditioned in a separate environment in a conditioned emotional response paradigm[8,50]. Immediately following radial-maze foraging trials on 6 consecutive days, rats were placed in a separate cubical testing chamber with an electrical grid floor (0.5 × 0.5 × 0.5 m) for adaptation, and allowed to drink water from a water bottle inserted through one wall. During the next 4 days, a tone (3 kHz for 1 s) followed immediately by unavoidable footshock (1 mA for 1 s) was presented at 1 h intervals for 6 trials, and freezing and lick suppression were observed. As noted, other non-specific experiential differences between animals were not directly controlled, but varied dependent upon the conditions extent in two different vivarium facilities in which the animals were housed; and also dependent upon the length of time required for single-unit isolation prior to long-term study.

Instrumentation and single-unit recording

The recording methods used have been described previously[56]. Driveable bundles of ten 32 μm nichrome microwire electrodes[23] were chronically implanted in the dorsal hippocampus of maze-trained rats under barbiturate anesthesia. Several days after surgery, the electrodes were advanced until one or more hippocampal complex-spike units were isolated extracellularly. Conservative criteria for single-unit isolation were used: the peak-to-peak amplitude of the unitary signal had to exceed that of all other signals (except decremental spikes within a complex-spike burst) on the electrode by at least a factor of 4:1 for inclusion in the study; i.e. individual signals from electrodes with multiunit activity were not included in the statistical analyses. Only the first spike of complex-spike bursts was digitized and used for compilation of rate data. The peak amplitudes of the negative spike of all unitary activity and of all decremental complex-spikes had to vary by less than 5% within and across sessions for inclusion in the study. (Note: the latter criterion was extremely conservative, as no units had to be excluded due to variability in unit amplitude across sessions. In our hands, if stable recordings were not obtained within a single session, we were unable to isolate the same unitary signal on the same electrode on subsequent sessions. Either unit signals with different waveform characteristics were observed, or more typically all identifiable signals were lost, requiring electrode advancement to isolate a new unit.) Complex-spike units were differentiated from the other major behavioral class of hippocampal units, theta cells, based upon Ranck's criteria[16,17,48], including a low spontaneous rate of activity and constant waveform during awake quiet immobility, and increased activity during slow-wave sleep. Complex-spike activity was also considered, but not used as a rigid criterion, since the proportion of complex-spike bursts to single spike firings varied

considerably between neurons, and even for the same neuron within sessions.

Unit activity in the behaving rat was amplified by DC-powered source-follower FET amplifiers attached to the chronic headblock, and differentially recorded against an adjacent hippocampal electrode and/or a cortical indifferent electrode. Unit signals were amplified with Grass P15 AC preamplifiers (gain × 10; bandpass 0.3–3.0 kHz) and BAK MDA-3 AC differential amplifiers (gain × 1000; bandpass 0.5–5.0 kHz), and displayed on a storage oscilloscope. Single-unit activity was hardware discriminated using standard time/amplitude window discriminators and stored online on a 6502-based microcomputer.

Place field measurements

As the rats explored the radial maze, a video camera centered overhead recorded their spatial behavior while filtered single-unit activity was recorded on the audio channels of a video cassette recorder. A small DC lamp was mounted atop the electrode assembly, and an imaging device digitized the Cartesian spatial coordinates of the light continuously[7,38]. Testing sessions for each unit lasted at least 60 min per day, at irregular intervals across as many days as the unit continued to show spontaneous activity in the waking rat. If spontaneous activity was not apparent, the electrode was again advanced in 30 μm steps until additional complex-spike units were isolated, or the experiment was terminated. Small electrolytic marking lesions were used to localize the electrode tip at termination, and single-unit recording sites were calculated from records of electrode advancement relative to the lesion site observed in Nissl-stained sections.

Place-field activity was analyzed by computer as follows. The Cartesian coordinates of the animal's spatial location were mapped each time a unit fired. Independently and simultaneously, the coordinates of all locations visited by a rat within a session were mapped. These sets of coordinates were divided into square grids, 50 mm on a side. Grids in which at least 5 s of behavior were not observed were excluded from analysis for any given experimental session, to minimize sampling errors in the observed firing rate. The mean rate of activity of each unit was calculated within these grids by dividing the number of spikes fired in each grid by the amount of time spent in each grid. Firing rates within a grid greater than 3 standard deviations above the mean firing rate for the entire environment were used to define the boundaries of place fields. This is a more conservative measure of the boundaries of a place field than is used by some researchers, but is quite reliable statistically[20,46].

