The Journal of Neuroscience, January 1995, 15(1): 173–189

Influences of Vestibular and Visual Motion Information on the Spatial Firing Patterns of Hippocampal Place Cells

Patricia E. Sharp, Hugh T. Blair, David Etkin, and Douglas B. Tzanetos

Department of Psychology, Yale University, New Haven, Connecticut 06520-8205

Hippocampal place cells show location-specific firing as animals locomote in an environment. A possible explanation for these place fields is that each cell is simply driven by environmental sensory inputs available in its field. This cannot provide the full explanation, however, since cells can maintain stable place fields even in the absence of reliable environmental orienting cues. This suggests the cells are also influenced by movement-related information, since this is the only available, ongoing indicator of current location when external orienting cues are not present.

Two candidates for the movement-related information are vestibular activation, and visual motion. To test for these influences, place cells were recorded as animals locomoted in a cylindrical apparatus that was made so that its wall (painted with vertical black and white stripes) and floor could be independently rotated, to provide visual motion and vestibular inputs, respectively.

The results showed that both these inputs could influence place fields. Sometimes they caused a predictable locational shift, so that the field rotated its location on the apparatus floor in a way that was compatible with the movement indicated by the vestibular and/or visual motion input. This updating was most reliably obtained when the two inputs were presented in combination. In other cases, the apparatus rotations caused unpredictable changes in firing characteristics, so that cells either stopped firing, or developed place fields that were altered in overall size, shape, and eccentricity. Interestingly, the probability of these changes increased with experience with the rotational manipulations, suggesting a learned component.

[Key words: hippocampal formation, place cells, hippocampal single units, spatial information processing, navigational systems, vestibular system, visual motion cues]

Hippocampal pyramidal cells show location-specific firing properties when animals navigate in an environment (O'Keefe and Dostrovsky, 1971; O'Keefe, 1976; O'Keefe and Nadel, 1978). Any one of these place cells fires only when the animal is in a particular, circumscribed region, and each cell has its own preferred region.

It is not yet fully understood how these cells generate the location-specific patterns. One idea is that each cell could simply be driven by sensory information available in its field (Zipser, 1985; McNaughton, 1989). This idea is compatible with the fact that rotation of a set of salient environmental cues causes an equal rotation of the preferred firing field (e.g., O'Keefe and Conway, 1978; Muller and Kubie, 1987).

Other findings, however, suggest that this local-view idea cannot provide the entire explanation. For example, once established, a firing field can be maintained as an animal travels repeatedly through an environment, even in the absence of salient environmental orienting cues (O'Keefe, 1976; Hill and Best, 1981; O'Keefe and Speakman, 1987; McNaughton et al., 1989; Quirk et al., 1990). Also, in a visually symmetrical environment, most place cells show asymmetric patterns, so that they do not show the same firing pattern in all locations that are visually identical to one another (Sharp et al., 1990).

Results like these suggest that the hippocampal cells must receive some kind of movement-related information (McNaughton, 1989), and there is empirical support for this (e.g., Hill and Best, 1981; Foster et al., 1989). Thus, as an animal travels from some location, A, to a different location, B, place cell activity changes, so that cells that fire in location A are turned off, and cells that fire in location B are turned on. In the presence of environmental orienting stimuli, the updated pattern can be explained by the difference in sensory stimuli between locations A and B. However, in the absence of environmental cues that differentiate between the two locations, the only available information for the updating seems to be that based on the animal's own movements.

This experiment was designed to gain insight about what kind of movement-related information might play this role. Two candidates are vestibular activation (for which there is already some evidence; Hill and Best, 1981), and visual motion (movement-induced optic flow). The experiments presented below were designed to provide activation of each of these two types of input, to determine whether they play a role in updating place cell firing patterns.

This was accomplished using a cylindrical recording chamber with a radially symmetric pattern of vertical black and white stripes on its wall. It was constructed so that the wall and floor could be rotated either separately or together, to deliver various combinations of vestibular and visual motion input. Thus, rotation of the wall alone provided isolated visual motion input. Alternatively, rotation of the floor provided vestibular input, and this rotation could be performed both in the dark (so that visual information was absent), as well as in the light (so that the vestibular information was combined with visual motion information).

Received Apr. 20, 1994; revised June 6, 1994; accepted June 16, 1994.

This work was supported by NSF Grant 9120131 to P.E.S.

Correspondence should be addressed to Patricia E. Sharp, Department of Psychology, P.O. Box 208205, Yale Station, Yale University, New Haven, CT 06520-8205.

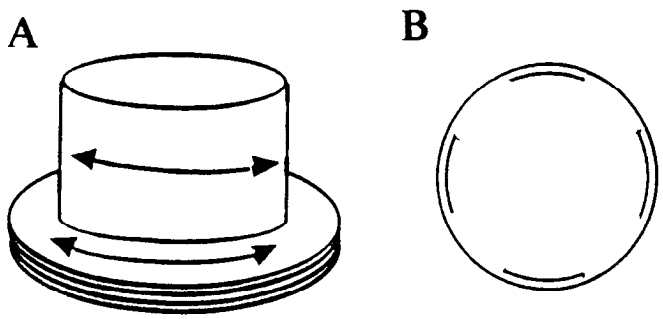


Figure 1. Behavioral apparatus used for all recording sessions. *A*, Diagram of the cylinder in which animals performed the pellet-chasing task throughout all recording sessions. *Arrows* indicate that the floor and wall could each be rotated independently, around a central axis. *B*, Overhead view of cylinder, showing the location of the alternating, vertical black and white stripes painted on the cylinder wall. These formed a symmetrical environment, in which any two locations that were 90° apart, and equidistant from the center were visually identical.

Materials and Methods

Experimental subjects

The subjects were 11 female, Long-Evans rats, weighing 200–250 gm at shipping. Animals were housed individually upon arrival, and had a 12 hr on (8:00 A.M. to 8:00 P.M.)/12 hr off, light/dark schedule.

Behavioral apparatus

All recording sessions were conducted in the apparatus shown in Figure 1*A*. The figure depicts a 50.5 cm high, 74.0 cm diameter cylinder mounted on a rotatable base. The cylinder rests just above the base on three equally spaced ball bearings (not shown) that are attached near the bottom of its outer wall, and that fit into a circular groove on the base. This arrangement means that both the cylinder and the base (which serves as the floor of the apparatus) can be easily rotated, separately or together, around the central axis.

The inner wall of the cylinder was painted to have a series of eight alternating black and white vertical stripes, as indicated in the overhead view in Figure 1*B*. Note that this environment is radially symmetrical, so that there is no single orienting cue available to "set" the place cell firing. Note, also, that any 90° rotation of the cylinder wall and/or floor leaves the apparatus in a configuration that is visually identical to that before the rotation.

The floor (base) was painted uniformly gray, with a water-resistant paint.

The entire cylinder was surrounded by a uniform, circular, black curtain that formed an enclosure 175 cm high and 137 cm in diameter at its widest, and then tapered off above this to a diameter of 57 cm, and a height of 213 cm. Illumination was provided by a single 100 W overhead light (located in an inverted position above the curtain), which spread a diffuse, uniform light over the cylinder floor. Also located above the curtain was an automatic dispenser for the remote-controlled delivery of food pellets. Pellets dispensed in this way dropped to a position near the center of the cylinder floor and scattered to random locations throughout the area of the cylinder. The cylinder was located in a room separate from the recording equipment. A speaker located centrally above the cylinder delivered a constant white noise, designed to mask any uncontrolled auditory cues provided by the laboratory environment.

Behavioral training

Prior to training, rats were placed on a food deprivation schedule with which they were reduced to 80% of their ad lib weight. They were then trained to search for 20 mg food pellets (BioServe, Frenchtown, NJ) that were thrown into a cylindrical apparatus (identical to the recording chamber described above) at pseudorandom locations, at approximately 15 sec intervals (Muller et al., 1987). During training, the cylinder was located in a separate room from the one in which cell screening and recording were later conducted. To begin each daily 15 min training session, each rat was placed into the cylinder at a fixed angular location adjacent to one of the white stripes. A total of six training sessions were given; for the first three sessions animals chased pellets in the chamber in groups of two or three, while the last three were administered individually. During this period rats developed a pattern of nearly constant locomotion that lasted throughout the sessions and resulted in the rat covering the entire cylinder floor repeatedly throughout the session, in an apparently homogeneous fashion.

Electrode implantation

After training, two driveable microrecording electrodes (one per hemisphere), consisting of six wires each, were chronically implanted. The six separate wires, cut at an angle of approximately 45°, consisted of Formvar-insulated, 25 μm diameter, nichrome wire (California Fine Wire Co., Grover City, CA). Prior to surgery, animals were anesthetized with a 0.18 cc injection of 65 mg/ml pentobarbital, and supplemental doses were given during surgery, if necessary, to maintain deep surgical anesthesia. The rat was placed in a Kopf stereotaxic frame, the skull was exposed, and three securing screws were placed in the skull over each of the frontal and cerebellar cortices. An approximately 2 mm hole was drilled in the skull over the dorsal hippocampus of each hemisphere, to allow electrode insertion. At surgery, the electrodes were placed with their tips 2.0 mm below the brain surface, so that they were well above the hippocampal cell layers, and could be gradually lowered through these layers after recovery. Sterile petroleum jelly was applied to the exposed brain surface, as well as the guide cannula surrounding the recording wires. The electrodes and securing screws were then cemented permanently to the skull by dental acrylic (Turotech, Wynnewood, PA). One of the securing screws was equipped with a connecting pin that protruded out from the dental acrylic, so that it could be used as a grounding wire during recording. Also cemented into the acrylic was a small connector, used for later attachment of the recording cable.

Unit isolation and data collection

After recovery from surgery, animals were given screening/recording sessions during which the activity from the electrode wires was sampled while the rat performed the pellet-retrieving task in the cylinder. If no single cell activity was present, the electrode bundles were lowered slightly (between 0.022 and 0.044 mm) and the wires were checked again (up to four repetitions of lowering and checking per day). Upon isolation of activity from a single cell(s), a recording session (see below) was initiated. Two wires could be recorded from at the same time, and the signal from each was passed first through a field-effect transistor in source-follower configuration that was mounted on the pin attached to each electrode wire. This signal then passed through a cable (affixed to the connector on the animal's head) to an amplifier (gain between 5000 and 20,000) and filter (300 Hz, high pass, and 10 kHz, low pass), and then to a computer, for automatic data collection. The software used for data collection and cell discrimination (Brainwave Corp.) collected an epoch of the digitized analog signal for every event from the amplifier that exceeded a user-set threshold. These events were then separated into bins, each of which captured the waveforms generated by spikes from one individual cell, through a cluster analysis routine that utilized information from eight different parameters extracted from each waveform. In this way, it was often possible to collect data from more than one cell simultaneously. Each waveform, along with a time stamp, and indication of which bin it belonged to, was automatically stored.

The waveforms for any one cell were considered as acceptable for inclusion in the data set if (1) there was judged to be a good signal-to-noise ratio at the time of recording, and the waveforms appeared uniform in shape; (2) there was a refractory period of 1 to 2 msec between spikes; and (3) there was a sufficiently strong spatial signal [spatial coherence (see below) ≥ 0.50].