Assessment of long-term stability

A simple variant of a Monte Carlo statistical test[14] was used to evaluate the stability of place-field activity, i.e. to determine whether a given place field remained in one place over time or whether it moved from session to session. As noted above, the data relating the Cartesian coordinates of a rat's position to place cell firing was reduced to an array of firing rates in square grids representing the surface of the radial-arm maze for each test session. The locations of the grids which made up a place field during any given session were readily comparable between sessions. The statistical test used determined whether the locations of the place fields observed on different sessions were significantly closer to one another than would be expected by chance. If place fields did not move apart across days, the distance between the grids making up the fields on different days should be small and relatively constant. If place fields exhibited random movement around the environment, generation of a large number of alternate sets of equal sized groupings of grids would be expected to yield some sets closer to the original place field than those actually observed on subsequent days of testing. Starting with those grids that made up a place field on a specified day, the distance between each of those grids and each grid visited on subsequent testing days was calculated. It was then determined if the grids included in the original place field were significantly closer to those of subsequent fields than to grids outside the place field on subsequent days. If the original and subsequent place fields were unrelated, i.e. if the location of the field moved between days, or if the electrode moved to a new unit with a place field in a different location, then many grids outside the subsequent place field would be significantly closer to the grids of the original field than would be the grids within the field observed on subsequent days. Absolute distance measures (in grid units) between the grids making up the place fields on each day were also calculated, and are shown in Table I. This absolute distance measure is an indicator of the displacement of a place field between sessions.

A Mann–Whitney *U*-test was used to test significance, because Monte Carlo methods demonstrated that the underlying distribution

TABLE I

The stability of the place-field activity of 10 hippocampal units held for 6 or more days is shown

The rate of activity in-field and out-of-field is the mean over daily sessions. The in/out ratio represents the ratio of mean firing rates. The mean distance (in radial-maze grid units) between the grids making up the place field on any day tested is shown, as is the mean distance between the grids representing the place field for one unit and the grids representing the place fields of other units isolated on the same electrode at other recording sites within a 75 μm electrode excursion (in all cases, the probability of the same place field being located at this greater distance was greater than chance ($P > 0.5$). The probability (P) that the fields were no closer than predicted by chance was determined between all combinations of sessions for a single unit. The P value is reported for the comparison of the first and the last recording session for each unit (the most long-term measure of stability), as well as the P value between the sessions where the fields differed the most, i.e. the P most closely approximated chance (the worst-case session for each long-term unit).

n (days)	*n (sessions)*	*Mean rate in-field (Hz)*	*Mean rate out-of-field (Hz)*	*In/out ratio*	*Mean distance between grids of a field*	*Mean distance to grids of adjacent cells*	*P first-last session*	*P worst session*
153	14	6.1 ± 0.4	0.5 ± 0.1	12.2 ± 0.7	1.64	7.28	<0.0001	<0.003
66	17	5.2 ± 0.3	0.7 ± 0.2	7.5 ± 0.2	1.45	11.37	<0.0004	<0.002
43	4	8.2 ± 0.1	1.0 ± 0.1	8.2 ± 0.2	3.08	9.24	<0.002	<0.01
32	5	9.4 ± 0.4	0.5 ± 0.1	18.8 ± 0.1	1.04	12.95	<0.0002	<0.004
30	4	9.6 ± 0.3	1.1 ± 0.1	8.7 ± 0.4	1.13	–	<0.0003	<0.0003
18	5	8.1 ± 0.2	0.6 ± 0.1	13.5 ± 0.3	1.13	8.16	<0.0005	<0.0005
12	4	8.6 ± 0.8	0.7 ± 0.1	12.3 ± 0.9	0.77	16.90	<0.0003	<0.002
12	3	9.4 ± 0.4	0.5 ± 0.1	18.8 ± 0.1	1.74	13.87	<0.0002	<0.004
11	3	7.3 ± 0.1	0.6 ± 0.1	12.2 ± 0.5	1.69	14.13	<0.0005	<0.0005
6	3	5.7 ± 0.2	0.7 ± 0.1	8.2 ± 0.4	2.61	14.47	<0.0003	<0.0003

of distances between grids was not normal and that the variance differed drastically depending on the specific size, shape, and location of the original place field. Monte Carlo methods also demonstrated that the Mann–Whitney U was a conservative test, since if the place fields on different days differed by more than a few grids, they would not be significantly closer than chance. A 6502-based computer program (Best, unpublished) was used to calculate the statistics.