The animal's moment-to-moment position in the cylinder was also sampled continuously throughout each session. For this, a video camera located above the cylinder monitored the location of two light-emitting diodes attached to the animal's head. One of these lights was positioned toward the front, while the other was toward the back of the animal's head. The video signal was sent to a camera tracking system (Brainwave Corp.) that extracted a digitized representation of the location of each of the two lights for transmission to the computer at a rate of 60 Hz. This information was time-stamped and automatically stored. During subsequent analysis, the animal's location at each sample time was calculated as the midpoint between the front and rear headlights.

Recording sessions

Prior to introduction of an animal to the cylinder for any screening/recording session, the floor and wall of the cylinder were each set separately to one of four randomly determined angular positions, each of which were 90° apart. This meant that for the cylinder wall, the vertical stripes were always in one of four visually identical positions, with one of the white stripes being closest to the spot in the curtained enclosure used for entry and exit. In this way, any uncontrolled cues that may have been unintentionally present on the cylinder wall or floor were in a nonconstant relationship to the laboratory framework and the animal's entry location. It was hoped that, in this way, the influence of any such uncontrolled cues would be minimized.

To begin each screening/recording session, the animal was transported into the curtained enclosure (surrounding the cylinder) in an enclosed carrying cage. The cage was then placed on the floor next to the cylinder, the top was removed, and the animal was attached to the recording cable while it was held by the experimenter. The animal was then placed down into the cylinder in a fixed location, so that the right side of its body was adjacent to the white stripe closest to the entry/exit position of the curtained enclosure. The experimenter then picked up the carrying cage, and immediately exited the curtains. The entry location, position of the experimenter during animal handling, and position of the carrying cage were constant over sessions.

Upon isolation of single cell activity, a recording session was begun, involving one of the eight experimental manipulation types described below. Each session consisted of an initial 20 min long premanipulation phase, a brief (20–60 sec) manipulation phase, and a subsequent 20 min postmanipulation phase. Throughout each of these phases the animal constantly performed the pellet-chasing task described above. During the manipulation phase computerized data acquisition was briefly interrupted. On a few occasions, during the premanipulation phase, it was noticed that the animal had defecated on the cylinder floor. In these cases the droppings were quickly removed by the experimenter, prior to conducting the manipulation. If the animal urinated prior to the manipulation, the session was discontinued. The experimenter never entered the curtained enclosure or the recording room during the postmanipulation phase of recording, since this would have constituted an unwanted orienting cue during a time when the effects of the experimental manipulation were being tested.

To end a screening/recording session, the experimenter entered the curtained enclosure with the carrying cage and placed it on the floor, picked up the animal and detached it from the recording equipment, and replaced it into the cage for return to the colony room. For this, the entry/exit, and experimenter positions were usually identical to that used to start all sessions. The only exceptions to this were cases in which there was a place field rotation (see below); in this case, the positions of the experimenter entry/exit, handling, and carrying cage were all rotated by the same amount as the place cell firing field.

The floor and lower wall of the cylinder were wiped with a wet rag between each screening/recording session.

Upon obtaining reliable single cell activity in a given animal, the full series of eight manipulations described below was conducted (provided the cell isolation remained suitable), at a rate of no more than one manipulation per day. Within each series, the presentation order was randomized across the set of all series conducted over animals, with the exception that care was taken to insure that each manipulation type occupied each presentation order number an approximately equal number of times. The electrodes were not moved within the series of eight manipulations, unless it was necessary due to loss of cell isolation. In this way it was sometimes possible to collect data from much or all of the entire set of manipulations on the same cell(s). After a series was completed, the electrodes were lowered in order to encounter a new set of cells, so that the same cells were not recorded in the same manipulation more than once.

For each manipulation type described below, a total of 14 sessions were conducted.

Experimental manipulations

Rapid rotation of the cylinder wall and floor, with the lights out (W+F OFF FAST). This manipulation was designed to provide isolated activation of the vestibular system. The manipulation was conducted during an approximately 60 sec period of darkness, during which the overhead light, as well as the headlights attached to the animal, were both extinguished in order to remove any visual cues. (Also, the door of the recording room was equipped with a set of black curtains that were dropped around its edges during this time, to prevent any light from entering the room.) The wall and floor of the cylinder were securely attached to each other prior to the start of the session, so that they would not move in relation to each other. During the lights out period the experimenter quietly entered the recording room (from the darkened outer laboratory), reached under the curtains enclosing the cylinder, and rapidly (over a 2–4 sec period) rotated the cylinder wall and floor by 90°, in either a clockwise or counterclockwise direction (counterbalanced across sessions), and then immediately exited the room. Recording was interrupted during the 60 sec lights out period, since the animal's headlights were not available to provide positional information.

It should be noted that since the animals were freely locomoting throughout the manipulation phase, there was no strict control over the delivery of the vestibular input provided by the manipulations involving floor rotation. Thus, if the animal happened to be located toward the edge of the cylinder at the time of the rotation, this might result in relatively more translational, rather than rotational movement of the animal (thus, possibly providing relatively strong activation of the static labyrinth receptors), while a more central location might result in relatively more angular rotation (thus more strongly activating the kinetic labyrinth receptors). Since the animals' headlights were necessarily turned off during these manipulations, it was not possible to observe the animals' behavior during the manipulations, so that no post hoc analysis of these variables could be conducted.

Slow rotation of the cylinder wall and floor, with the lights out (W+F OFF SLOW). This manipulation was identical to the W+F OFF FAST manipulation described above, except that the wall and floor were rotated very slowly (over the entire 60 sec lights out period), so that the speed of rotation would presumably be below that necessary for vestibular system activation. This was done as a control condition for the W+F OFF FAST manipulation, since the effects of any uncontrolled cues (such as noise or odors from the laboratory environment) would be the same in the two manipulation types, and they would, presumably, differ only in whether vestibular stimulation was provided.

Rapid rotation of the cylinder wall and floor, with the lights on (W+F ON FAST). This rotation was identical to the W+F OFF FAST manipulation described above, except that the lights were not extinguished. This was designed to test the effects of vestibular information indicating a *rotation*, while in the presence of accompanying visual information indicating *no rotation*. That is, since the stripes on the wall rotate along with the animal, there is no experimenter-induced visual motion. Thus, it was reasoned that this manipulation could provide an indication of the relative strength of the influences from the two types of sensory input.

Rapid rotation of the cylinder wall (alone), with the lights on (W ON FAST). For this, the cylinder wall was quickly (over a 2–4 sec period) rotated, in either a clockwise or counterclockwise direction (counterbalanced), while the cylinder floor was securely fixed. This provided isolated visual motion information indicating a 90° rotation. Note that after the rotation, the final location of the black and white stripes was visually identical to that before the manipulation, so that static visual cues could not be responsible for any changes in the locational preference of a cell.

It should, again, be mentioned that the behavioral paradigm used here prevented precise control over the details of the stimulation delivered by these rotations. Thus, for example, the total retinal displacement of the image of any one stripe would be dependent on the animal's distance from that stripe at the time of the rotation.

Rapid rotation of the cylinder floor (alone), with the lights on (F ON FAST). For this, the cylinder floor was quickly (over 2–4 sec) rotated by 90°, either clockwise or counterclockwise (counterbalanced), while the wall was held in place. Note that in this case, both vestibular and visual motion information indicated the same direction of rotation. Thus, this manipulation allowed for examination of concordant vestibular and visual motion influences.

Rapid rotation of the cylinder floor (alone), with the lights off (F OFF FAST). This manipulation was identical to the F ON FAST manipulation, except that it was conducted during a 60 sec period of darkness. This served as a control condition for the effects of visual motion in the F ON FAST condition, since all uncontrolled cues were the same in the two cases.

Rotation of the cylinder wall by 45°, with the lights out (W 45 OFF). Here, the cylinder wall was rotated 45° in a clockwise or counterclockwise (counterbalanced) direction, during a 60 sec period of darkness.

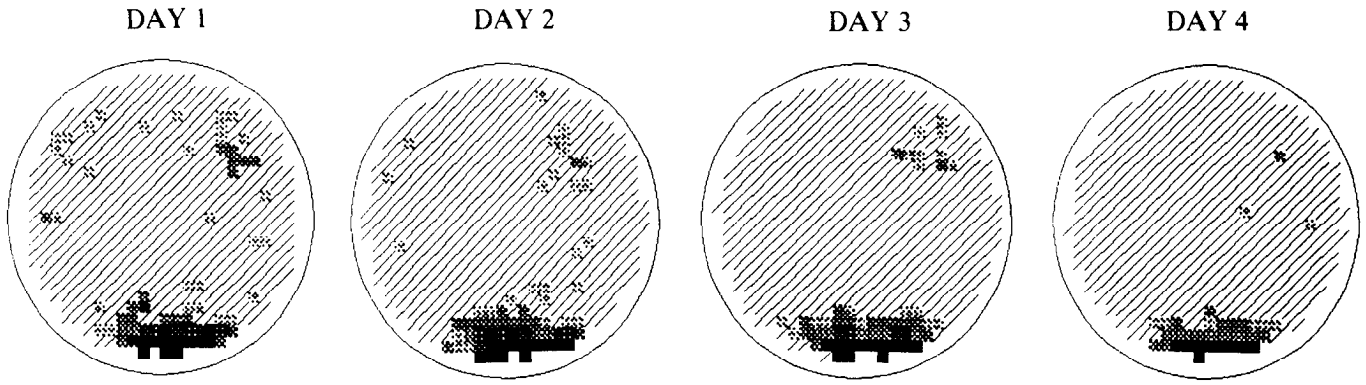


Figure 2. Spatial firing characteristics of a hippocampal cell in the visually symmetrical recording chamber. Firing rate maps for the initial (prerotation), 20 min phase of each of a series of sessions from one place cell recorded over several consecutive days. For map construction, the cylinder floor was divided into 2.9 cm by 2.9 cm pixels, and the firing rate for each pixel was obtained by dividing the total number of spikes that occurred when the rat was in that location by the total time spent in that location. These values were then used to compute a mean and SD over all the pixel rates. The relative rate for each pixel is depicted using one value of a five-valued gray scale so that pixels that are more than 2 SDs above the mean pixel rate for that cell are given the darkest value, those between 1 and 2 SDs above the mean have the next-highest value, those within 1 SD above the mean use the next value, those within 1 SD below the mean receive the next value, and those that are 1 or more SDs below receive the lowest value. Pixels that the animal visited, but in which the cell did not fire, are indicated by a *diagonal line*. Blank white pixels indicate regions that were not visited during the session. This cell was typical of many in the study, in that it had a relatively small, unitary firing field that was stable over days.

Note, this meant that during the postmanipulation phase, every region that had previously been occupied by a black stripe was now occupied by a white stripe, and vice versa. Since the floor was stable, and the lights were out during the rotation, there should have been no vestibular or visual motion information provided by this manipulation. Thus, this served as an indicator of whether the stripes, as static cues, played a role in influencing the firing properties of these cells.

Sixty seconds of darkness (LIGHTS OUT). For this, there was simply a 60 sec period during which the lights were extinguished, just as for the other manipulation types involving a dark period. This control manipulation was thought necessary, since it was possible that either the 60 sec period of no visual information, or just the presentation of sudden changes of illumination, might cause changes in the cells' fields.