RESULTS

Recordings from 61 complex-spike units (from 21 rats) that had clear place fields on the maze and for which unit isolation met our criterion for at least 1 h showed reliable place fields within daily recording sessions, with place-field activity occurring during each visit by a rat to a given unit's place field. No differences in mean firing rates (averaged across the entire environment) or in-field firing rates were observed between 5 min samples taken at the beginning, middle, and end of daily testing sessions ($P > 0.5$). These findings replicate earlier reports[38,46] that place-field activity is stable over the short-term in a fixed spatial environment.

Ten complex-spike units recorded on 6 different electrodes in 5 different rats were followed for periods substantially longer than 1 day. All showed reliable place-field activity during testing on 6 or more days (see Table I). Both within and across sessions, mean firing rates and within-field firing rates were stable and characteristic of a given unit. The firing rate within the place field was generally an order of magnitude greater than the background firing rate. Fig. 1 shows the recording sites of all these units, plus that of units that were isolated on the same electrode before or after isolation of the long-term units. All were in *stratum pyramidale* of CA1 of the hippocampus (see Fig. 1B for histological identification of a typical recording site).

In every case in which our electrodes were not moved and unit activity was not lost, the location of the place fields between recording sessions was significantly closer than chance (lowest $P < 0.01$, see Table I). Comparisons of the first and last day of isolated cellular activity at each electrode site revealed significantly closer than chance place fields. Comparisons of all possible pairs of observed fields from each single-unit revealed that they were

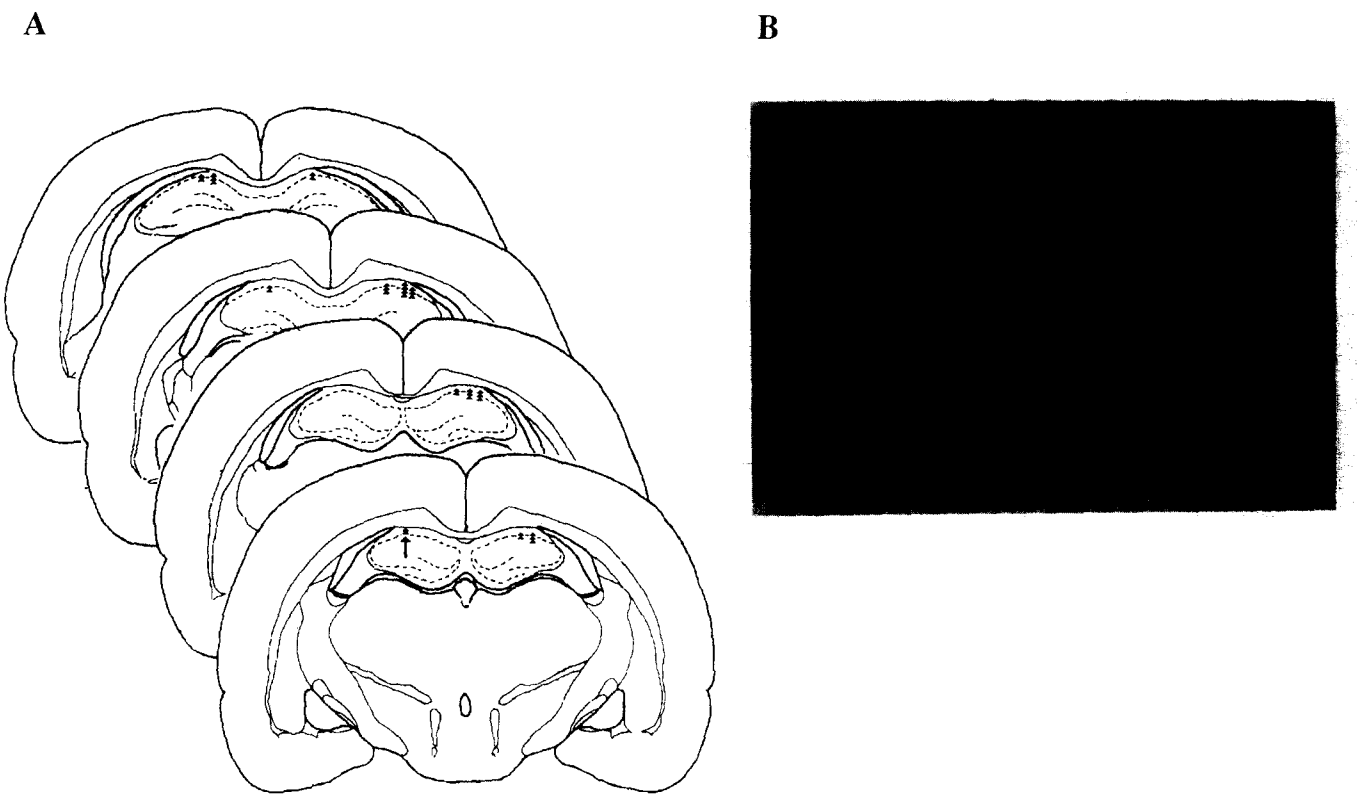

Fig. 1. Histological identification of recording sites. A: electrode sites in rat dorsal hippocampus from which place-field activity of single units was recorded. Locations of recording sites for long-term units and those preceding and/or following them are identified by black triangles. The large arrow points to the recording site shown in 1B. All recording sites were within or adjacent to the CA1 pyramidal cell layer. B: photomicrograph of recording site for a single electrode within a loose bundle of chronically implanted microwires is shown, at the most ventral excursion of the electrode within the dorsal blade of the CA1 subfield of the rat hippocampus. Single-unit activity from this site was recorded for 32 days. An adjacent electrode's marking lesion is marginally visible to the right of this lesion, and was plainly visible in adjacent serial sections. In general, microwires within each Kubie[23] electrode bundle recorded from sites within a 300 μm radius of one another.

significantly closer than chance between sessions, regardless of the intervening interval. It must therefore be concluded that the field locations did not move, but instead were extremely stable, over time. In each case in which the electrode was moved and a new unit was isolated, the new field was not closer than chance to the old field ($P > 0.5$). In cases where two or more units could be discriminated simultaneously from a single-electrode recording, each unit had a unique place field, bearing no discernible relationship to that of the other unit studied. As seen in Table I, the minimum absolute distance between the place fields of successively isolated units on the same electrode was always greater than maximum absolute distance between the place fields observed for the same cell across multiple sessions.

Five of the long-term units were isolated by advancing the electrode ventrally from a previously encountered complex-spike unit with its own clear place field. Additionally, final recordings from 5 of the long-term units were terminated by electrode advancement and isolation of a new unit. In each case, electrode advancement resulted in identification of a new place field, with its location not predicted by the location of the previous field (see Fig. 2). That is, each time a recording electrode was advanced and a new unit was isolated, the distance between the fields encountered before and after electrode advancement was not significantly smaller than chance ($P > 0.5$). In our experience, both for units followed over long periods and for those that were lost after 1 h or less of isolation, the place fields of the next unit isolated on the same electrode in the awake rat (typically after ventral electrode excursions of <75 μm within the same blade of the hippocampus) were invariably different from that of the preceding unit. The location of the place field of each unit remained fixed to a given set of spatial coordinates for as long as we were able to maintain single-unit isolation.