Data presentation and analysis

Firing rate maps. The time of occurrence for the spikes of a given cell, along with the position data, were used to construct a firing rate map for each cell (Muller et al., 1987). For this, the cylinder was divided into 2.9 by 2.9 cm pixels, and the total amount of time spent in each pixel, along with the total number of spikes that occurred when the animal was in that pixel, were used to calculate an average rate for each. The relative rate in each pixel was indicated in the map using a gray scale, the value of which was chosen based on the mean and SD of the pixel rates for that cell (see the Figure 2 caption for details). A diagonal line was used to indicate pixels that the animal visited, but in which the cell did not fire. A blank white pixel means that the animal did not visit that location during the session.

Rate. The overall rate (in Hz) for each cell was calculated by dividing the total number of spikes in the session by the total session time (in seconds).

Spatial coherence. This measure provides one way of quantifying the strength of the spatial signal for a cell, and is very similar to the spatial coherence measure developed by Kubie et al. (1990). The measure has been described elsewhere (Sharp and Green, 1994); briefly, it consists of a spatial autocorrelation, in which a correlation coefficient is calculated between the rate for each pixel, and the average rate of the eight surrounding pixels. Thus, high, positive values for R result if the rate for each pixel can be better predicted by knowing the rate of the neighboring pixels. This means the R value serves as a measure of any consistent, graded, location-related variations in rate. This measure was used to help determine whether a cell was acceptable for inclusion in the data set, since it was desirable to use only cells with a strong spatial signal. Only cells with a spatial coherence value ≥ 0.50 were included.

Correlations between firing rate maps. Two pieces of information were important for determining the effects of the various rotational and lights out manipulations on the place cell firing fields. One was the degree of similarity between the pre- and postmanipulation fields, to see whether the manipulations caused changes in the overall spatial firing characteristics of the cells. The other was the degree of rotation (if any) induced by a particular manipulation. To obtain these two pieces of information, the firing rate maps from the pre- and postmanipulation phases of each session were subjected to a rotational cross-correlation analysis. For this, a pixel-by-pixel correlation was conducted between the premanipulation map, and each of a series of 60 rotated versions of the postmanipulation map. Each successive map in the series was rotated by 6° in relation to the last. Thus, a correlation coefficient was calculated for the rate values of the pixels of the pre- and postmanipulation maps at each possible rotational angle to each other, from 0° to 360° (in 6° degree increments). This yielded two values: (1) MAX R, which was the highest R value obtained at any of the 60 angular rotations, and served as the measure of field similarity, and (2) DEG MAX R, which was the degree of rotation at which that MAX R value was obtained, and defined the degree of rotation of the place field.

Histological examination and reconstruction of cell location

After recording, animals were perfused transcardially under deep anesthesia with a formyl saline solution. Prior to this a small current (30 μA × 5 sec) was passed through one wire of each electrode, in order to mark the location of the electrode tips. The brains were then sectioned in the coronal plane at 40 μm intervals, mounted, and stained with both cresyl violet and Prussian blue. All electrode tracks were shown to have passed through the hippocampal cell layers.

Results

Cell sample

Data were collected from a total of 102 hippocampal complex spike cells (Ranck, 1973) in the 11 experimental animals. The average spike width (measured between the initial departure from and subsequent return to baseline), at the filter settings used here was 339.57 μsec (±6.77), while the average amplitude (from the peak negativity to the peak positivity) was 371.78 μV (±24.13). The average firing rate and spatial coherence (based on the initial, premanipulation phase of each session) were 0.94 Hz (±0.08) and 0.73 (±0.01), respectively.

It was sometimes possible to electrically isolate more than one cell at a time on the same electrode bundle, so that the effects of a given rotational manipulation could be observed in multiple cells simultaneously. The average number of cells per

recording session was 1.24. For the purpose of statistical analysis, all measures for cells recorded simultaneously (i.e., during the same manipulation) were averaged together, so that, as a group, they contributed only one data point to the overall analysis. This averaging into one data point was thought necessary, since it was reasoned that cells recorded simultaneously could not be counted as independent observations. Thus, slight differences between manipulations in speed of rotation, or the animal's position during rotation, might cause all the cells during a given manipulation to show the same outcome. (As described below, simultaneously recorded cells did, in fact, tend to show the same response.) In cases in which several cells were recorded simultaneously, the results from that particular session would be unduly weighted in the overall analysis, if each cell were counted as an individual data point.

It was often possible to hold the electrical isolation for a given cell (or set of cells) over multiple days, so that the effects of different manipulations could be observed on the same cell(s). The average number of sessions per cell was 1.35. In no case was the same cell tested in the same manipulation more than once.

As described in Materials and Methods, animals were given repeated series of manipulations, with each series consisting of the full set of eight rotational (or lights out) manipulations (with presentation order randomized within each series). This procedure meant that in many cases the same animal (though not the same cell) contributed more than one data point to the same manipulation type. The average number of animals contributing to each manipulation type was 6.75. In no case did the data from one animal contribute more than four data points to any one manipulation type, and the smallest number of animals contributing to any one manipulation was five. Figure 3 (described below) depicts the distribution of data points contributed by each individual animal, and also shows that the different cells of an individual animal did not necessarily show the same response to repeated tests on the same manipulation. With the exception of one animal (described below) we could not discern consistent traits in the data patterns of individual animals.

Firing fields in the visually radially symmetric environment

Figure 2 shows the firing rate map from the initial (premanipulation) phase from each of a series of sessions for a cell that was retained over several days of recording. This field is typical of many in the study, in that it showed a small, unitary firing field that was stable over days. Interestingly, no tendency was observed for the cells to show a symmetrical firing field, despite the symmetrical nature of the visual surroundings. Note also, the field retained the same position over days, even though the cylinder wall and floor were each rotated to one of four possible randomly-chosen angular settings (see Materials and Methods), prior to the start of each session. This suggests that any uncontrolled cues (such as odors) that may have been unintentionally provided by portions of the cylinder floor and wall were not the major influence determining the field location. Rather, it appears that the animals' entry location (which was constant over sessions) may have been the main determinant of field location for the initial, premanipulation phase of these sessions. This control of field location by entry location is compatible with previous observations in another type of visually symmetrical environment (Sharp et al., 1990).

Of the 19 cells that were retained over more than 1 d, only one failed to show this stability of the angular location of its firing field, and it did so in only one session. This was true even though, as discussed below, fields often changed within a session as a result of the rotational manipulation, so that the postrotation field was quite different, either in angular location, or more global properties, after the manipulation. Nonetheless, the fields were returned to their original firing pattern when reintroduced into the chamber the following day.

Effects of rotational manipulations

Graphical representations of the overall data pattern for each manipulation are shown in Figures 3 and 4. Figure 3 shows a series of scatter plots (one for each manipulation type) in which each data point represents the results of the cell(s) recorded during one session. Here, degree of rotation of the postrotation firing field in relation to the prerotation field (DEG MAX R) is plotted as a function of the correlation coefficient for the comparison of the two maps at that degree (MAX R). Thus, the ordinate provides a scale for the similarity of the field before versus after the rotational manipulation (at the optimal angular orientation), while the abscissa shows how much the field rotated. For the purpose of this display, the directional sign of the firing field rotation was determined *in relation to the direction (clockwise versus counterclockwise) of the apparatus rotation* (recall that this variable was counterbalanced within manipulation types), so that a field rotation in the same direction as the apparatus rotation was coded as positive, while a rotation opposite in direction to that of the apparatus was negative. The only exception to this was the lights out manipulation, in which there was no apparatus rotation; in this case, counterclockwise rotations were coded as negative, while clockwise rotations were assigned a positive value. In cases in which multiple cells were recorded during the same session, the data were averaged to yield a single data point, which was used both for the visual displays in Figures 3 and 4, as well as for all statistical analyses. This averaging of data from the cells within a session was possible, since, in general, any set of cells recorded simultaneously yielded the same qualitative result (see below). In some cases, cells stopped firing after a manipulation (see below), and for these cells the data could not be included in Figure 3, since no measure could be obtained for degree of rotation.

It should also be noted that in Figure 3, the data from each individual animal are coded using a different symbol. Thus, any one symbol type shows the data for all the manipulations performed on one particular animal. This provides for visual assessment of the number of data points contributed by each animal to each manipulation type, as well as any tendency for the cells from a given animal to show a consistent type of response. It can be seen that the cells in an individual animal did not tend to show the same response to a given manipulation on different occasions. Thus, for example, the animal whose data are represented by ◆ was tested on the W+F ON FAST manipulation on three different occasions, and showed a different type of qualitative response each time.

Examination of Figure 3 shows that there is substantial variation in the MAX R values obtained for cells within each manipulation type. Some of this variability is presumably due to individual differences between the cells in the robustness of their firing fields across local time slices. However, differences across manipulations in this measure may partially reflect the tendency of particular manipulations to have a disruptive effect on the firing field, and thus may provide some insight into the strength

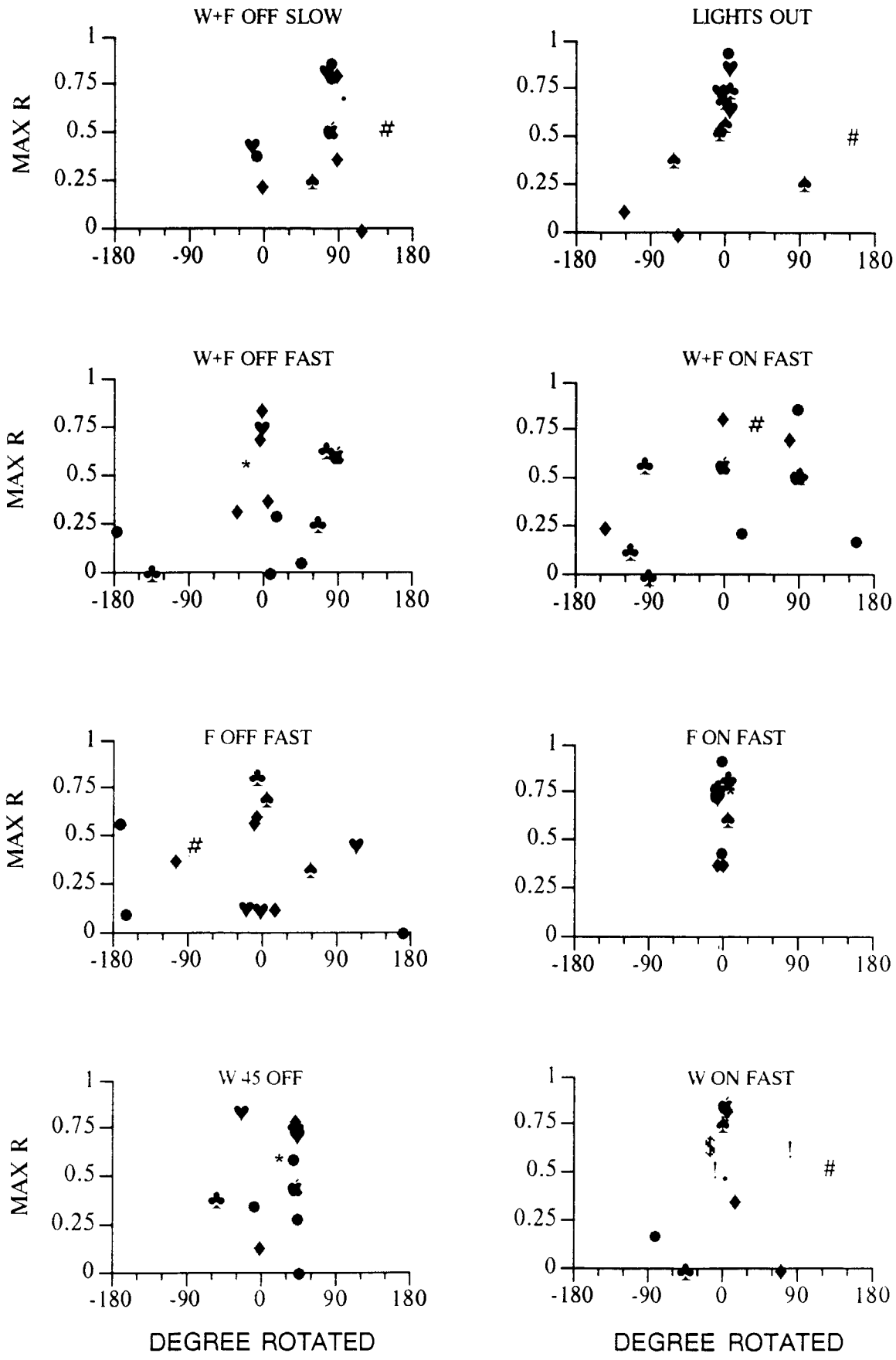


Figure 3. Maximum correlation coefficients (*MAX R*) for the pre- versus postmanipulation firing rate maps for all experimental sessions, displayed as a function of degree of firing field rotation (DEG MAX *R*) between the two maps. Each data point represents the data from the cell(s) recorded