The best case was a unit that was observed to have the

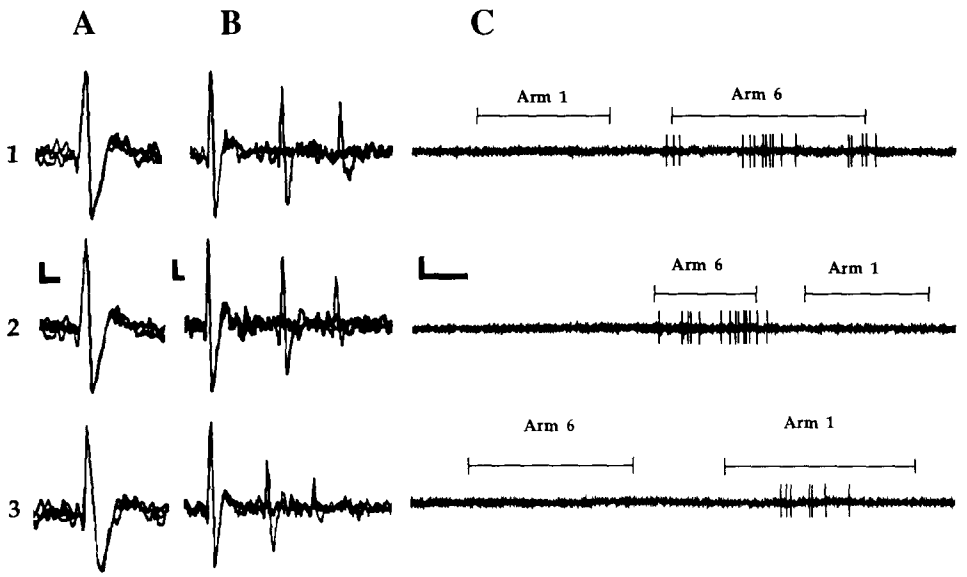

Fig. 2. Extracellular single-unit recordings from a single electrode's excursion through the dorsal blade of CA1. This electrode yielded good quality unit signals over nearly 8 months of chronic implantation. Three sets of records (A, B and C) are shown, demonstrating the stability of unit isolation and of place-field activity. 1, records from the 1st day of recording from one unit; 2, records from the last (the 153rd) day of recording from the same unit, with no intervening advancement of the electrode bundle; 3, records from the 1st day of recording from the next unit isolated on this electrode, after advancing the electrode bundle. The place-field activity of these units is similarly shown in Fig. 3, on days 8–160 and on days 162–205, respectively. A: 10 successive traces of analog-delayed negative-spike triggered single-unit activity are shown. Spike height (peak-to-peak voltage) and spike width (time between peaks) was constant for the same unit but differed between units, although the variability between complex-spike units meeting our rigid criteria for isolation was relatively small. B: 10 successive digitized oscillographic traces of analog-delayed spike-triggered single-unit activity at a slower sweep speed, showing at least one example of complex-spike activity (with decremental spike amplitude) for each unit. A constant interspike interval within these complex-spike bursts was observed for any given unit, and different units were characterized by different interspike intervals. Although the physical parameters (e.g. peak-to-peak voltage, spike width, and interspike interval) of the two sets of waveforms from the 1st and the 153rd day of recording from this unit were constant and were different from the third set of waveforms obtained after isolation of a new unit, this physiological evidence alone was not sufficient for identification of the recorded units as the same or different cells. C: linographs of extracellular recordings from the same units as the rat traversed two arms of the radial-arm maze. The location of the rat on the maze is indicated above the unit records. Note the responses of both units, with relatively silent background activity shifting to rapid firing as the rat entered the place field. The place field of the long-term unit was significantly closer to its initial position on the 153rd day than would be predicted if it moved randomly about the maze ($P < 0.0001$). The place fields of the two different units were not significantly closer than chance to each other ($P > 0.05$). Thus, the different units could be distinguished from one another on the basis of their spatial correlates. The calibration bars for A and B are 500 μs and 50 μV, and for C are 1 s and 150 μV. Negative is up in these traces.