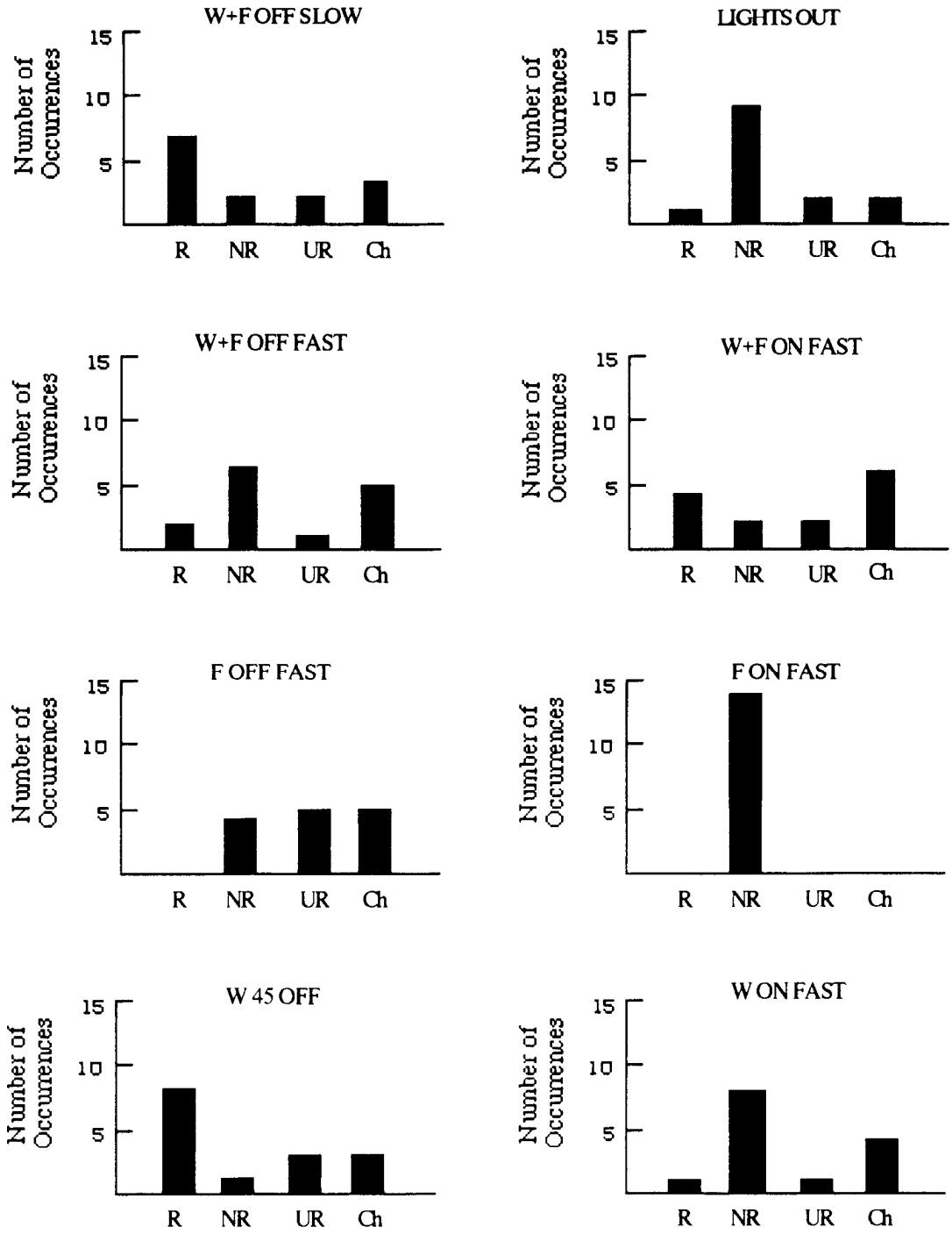


Figure 4. Frequency of occurrence of firing field rotation (*R*), nonrotation (*NR*), unpredicted rotation (*UR*), and changed field (*Ch*) for the data points (from Fig. 3) in each manipulation type. See Results for exact definition of each categorical term.

of the various types of sensory inputs (vestibular, visual motion, etc.) in their influence on these cells (see below).

Based on the measures used for Figure 3, the data points were placed into nominal categories, and histograms for each manipulation, based on these categories, are shown in Figure 4. These categories were (1), rotation (*R*), which included all cells for which the postrotation firing field rotated to a position within ±20° (in the same direction) of the apparatus manipulation, and

←

during one session, and the data for each manipulation type are plotted separately, as indicated. The sign of the rotation was determined in relation to the direction of rotation (clockwise vs counterclockwise) of the apparatus (the cylinder wall and/or floor), so that a firing field rotation in the same direction as the apparatus rotation was coded as positive, while one in the opposite direction was negative. For the LIGHTS OUT manipulation, which did not involve an apparatus rotation, clockwise rotations were coded as positive, while counterclockwise rotations were negative. The data for each of the 11 experimental animals is displayed using a different symbol, to illustrate the contributions of individual animals. As described in the text, there was variation across the manipulation types in the pattern of MAX *R* and DEG MAX *R* values.

whose MAX *R* value was above 0.25; (2), no rotation (*NR*), which included all data points whose postrotation field remained within ±20° of the prerotation field, and for which the MAX *R* was above 0.25; (3), unpredicted rotation (*UR*), which included all cells for which the MAX *R* value was above 0.25, but which did not fall into either the *R* or *NR* categories; and (4), changed field (*Ch*), which included all data points yielding a MAX *R* value of 0.25 or less (including cells that stopped firing after the manipulation, and that were assigned a MAX *R* of 0.0). Note that for the LIGHTS OUT manipulation there was no apparatus rotation, so that by the definition given here, there could be no data points in the *R* category. However, this manipulation was intended to serve as a control condition to indicate the probability of 90° rotations that might result simply from the 60 sec lights out period. Thus, for the LIGHTS OUT manipulation, a rotation of 90° (±20) *in either direction* was included in the *R* category.

The MAX *R* value of 0.25 used to divide the cells designated as having changed (*Ch*) from those in the other categories (*R*, *NR*, and *UR*) was chosen for two reasons. First, it corresponds to the value at which the *r* statistic is significant at the 0.005 level for the df > 100 provided by these maps, and, second, it corresponded to the level at which any two maps appeared, upon visual inspection, to have firing fields that were not recognizably the same. As is clear from Figure 3, however, some cells showed intermediate values on this variable, so that the pre- and postmanipulation fields were clearly similar, but not identical.

As mentioned above, it was often the case that more than one cell was recorded simultaneously during the same manipulation. Of these 23 cases of multiple cell recording, there was *no* case in which a cell fell into either the *R* or *NR* category for which a simultaneously recorded cell(s) did not also fall into the same category; that is, there was concordance for all simultaneously recorded cells that fell into either of these two categories. The only cases of discordance were from five pairs of simultaneously recorded cells in which one was classified into the *UR* category, while the other went into the *Ch* category. In these cases the average of the MAX *R* values was used to determine which category the data point representing the two was classified into (*Ch* for an average MAX $R \leq 0.25$ or *UR* for a value > 0.25).

The perfect concordance for cells in the *R* and *NR* categories suggests that there is a strong tendency for all the place cells to show the same qualitative response to a manipulation on any one occasion. Accordingly, the lack of concordance for only the *UR* and *Ch* categories suggests that it may be appropriate to view cells showing an unpredicted rotation as possibly being examples of cells that changed their fields, but for which the new field is similar enough to the old one so that there happens to be an angular location at which its correlation with the premanipulation map reaches 0.25.

It is interesting that cells recorded during the same session generally showed the same type of response, even though, for most manipulation types, it was possible for cells recorded in different sessions to show different responses to the same manipulation type. One possible explanation is that, as mentioned above, each instance of a given manipulation type could have provided a somewhat different pattern of sensory input, due to slight differences in details of the rotation, such as speed, smoothness, or the animal's behavior. These details may be critical to determining the cells' responses, and they would, of course, be identical for all cells recorded simultaneously. Alternatively, it is also possible that the cells that fire in any one environment are linked together through synaptic connections that make it so that, for example, a rotated field for one cell induces other cells to also fire in a rotated location.

Examination of Figures 3 and 4 shows that there were differences in the overall data pattern across the different manipulations, and the details of these differences are discussed below in relation to their implications for the influence of each of the stimulus modalities tested.

Evidence for vestibular influences on place fields. One test of the effects of vestibular information was provided by the manipulation in which the wall and floor were quickly rotated by 90°, while the room lights were off (W+F OFF FAST). Figure 5 shows the results from a typical cell recorded during this type of session. It can be seen that after the rotational manipulation, the firing field was nearly identical to that before the manipulation. Note that, since both the wall and floor were rotated, this means that after the rotation the cell fired in a new location *in relation to the cylinder floor and wall*. This suggests that the vestibular information did, in fact, influence this cell's firing field location, since it stopped firing in the spot *on the cylinder floor* where its field was located before the manipulation, and retained a constant relationship to the larger laboratory framework. Thus, it appears that the vestibular information provided during the rotation caused the hippocampal system to accurately detect that the animal had been moved.

As can be seen in Figure 3, most of the DEG MAX *R* values for the W+F OFF FAST manipulation are near zero degrees, with relatively few near 90°. Also, some of the cells have very low correlation values, indicating that their field changed after the manipulation.

Figure 4 shows that the plurality of data points for the W+F OFF FAST manipulation fell into the NR category, suggesting that vestibular stimulation provided by the 90° rotation in this condition was sufficient in the plurality of cases to inform the hippocampal place cell system that the animal had moved.

One difficulty with this interpretation, however, is the possibility that there was some uncontrolled extra-apparatus cue (such as equipment noise, or odors) that actually controlled the firing of the cells, and enabled them to maintain stable fields (in relation to the laboratory framework). A control for this possibility is provided by the manipulation in which the wall and floor were rotated slowly, with the lights out (W+F OFF SLOW). Figure 6 shows a result that is typical for this manipulation, in that the firing field rotated by 90° (in the same direction as the apparatus). Thus, although a possible influence of uncontrolled laboratory cues cannot be ruled out, it seems that these cues were at least not the predominant influence for this cell.

Examination of Figures 3 and 4 reveals that most of the cells in this slow rotation condition showed this rotation. A Mann-Whitney *U* test conducted on the DEG MAX *R* values for all data points in the *R*, *NR*, and *UR* categories for the W+F OFF FAST versus W+F OFF SLOW manipulations indicated a significant difference between the two groups ($U = 24.5, p < 0.025$).

The fact that a few cells in both manipulation types showed changed fields and unpredicted rotations suggests that both these manipulations provided a disruptive influence on the place fields, possibly having to do with the sudden changes in illumination involved in turning off the lights for 60 sec. The slightly higher relative incidence of changed fields in the W+F OFF FAST

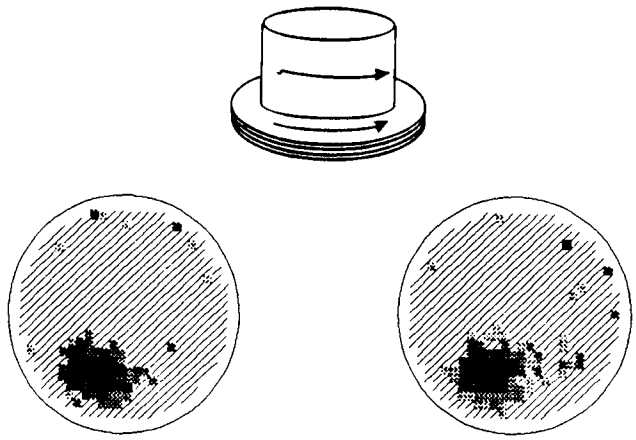


Figure 5. Firing rate maps (constructed as in Fig. 2) from a typical cell recorded during a W+F OFF FAST manipulation. The *left map* shows the firing rate data for the 20 min period before the rotational manipulation, while the *map on the right* shows the 20 min postmanipulation phase. The *inset* provides a diagrammatic representation of the rotational manipulation. In this case, both the cylinder wall and floor were quickly rotated in a counterclockwise direction, during a 60 sec period in which the room lights were extinguished. This field is typical of that of the plurality of cells recorded during this manipulation, in that it showed no rotation (NR in Fig. 4) as a result of the manipulation. The MAX R value for this session was 0.66, while the DEG MAX R value was −6°.

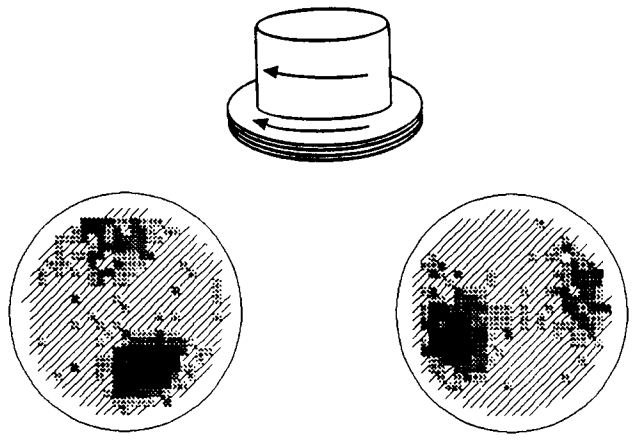


Figure 6. Firing rate maps (constructed as in Fig. 2) from a typical cell recorded during a W+F OFF SLOW manipulation. The *left map* shows the firing rate data for the 20 min period before the rotational manipulation, while the *map on the right* shows the 20 min postmanipulation phase. The *inset* provides a diagrammatic representation of the rotational manipulation. In this case, both the cylinder wall and floor were slowly rotated in a clockwise direction, during a 60 sec period in which the room lights were extinguished. This field is typical of that of the plurality of cells recorded during this manipulation, in that it rotated (R in Fig. 4) as a result of the manipulation. The MAX R value for this session was 0.81, while the DEG MAX R value was 78°.

manipulation suggests the possibility that vestibular information that is not accompanied by other kinds of movement-related information (such as visual motion, and motor commands) with which it would normally be correlated, tends to cause a disruption of the place cells' spatial signal (see below for further discussion of this point).

Evidence for the influence of visual motion on place fields. The simplest test for the influence of visual motion cues is provided by the rotation of the wall alone, which provides visual motion in the absence of vestibular stimulation. Figure 7 shows the results of this manipulation for a typical cell, and it can be seen that no rotation resulted from this treatment. Note that, in this case, since the floor of the apparatus did not move, the no-rotation result here (in contrast to that for the wall + floor manipulations) indicates that the manipulation had no apparent effect on the location of the firing field for this cell. Thus, the visual motion input was not sufficient to cause the hippocampal place cell system to behave as though the animal had moved.

Examination of Figures 3 and 4 reveals that eight of the cells tested in this condition showed no rotation, while one cell did rotate, one cell showed an unpredicted rotation, and four cells showed changed fields. Overall, these data suggest that there is little effect of visual motion information alone, except to cause field changes in a minority of cells.

Evidence for the combined influence of visual motion and vestibular inputs on place fields. A further test of whether visual motion cues can influence place cell firing patterns was provided by the manipulation in which the floor alone was rotated while the lights were left on (F ON FAST). In this case, both visual motion and vestibular information indicate that a particular rotation has taken place. Results from a typical cell in this manipulation are shown in Figure 8. This cell did not rotate, and this indicates that the combined information was sufficient to allow correct detection of the movement of the animal during this rotation. Thus, since the floor was moved in this manipulation, the cell had to stop firing in its original location *in relation to the apparatus floor*, and fire in a new location, 90° away.

Examination of Figures 3 and 4 reveals that this result was

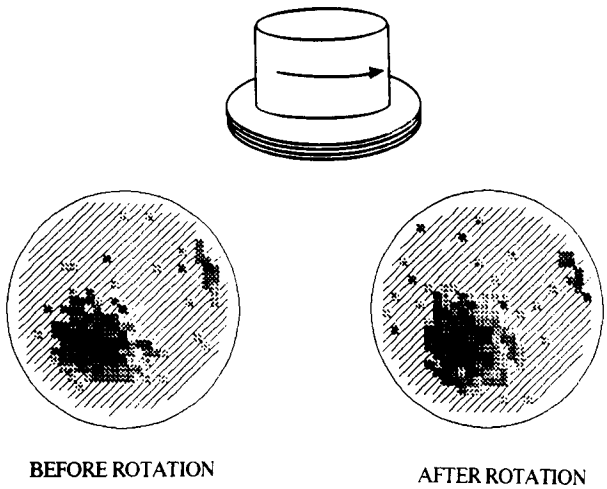


Figure 7. Firing rate maps (constructed as in Fig. 2) from a typical cell recorded during a W ON FAST manipulation. The *left map* shows the firing rate data for the 20 min period before the rotational manipulation, while the *map on the right* shows the 20 min postmanipulation phase. The *inset* provides a diagrammatic representation of the rotational manipulation. In this case, the cylinder wall was quickly rotated in a counterclockwise direction (while the room lights remained on). This field is typical of that of the majority of cells recorded during this manipulation, in that it did not rotate (NR in Fig. 4) as a result of the manipulation. The MAX R value for this session was 0.77, while the DEG MAX R value was −6°.

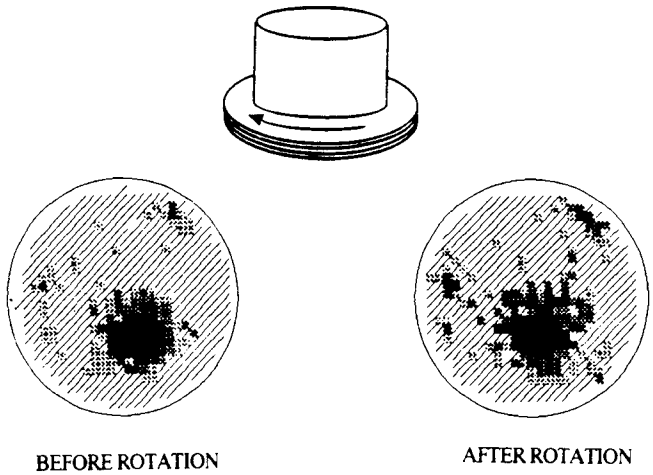


Figure 8. Firing rate maps (constructed as in Fig. 2) from a typical cell recorded during a F ON FAST manipulation. The *left map* shows the firing rate data for the 20 min period before the rotational manipulation, while the *map on the right* shows the 20 min postmanipulation phase. The *inset* provides a diagrammatic representation of the rotational manipulation. In this case, the cylinder floor was quickly rotated in a clockwise direction (while the lights remained on). This field shows the same pattern as all of the fields recorded during this manipulation, in that it showed no rotation (NR in Fig. 4) as a result of the manipulation. The MAX R value for this session was 0.77, while the DEG MAX R value was 6.

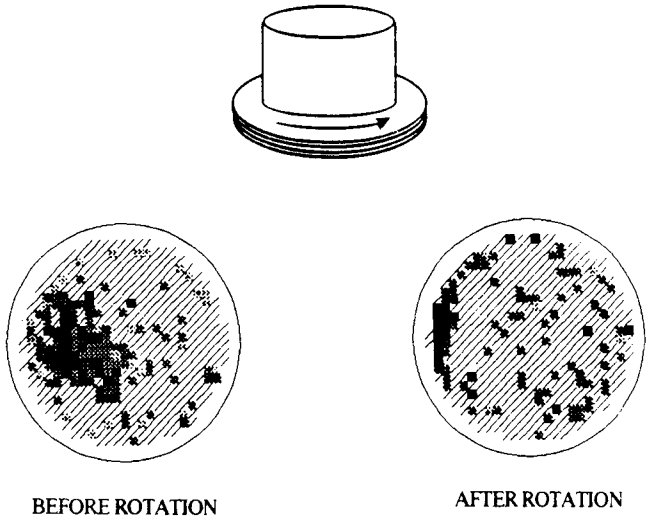


Figure 9. Firing rate maps (constructed as in Fig. 2) from a typical cell recorded during a F OFF FAST manipulation. The *left map* shows the firing rate data for the 20 min period before the rotational manipulation, while the *map on the right* shows the 20 min postmanipulation phase. The *inset* provides a diagrammatic representation of the rotational manipulation. In this case, the cylinder floor was quickly rotated in a counterclockwise direction, during a 60 sec period in which the room lights were extinguished. This cell shows a pattern exhibited by many of the cells recorded during this manipulation, in that it showed a change (*Ch* in Fig. 4) in its overall firing characteristics, as a result of the manipulation. The MAX R value for this session was 0.05, while the DEG MAX R value was −0.24.

obtained for every cell recorded in this manipulation. This is the only manipulation for which there were no unpredicted rotations or changed fields, and this pattern of results suggests that the combined, concordant vestibular and visual motion information was a very reliable way to influence the cells' firing patterns.