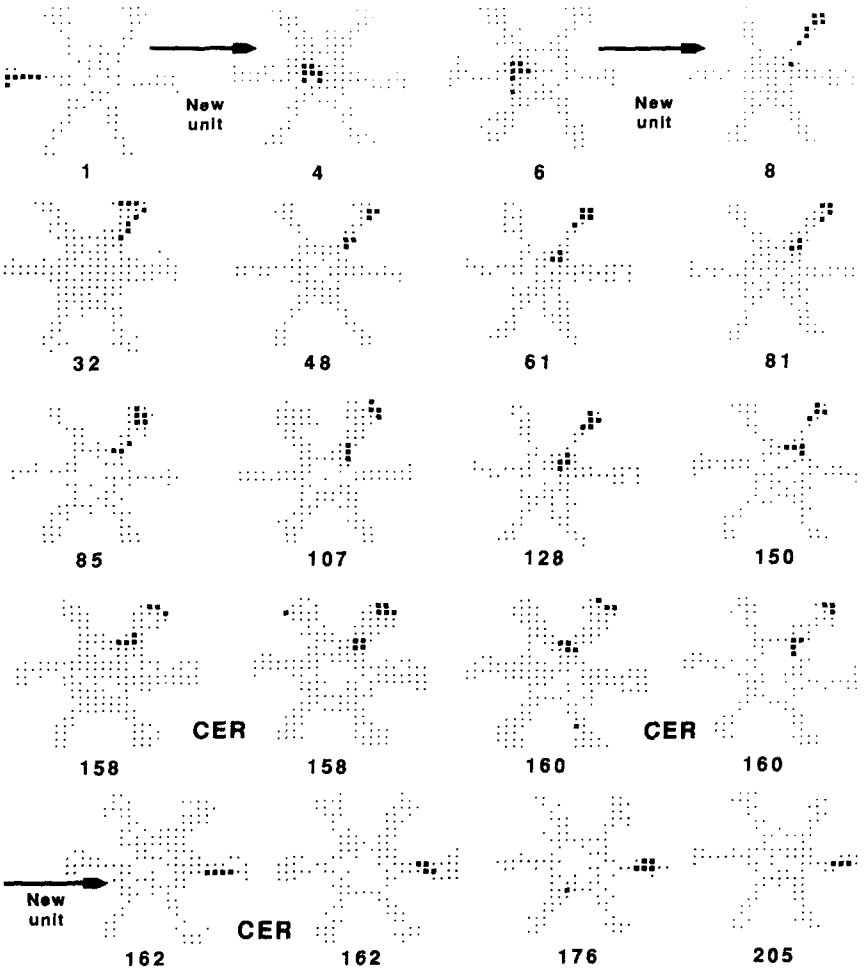

Fig. 3. Examples of the place fields of 4 CA1 pyramidal units, recorded sequentially from a single electrode track through the dorsal hippocampus of a single rat, mapped on a radial-arm maze. Place fields (defined by unit firing more than 3 standard deviations above the grand mean firing rate) are indicated by dark boxes, and background activity in all other areas visited by small dots. The electrode was advanced 30 μm following the initial recording session to isolate a new unit, whose field is shown over 3 days of recording. The electrode was then advanced ventrally, and a new unit was isolated. The place-field activity of this unit is shown for multiple sessions over 153 days of recording. Note the stability of its place-field's location on arm 6 of the maze over time. In the intervals indicated, conditioned emotional response (CER) training occurred in a separate environment. Response plasticity was demonstrated by this and the following unit, as both suppressed their firing during the 100 ms interval following onset of the conditioned stimulus. After the electrode was again advanced, a fourth unit was isolated. Its place-field's location on arm 1 was stable for the next 43 days. Unit isolation was then lost, and subsequent attempts to isolate units on this same electrode were unsuccessful.

same place field during 14 independent sessions over a 153 day period (see Fig. 3). Grids containing place fields are shown as large squares, while all other explored grids in which the unit firing rate was below this criterion are shown as small dots. The place field, with two centers of high firing rate activity, remained stable on the northeast arm of the radial maze. At the end of the 153 day period, the recording electrode was advanced until a new unit was isolated, in order to permit a more compelling argument that the activity had been from a single unit rather than from a cluster of cells with similar place fields. The new unit had a place field that was not significantly closer than chance to the 153 day field ($P > 0.5$), but that similarly remained stable over the next 43 days.

In one case, different units were isolated simultaneously on two different electrodes in the same bundle in the same rat (see Fig. 4). The place fields of these two units were unrelated to each other ($P > 0.5$). When the electrode bundle was advanced 30 μm ventrally, new units were again isolated simultaneously on both electrodes, with place fields that were again different both from each other and from the fields of the previous units on the same electrode ($P > 0.01$). Both of these new and disimilar place fields also demonstrated long-term stability for as long as single-unit isolation could be maintained.