One caveat in relation to the above interpretation is that these results could possibly be explained by the presence of uncontrolled cues that could be present on both the apparatus walls, as well as in the extra-apparatus laboratory environment. Thus, in this manipulation, nonrotation of the firing field means that the field retains its prerotation relationship to both the cylinder wall (which could have unintentional odorous spots, or other irregularities), as well as the laboratory framework. It could be that the combination of these cues, along with the vestibular information provided by the manipulation, was sufficient to stabilize these cells, and no influence of visual motion need be postulated. As a test for this possibility, the results from the F ON FAST manipulation can be compared with those of the F OFF FAST manipulation. These two manipulations were identical, except for the fact that in the latter case the lights were out during the manipulation, thus removing any visual motion information. Figure 9 shows results from a cell that exhibited one of the common responses to this manipulation, in that the firing field characteristics were changed (*Ch*) after the manipulation. As can be seen by examination of Figures 3 and 4, the F OFF FAST manipulation resulted in a nearly equal distribution of outcomes into the no rotation, unpredicted rotation, and changed field categories. Thus, the combination of possible uncontrolled cues and vestibular information was not sufficient to stabilize these cells in the unrotated position. A χ^2 test of the difference in the pattern of results for these two conditions for the data in Figure 4 revealed a significant difference ($\chi^2 = 13.05$, $df = 2, p < 0.01$). This outcome suggests, first, that visual motion information was able to combine with vestibular information (and also, possibly, information from uncontrolled cues) to control the location of the cells' firing fields (as in the F ON FAST condition), and, second, that vestibular information that is unaccompanied by corroborating visual motion information can cause cells to change their firing fields, or show an unpredicted rotation.

Some additional support for this conclusion is provided by comparing the two wall + floor fast rotation types (W+F OFF FAST and W+F ON FAST). Note that in this case, visual motion information available during the rotation with the lights on is "contradictory" to the vestibular information. That is, vestibular signals presumably indicate that a rotation has taken place, while the visual motion information suggests the opposite, since the walls move around with the animal. Note that the W+F ON FAST condition results in slightly more rotations than the W+F OFF FAST condition (Fig. 4). Although a statistical test of differences between these two groups in degree of rotation for all data points in the *R*, *NR*, and *UR* conditions was not significant ($U = 27, p > 0.10$), this small trend is compatible with the suggestion that visual motion information, in this case, worked against the influence of the vestibular information that suggested that the animal had moved.

Also, this comparison makes it seem unlikely that the relatively large number of changed fields and unpredicted rotations in the F OFF FAST condition (as compared to F ON FAST) was caused simply by a disruptive influence of the lights being momentarily turned off. This is true since there were actually more changed fields and unpredicted rotations in the W+F ON FAST than for the W+F OFF FAST manipulation. Also, this conclusion is supported by the fact that the LIGHTS OUT condition produced only two instances of changed fields.

Evidence for the influence of static cue information. Rotation of the wall alone by 45° with the lights out (W 45 OFF) provided an indication of whether the black and white stripes themselves, as static environmental cues, had any effect on these cells, since this resulted in a switch in the absolute locations of black and white areas. Figure 10 shows an example of a typical cell tested under these conditions. This cell rotated by approximately 45° in the direction of the wall rotation, suggesting that even in the absence of any vestibular or visual motion information, the cells changed their firing location due to the change in location of these static cues. Examination of Figures 3 and 4 shows that most of the cells showed this response (where *R* in this case is defined as any rotation of 45° (±20) from the prerotation field, in either direction). Unexpectedly, most of the cells in this condition rotated *in the same direction* as the wall (see Fig. 3). This bias in the *direction* of rotation was not predicted in advance, since the data from the other manipulations provided no evidence that the cells could discriminate one particular black or white stripe from another, and it was reasoned that conducting the 45° rotation in the dark would prevent the animals from knowing which direction the stripes had traveled. These data suggest, however, that the cells had some information that enabled them to discriminate between different stripes of the same color, so that they tended to retain the same relationship to them after the manipulation. One possibility is that there were uncontrolled cues on the cylinder wall (such as odors), while another possibility is that the cells somehow received information about the direction of movement itself, possibly through the vibrissa brushing against the wall during the rotation.

Evidence related to the effects of a 60 sec period of darkness. The LIGHTS OUT manipulation provided a control for the effects of simple exposure to a period of darkness. Examination of Figures 3 and 4 reveals that most of the cells showed no detectable change in their field, although there were four instances in the combined *UR* and *Ch* categories. These results suggest that either a transient loss of visual information, or some other disruptive influence of these changes in illumination are occasionally capable of causing these fields to either rotate or change.

Note that the distribution of outcomes seen in Figure 4 for the LIGHTS OUT is nearly identical to that for the W+F OFF SLOW manipulation, except that the values in the *R* and *NR* categories are reversed. This suggests that these two manipulations were, as planned, identical in their effects on the hippocampal system, since the greater incidence of the *R* versus *NR* outcome for the W+F OFF SLOW manipulation presumably reflects the fact that the apparatus and animal were rotated so slowly that it was undetected by the hippocampal system.

Overview of firing field changes as a function of vestibular and visual motion information

Figure 11*A* provides an overview of the percent of fields that shifted their location *in relation to the apparatus floor* by 90° in the predicted direction as a result of various combinations of vestibular and visual motion influences provided by the set of rotational and lights out manipulations. (The WALL 45 OFF manipulation is excluded from this analysis, since it involved a manipulation of static, rather than movement-related cues.) Since the term "shifted" is used here to mean that the cell came to fire in a 90° (±20) rotated position *in relation to the apparatus floor*, this means that for all cases in which the manipulation included a rotation of the apparatus floor, shifting refers only to those cells that showed no rotation (NR category in Fig. 4), while for those manipulations *not* including a rotation of the apparatus floor (i.e., the wall alone and lights out manipulations) shifting refers only to those cases in which the field did rotate (*R* category in Fig. 4). The term "shifted," defined in this way, is useful because it serves as a measure of whether the movement-related information provided by a particular manipulation was treated by the hippocampus as an indication that the animal had moved, thus causing an appropriate updating, or shift, of the ongoing locational signal. Thus, in any manipulation involving a floor rotation, if the accompanying vestibular stimulation allowed the hippocampal system to accurately reflect the movement of the animal, then the cell's place field will remain fixed in relation to the outer, laboratory framework, but will necessarily shift in relation to the apparatus floor. Alternatively, in the case of a wall-alone rotation, if the movement of the stripes in one direction causes the hippocampus to act as though the animal had moved 90° in the opposite direction, then the field will necessarily shift its relation to both the floor and the laboratory framework. In any case, a shift in the relation between the firing field and the floor indicates that any movement-related information provided by a manipulation was successful in "convincing" the hippocampal system that the animal had moved.

Also included in Figure 11*A* is a set of data points that were derived from the available sessions in order to measure the place field behavior when no manipulation was performed (NO MANIP in Fig. 11*A*), that is, to measure any tendency of the place fields to show spontaneous changes. Since the original experimental design did not include sessions in which no manipulation was performed, it was necessary to derive this data by performing a rotational correlation analysis on consecutive 10 min sections of either the pre- or postmanipulation phases

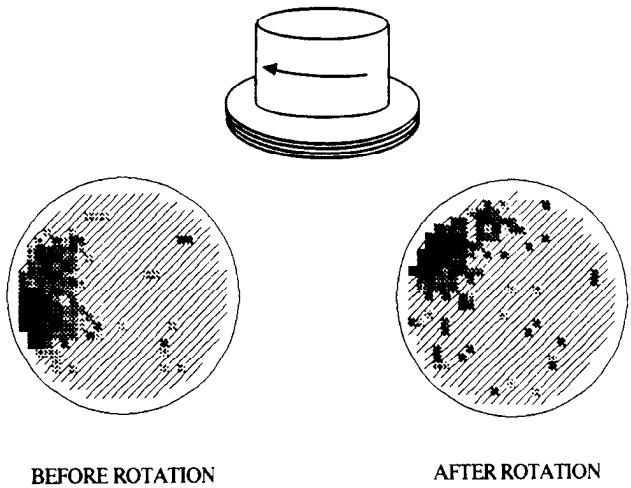


Figure 10. Firing rate maps (constructed as in Fig. 2) from a typical cell recorded during a W 45 OFF manipulation. The left map shows the firing rate data for the 20 min period before the rotational manipulation, while the map on the right shows the 20 min postmanipulation phase. The insert provides a diagrammatic representation of the rotational manipulation. In this case, the cylinder wall was rotated 45° in a clockwise direction, during a 60 sec period in which the room lights were extinguished. This cell shows a pattern exhibited by the majority of cells recorded during this manipulation, in that it showed a 45° rotation of its field (*R* in Fig. 4) as a result of the manipulation. The MAX *R* value for this session was 0.78, while the DEG MAX *R* value was 42°.

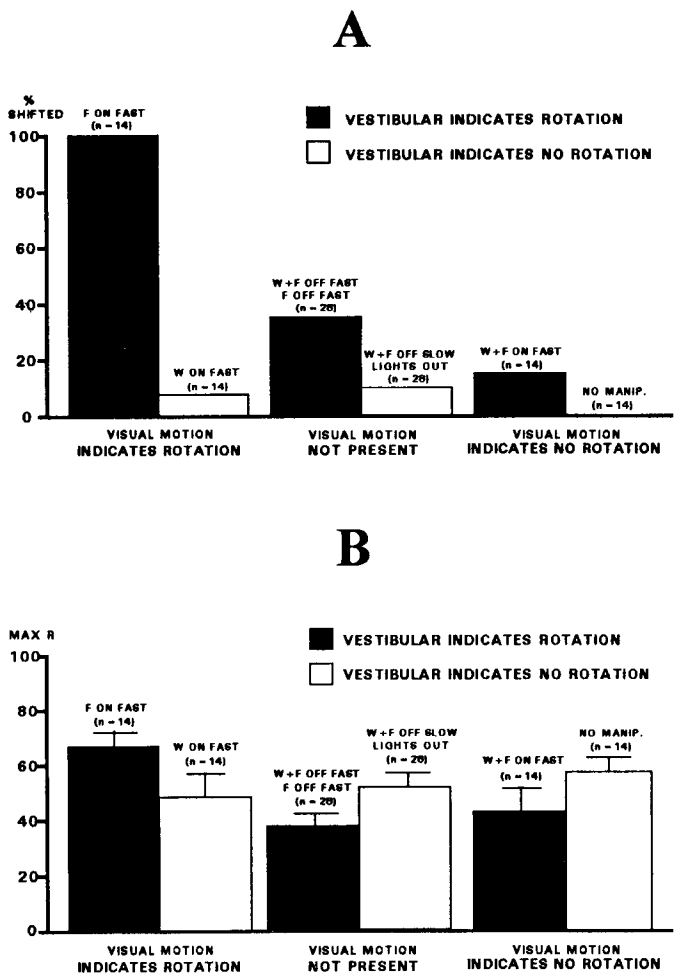


Figure 11. Overview of the combined effects of vestibular and visual motion inputs. *A*, Percentage of observations for which cells were "shifted" (rotated by 90° in the predicted direction from their initial position *in relation to the apparatus floor*) for each category of manipulation type. Manipulations were categorized by whether visual motion information indicated rotation, provided no information, or indicated no rotation, and within each of these categories, the manipulations were further divided into those in which vestibular information indicated rotation (*solid bars*) or no rotation (*open bars*). The NO MANIP category was derived from data collected within either the premanipulation or postmanipulation phase of individual sessions (see Results for details). The data pattern indicates that both vestibular and visual motion input played a role in shifting the fields, and that they interacted, so that combined, corroborating vestibular and visual motion cues shifted the fields most reliably. *B*, MAX *R* (±SE) values for the data from each manipulation type, organized into the same categories as for Figure 11*A*. The data pattern indicates that field changes were induced most strongly by manipulations in which vestibular and visual motion information were not in concordance, in that only one indicated a rotation.

of the available set of sessions. Thus, a set of sessions was first randomly chosen from the larger set of sessions for the seven manipulation types included in Figure 11 (with two sessions being chosen from each manipulation type), and the usual rotational correlation analysis was performed, except that instead of the analysis being conducted on the pre- versus postmanipulation maps, it was conducted on the first, versus the last 10 min period of either the pre- or postmanipulation phase (with the pre- vs post variable counterbalanced across session type). Note that for this NO MANIP category, as well as for the LIGHTS OUT manipulation, there is no predicted direction of rotation, so that, by the definition given here, there could not be any shifted category. However (in accordance with the reasoning presented above), since these conditions were intended as a control for spontaneous 90° rotations, it was decided to define a 90° rotation in either direction as a "shift" for these LIGHTS OUT and NO MANIP categories.