Behavioral conditioning has been reported to affect hippocampal single neuron activity, with many pyramidal cells exhibiting a 'neural model' of the conditioned

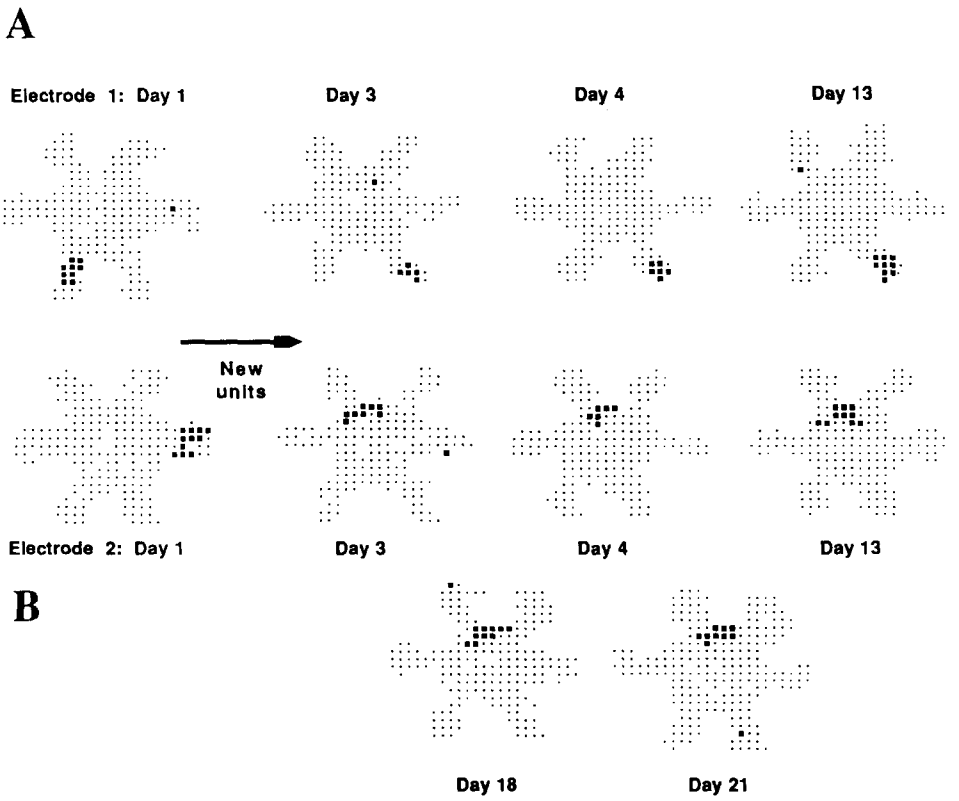

Fig. 4. Place-field activity of CA1 pyramidal units from a freely behaving rat, recorded simultaneously on two different electrodes from the same bundle. The electrode bundle was advanced ventrally through CA1 after the first day of recording, and new units were isolated on each electrode. Note that the place fields of these new units were different from those originally observed, and were different from those present on the other electrode, although all recording sites were within a few tens of microns of each other. These new place fields were stable as long as unit isolation could be maintained (10 days for the place field shown in A, and 18 days for the place field shown in B).

response[4]. In parallel experiments[7] to those reported here, hippocampal pyramidal cell activity was altered by conditioned emotional response (CER) training, with 6 of 23 units tested showing enhanced firing rates during the first 100 ms of tone presentation after 20 training trials and 11 of 23 showing a decreased firing rat over the same interval[7]. Of the 4 long-term units tested (i.e. those followed for a week or more of maze testing) that exhibited conditioned responses in the CER paradigm, 1 exhibited an increased firing rate during tone presentation and 3 exhibited decreased firing rates during the tone stimulus. As seen in the example in Fig. 3 (on Days 158, 159, and 162), however, despite this induced plasticity in neuronal activity in the separate CER training environment, place-field activity was not altered on the radial-arm maze. Neither the mean firing rates of the units (i.e. averaged across the entire environment), the within-field firing rates, nor the location of the place fields in the spatial environment were altered by experience in the CER environment during subsequent testing. Other non-specific differences in hippocampal afferent activity resulting from differential experiences outside the fixed spatial environment also had no effect on place-field activity on the maze, as demonstrated by the lack of place-field movement across sessions described above.

DISCUSSION

In no case have we found evidence that the place-field correlates of single hippocampal pyramidal cells change over time in a stable spatial environment. This conclusion would be hard to maintain if an extracellular recording electrode tended to shift from one neuron to a neighbor with the same or a similar place field. Our data indicate that this is not the case. Electrode advancement invariably resulted either in the loss of hippocampal single-unit activity or in the isolation of a new hippocampal unit with a dissimilar place field.

The amplitude of the unit signal and the frequency of spontaneous activity for individual units reported did not vary significantly over time, which suggests that multiple units were not involved. Although in this experiment stable biophysical measures were a requisite requirement for long-term recording, and electrode movement (purposeful or artefactual) typically resulted in changes in the electrophysiological signal recorded, it has been shown

that single units can be followed over electrode excursions of up to 50 μm before the signal degrades sufficiently for loss of isolation[11,19,25]. Typically, signals from more than one unit are observed during electrode excursions over this range, with one unit decreasing in amplitude as it becomes more distant from the recording electrode and a new unit increasing in amplitude as the recording electrode nears it. At certain points, the unit signals from two distant cells are equal in amplitude. Therefore, as noted in studies of neuronal activity in sensory systems, a stable unitary signal is necessary but not sufficient to verify that a given unit is being followed over time[12,52]. The combination of stable biophysical measures with other robust and stable measures, such as sensory correlates, or in this case place-field correlates, however, validates the premise that a single unit has been followed over long periods[34].

Our data and those of others indicate no obvious topographical organization of hippocampal place cells, unlike the organization common to sensory systems. Within a given hippocampal region, such as dorsal CA1, the place fields of adjacent neurons do not correspond to adjacent places within an environment. As shown in Table I, each time an electrode was moved in the awake rat to isolate an adjacent neuron, the place fields observed moved relative to that of the place field of their last isolated neighboring cell. In fact, the neurons adjacent to a place cell are more likely to be behaviorally silent within a given spatial environment than to have a spatially close place-field[56]. Each unit isolated per single electrode track through the dorsal CA1 blade of the hippocampus in the present study had a place field that was not significantly closer than chance to that of other units recorded on the same electrode. Several studies suggest that neurons located throughout the hippocampal circuit may be involved in cognitive processing of spatial information[6,31,33,35,49]. Whether the place-field responses of neurons elsewhere in the circuit are as stable as those reported here for dorsal CA1 pyramidal cells is an open question, as is the topography of place-field organization within these other regions.

Hippocampal neuronal activity changes rapidly in response to such a wide variety of manipulations that it has been adopted as a model system for studying the effects of experience on the CNS. These changes include the *sprouting* of afferent terminals following selective deafferentation[28,53]; *kindling*, the induction of seizure activity after repeated presentation of subthreshold stimuli[18,58]; *long-term potentiation*, a sustained increase in the population EPSP after repetitive suprathreshold stimulation[1,2,3,9,51,55]; and reliable *associative changes* in multi- and single-unit activity during and after associative conditioning[4,7,15,21,44]. Students of synaptic potentiation are aware that the term 'long-term', as generally applied, refers to time intervals in the range of several hours, and rarely exceeds several days. The current findings perhaps more properly extend the term to cover time spans ranging up to several months. For a brain structure to play an important role in experiential modification of behavior, once a change in neuronal response occurs, it must be maintained for periods of time appropriate to behavioral needs. It is therefore not difficult to assume stable representations lasting for periods approaching the life span of the organism. The present study demonstrates such stability of the place-field correlates of hippocampal neurons, which is the first known demonstration of a behavioral correlate of such long duration.

The activity of hippocampal pyramidal units meet a necessary requirement for neural elements subserving cognitive mapping in the rat central nervous system[43], a consistency of the map in stable spatial environments. If the map were to change in a spatially non-specific manner, or to jump from one stable state to another over time without reference to the external environment, its utility would perhaps be reduced. In an environment in which places (or their identifying cues) do not change over long periods of time, the representation of these places in the brain via the place-field activity of pyramidal cells also remains unchanged. As seen in other studies, modifications in the environment can and do produce plasticity in these place-field responses[14,37,40]. A combination of stability in the absence of environmental changes and plasticity in response to those changes appears to characterize this system. Experiences outside the fixed spatial environment had no observable impact on place-field activity occurring within the environment, which indicates that non-specific effects of experience do not induce plasticity in this particular behavioral correlate of pyramidal cell activity. One emergent hypothesis suggests that the neuronal plasticity inherent in learning is interactive with and indeed may require the stability inherent in memory. As shown, the hippocampus is a site of both plasticity and of long-term stability of neuronal responses. The place-field activity of a given hippocampal neuron in an unchanging spatial environment is stable across all periods of time that it can be accurately measured, which at present is limited to 153 days.

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