In Figure 11 the data for each manipulation are located along the abscissa based on whether the manipulation provided visual motion information that could indicate a movement of the animal. For example, the W ON FAST and F ON FAST conditions were both manipulations in which the stripes on the cylinder wall were moved in relation to the animal, as must happen during the animal's own movements through the apparatus. Accordingly, these manipulations are both ones in which visual motion indicates rotation. In contrast, the W+F ON FAST and NO MANIP conditions involved no experimenter-induced rotation of the stripes in relation to the animal, so that, in this case, visual motion information indicated no rotation. Any manipulation during which the lights were out was one in which, presumably, visual motion provided no information. In addition, all manipulations that involved a rapid floor rotation provided vestibular information indicating a rotation of the animal had taken place (solid bars), while those during which the floor was stable (or rotated very slowly) provided information that no rotation had taken place (open bars).

Evidence suggesting a role for visual motion in shifting these fields can be obtained from examination of the three categories for which vestibular information indicated rotation (solid bars). As discussed above, when visual motion information indicating the rotation was also present (as in the F ON FAST condition) there was a 100% shifting of the fields. In contrast, when visual motion information was not present (W+F OFF FAST and F OFF FAST conditions), the percentage of fields showing a shift was at an intermediate level. Finally, when visual motion was present, but indicated no rotation (W+F ON FAST) there was an even lower level of shifting. A χ^2 test of the frequencies in these three categories indicated that this pattern was significantly different from that predicted by chance ($\chi^2 = 12.45$, df = 2, $p < 0.01$).

Evidence suggesting a role of vestibular information in shifting the fields is provided by comparison of the black versus white bars within each visual motion condition. In every case, the percentage shifted is higher for the condition in which vestibular input is provided, when compared to the companion condition in which it is absent.

Finally, data in the NO MANIP category showed that there were no instances of spontaneous shifting, and this is compatible with the well known observation of stability for place fields (e.g., Best and Thompson, 1989).

The overall data pattern shown in Figure 11*A* suggests an interaction between vestibular and visual motion information, so that shifting is observed most reliably when the two kinds of information are presented together, in such a way that they both indicate the same movement of the animal through space.

Examination of Figures 3 and 4 suggests that there were differential effects of the various manipulations on the MAX *R* measure, suggesting that some manipulations were more likely than others to cause disruptions of the cells' firing fields (since MAX *R* can be interpreted as a measure of firing field stability). To examine the effects of vestibular and visual motion information on this variable, the average MAX *R* values (±SE) for the same manipulation types used in Figure 11*A* are displayed

in Figure 11*B*, using the same organizational scheme. Recall that higher values for MAX *R* correspond to a higher degree of similarity (at the optimal angular rotation) of the premanipulation and postmanipulation firing fields, while lower values suggest at least a partial change in the field characteristics.

The highest values in Figure 11*B* are for the two manipulation types in which vestibular and visual motion information were in concordance, so that either both indicated rotation (F ON FAST) or both indicated no rotation (NO MANIP). It is noteworthy that these two are similar on this measure, since, in one case no manipulation was conducted (during the time period examined), while, in the other case the animal was subjected to both a vestibular activation, as well as a sudden relative movement of salient environmental stimuli. Thus, it might have been reasoned that field disruptions could be caused equally well by any salient environmental perturbation; however, the fact that the F ON FAST manipulation showed high MAX *R* values is not compatible with this idea.

The lowest values in Figure 11*B* were for cases in which either visual motion or vestibular information indicated a rotation, but for which the other input type did not provide corroborating input. Thus, for example, an average MAX *R* value of 0.39 was obtained for those sessions in which vestibular information indicated a rotation, but for which visual motion was not present (W+F OFF FAST, F OFF FAST). Finally, an intermediate value was obtained for those manipulation types in which vestibular information indicated no rotation, and for which visual motion information was transiently removed (W+F OFF SLOW and LIGHTS OUT).

Thus, the overview provided here suggests that the highest MAX *R* values result when there is concordance between visual motion and vestibular information, while lower values result when there is discordance between these two.

Effects of repeated manipulations

Since many of the animals received numerous, successive manipulations (sometimes including multiple exposures to the same manipulation) it was possible that this experience changed the nature of the cells' responses over time. To test for this possibility, the effect of presentation order was examined to see whether this variable influenced the percent of cells that (regardless of particular manipulation type) either (1) "shifted," defined, as above, as a postmanipulation firing field rotation *in relation to the apparatus floor* of 90° (±20) in the predicted direction; (2) showed "No Shift," in that they retained the same relationship to the apparatus floor; (3) showed an unpredicted rotation (*UR*, as in Fig. 4); or (4) changed their field (*Ch*, as in Fig. 4). Figure 12 shows the percentage of data points falling into each category as a function of which manipulation (collapsed into sets of five consecutive manipulations) it occurred on. That is, any data point included in manipulation block 1, for example, came from an animal for which that manipulation was one of the first five manipulations of any kind received by that animal.

It can be seen that the Shifted and Not-Shifted categories showed a slight, steady decline over sessions, while the *Ch* category showed a dramatic increase. (It should be noted, however, that the data points representing the last two manipulation blocks are based on relatively few observations; together, they represent less than 18% of the total number of sessions, since most animals did not receive this many manipulations.) A χ^2 test conducted on the frequency data represented in Figure 12 was highly significant ($\chi^2 = 46.62$, df = 15, $p < 0.001$). This suggests that the cells are more likely to show changes in their fields as a result of experience with these manipulations.

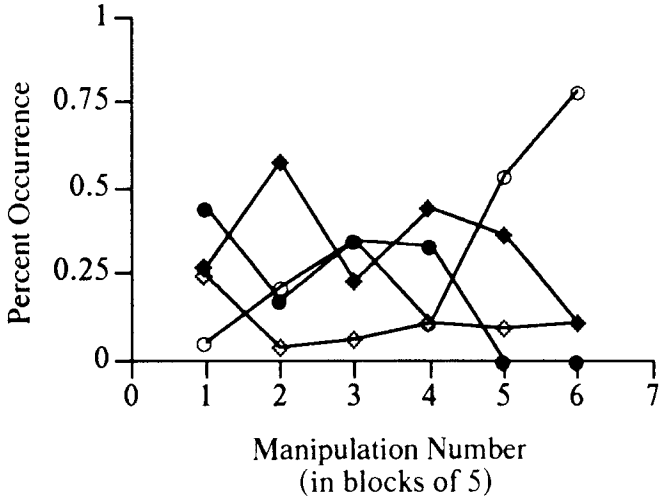


Figure 12. Effects of experience on place cell responses to experimental manipulations. The percent occurrence of each type of response to the experimental manipulations (collapsed over manipulation types) is displayed as a function of manipulation number (collapsed into blocks of five). Thus, for example, any data point represented in the first manipulation block is from a session for which that manipulation was one of the first five manipulations of any kind experienced by that animal. Responses have been categorized into (1) shifted (◆), which refers to cases in which the cell changed its field location *in relation to the apparatus floor* by 90° (±20) in the predicted direction; (2) not shifted (●), which includes fields that remained within 90° (±20) of the premanipulation field; (3) unpredicted rotations (◇; *UR*, as in Fig. 4); and (4) changed fields (○; *Ch* as in Fig. 4). See Results for more details. The percentage of sessions in which the cells showed a changed field increased over consecutive session blocks, while the percentage of responses in the shifted and not shifted categories declined slightly.

Examination of field changes

As already discussed, it was sometimes observed that cells responded to manipulations by showing a change in their firing field that could not be classified as simply an angular relocation of the premanipulation field, but, rather, consisted of a firing pattern that was different in its overall characteristics, such as field size, field shape, and distance from the wall. These cases were operationally defined as those for which the MAX *R* value was 0.25 or less, and they were placed into the *Ch* category defined above. It was not necessarily the case that the changed pattern consisted of a well-defined spatial firing field; in 67% of the *Ch* cases, the change consisted of either a cessation of firing, so that no meaningful measure of MAX *R* could be obtained (in which case a MAX *R* value of 0.0 was assigned), or a sparse, nonspatial, distributed pattern. The field changes were unpredictable, in that it did not seem possible to guess in advance (based on the cells' premanipulation firing characteristics) whether or not a cell would change its field, or what the nature of the change might be. As already discussed, however, some manipulations were more likely than others to cause a changed field.

One interpretational difficulty involving these changed fields is that they could possibly have resulted artifactually from mechanical disruption of the physiological preparation during the rotational manipulations. That is, it is possible that during a

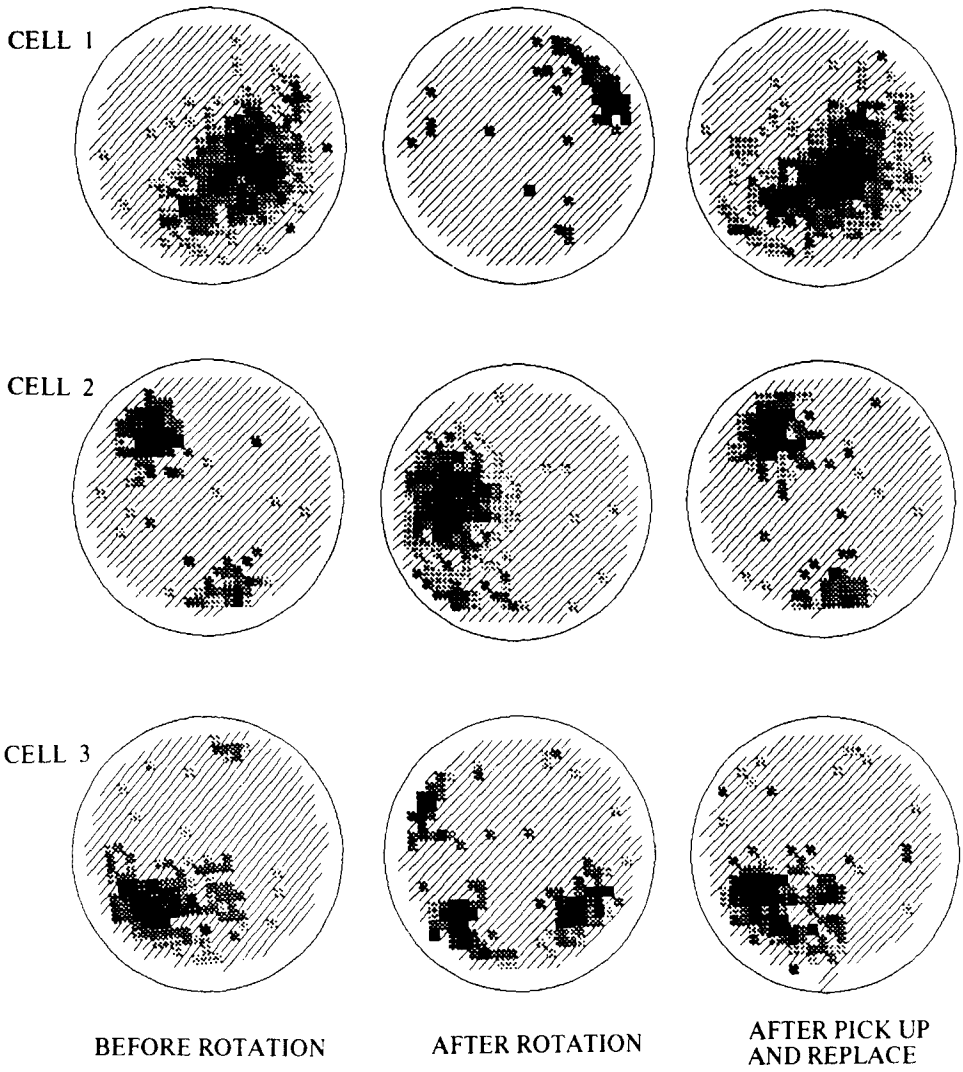
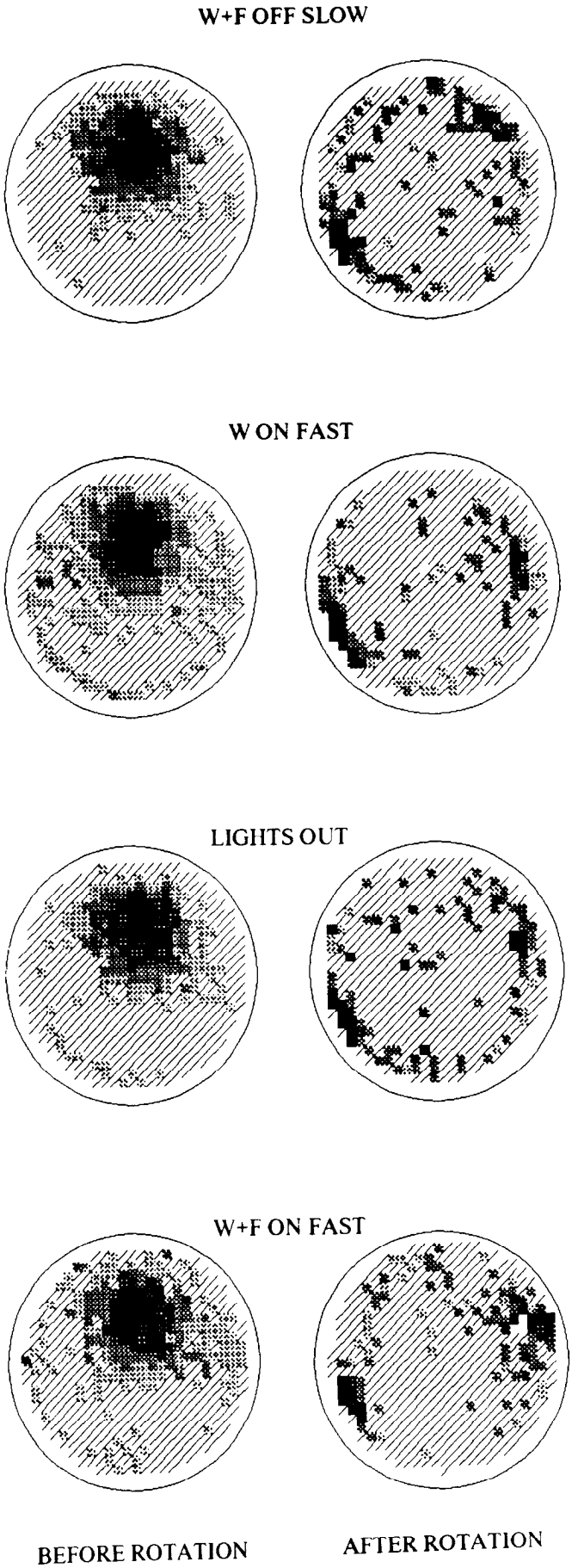


Figure 13. Firing rate maps (constructed as in Fig. 2) from a set of three cells (recorded in three different sessions, from two animals) which showed a changed field in response to a manipulation. The *left map* in each row shows the firing rate data for the 20 min premanipulation phase of the recording session, while the *middle map* of that row shows the postmanipulation data for the same session. The *right map* shows the firing rate data for an additional 20 min session (conducted immediately after the initial session) for the same cell after the animal had been picked up briefly, and then quickly replaced into the cylinder (from the standard start position). For each cell, the original firing field was reinstated after the replacement of the animal back into the cylinder.

rapid rotation there is a sudden pull on the cable (due to sudden movement of the animal and/or apparatus) and this causes a slight movement of the recording electrode wire in relation to the cell. This slight movement could result in either a loss of the electrical isolation of the cell (thus causing an apparent cessation of firing) or a change of the electrode over to another cell, which might be mistakenly identified as the original cell. To check for this possibility, in six cases a test was conducted to see if it was possible to return the cell to its original firing characteristics. For this, an additional recording period was conducted at the end of the regular recording session. At this time, the animal was picked up from the cylinder in the usual fashion, but instead of being returned to the home cage, it was replaced into the cylinder (in the same position as that used to start a regular session) for an additional 20 min recording session. It was reasoned that this reinstatement of the conditions used to initiate the recording sessions (i.e., handling and placement into the cylinder) might reinstate the original premanipulation firing field. In each of the six cases in which this picking up-and-replacement was conducted, the firing field did, in fact, return to its initial pattern. Figure 13 shows examples from three of these cells. This result suggests that, at least in most cases, the apparent field changes induced by manipulations were not just due to changes in the cell isolation resulting from mechanical disruptions, but were truly changes in the firing patterns of these cells. Further support for this conclusion comes from the fact that cells with changed fields were always found to be back to their original pattern when recorded in a new session the following day.

As discussed above, the probability of a cell showing a change in firing field increased as a function of the number of previous manipulations experienced by that animal. This suggests that there was a learned component to the changed fields. In one animal, field changes became the predominant response to almost all manipulations. This pattern began on manipulation number 16, and this animal's data were not included in the main experimental data set after this time. However, further tests were conducted to examine the nature of this behavior. Figure 14 shows the results from one cell that was recorded from this animal over a series of days, during which various manipulations were performed. Interestingly, this cell showed the same transformation from a unitary, rounded field, to a pair of smaller, more eccentrically located fields as a result of each manipulation. This suggests that some general, common aspect of the various manipulations somehow enabled this firing pattern to be instantiated on each occasion. Surprisingly, the angular orientation of the postmanipulation field was the same in each session. Thus, the postrotation field always showed the same



spatial relationship to the extra-apparatus (laboratory) framework, despite the fact that some of the manipulations (e.g., W+F OFF SLOW) were ones that tended to cause rotations in postmanipulation fields for most cells. This suggests that this angular orientation for this particular cell was unusually influenced by some uncontrolled, extra-apparatus cue(s).

Other cells, subsequently recorded from this same animal, similarly showed changed fields after many of the manipulations. As with the cells discussed above, it was possible to reset these cells to the initial, premanipulation pattern by picking the animal up, and replacing it into the cylinder. Interestingly, the only manipulation type that did not cause a changed field type for this animal was the F ON FAST manipulation. It will be recalled that this manipulation resulted in no field changes for any of the other cells in the study.

Discussion

Influences of vestibular, visual motion, and static environmental information. These data provide a demonstration that both vestibular activation (caused by rapid rotation of the apparatus floor), and visual motion (caused by rapid movement of vertical stripes) can influence the firing patterns of hippocampal place cells. In some cases these manipulations caused a predictable shift in the location of the firing field, while in other cases they caused a change in the overall spatial firing characteristics of the cell.

Evidence for the influence of vestibular inputs was most clear in the comparison of the W+F OFF FAST and W+F OFF SLOW manipulations. These two were identical, except that the rapid rotation was assumed to activate the vestibular system, while the slow rotation was assumed to be below this threshold. A slow 90° rotation typically resulted in a similar rotation of the place cell firing field. The probable explanation for this is that the system simply did not detect the rotation, so that, as the apparatus gradually moved, the field moved along with it. In contrast, the W+F OFF FAST manipulation most often resulted in no rotation of the field, so that it retained a constant spatial relationship to the outer, laboratory framework. This suggests that the vestibular information provided by the rapid rotation allowed a correct detection of the rotational movement.

The evidence suggesting a role for visual motion in influencing place fields is less straightforward, although the overall data pattern suggests that this information also reaches the hippocampal circuitry. The most clear test was the wall-alone rotation (W ON FAST). In this case, only one cell rotated its field (the expected result if visual motion played a powerful role in setting the location of place cell fields), while the majority of cells showed no apparent influence of this manipulation.

←

Figure 14. Firing rate maps (constructed as in Fig. 2) for a cell recorded over several days from an animal in which a pattern had developed, such that all cells examined showed a changed field (Ch in Fig. 4) in response to most manipulations. This animal's data were not included in the main experimental data set after this pattern developed, and the maps shown here are from a series of sessions conducted after this. The *left map* in each row shows the data from the 20 min premanipulation phase of each session (manipulation type indicated above each pair of maps), while the *right map* shows the data from the postmanipulation phase. This cell showed the same altered firing pattern to each of the manipulation types shown. The MAX R values for the pre- versus postmanipulation maps for the four manipulations (from top to bottom) were −0.04, 0.03, −0.03, and 0.06, respectively, while the DEG MAX R values were 60, 66, −48, and 72.

When tested in combination with vestibular inputs, however, there was an indication of a more subtle influence of visual motion cues. Thus, rapid rotation of the floor alone, with the lights on (F ON FAST) resulted in a no-rotation (*NR*) for every cell tested. These results were significantly different from those for the F OFF FAST manipulation, for which only four cases of *NR* were observed, while there were five observations in each of the *Ch* and *UR* categories. This suggests that the combined, corroborating vestibular and visual motion information provided by the F ON FAST manipulation was a very powerful way to inform these cells about the animals' motion through space.

A possible alternative explanation is that the difference between these two conditions was due simply to disruptive effects of sudden changes in illumination in the F OFF FAST condition. This idea is not supported, however, by the fact that the LIGHTS OUT condition caused relatively few instances of *Ch* and *UR*. Also, the W+F ON FAST manipulation resulted in a relatively high percentage of *Ch* observations. In that case, the lights were left on, just as in the F ON FAST condition, but, since the cylinder wall rotated along with the floor (and animal), there was no visual motion information to corroborate the vestibular activation suggesting rotation; in fact visual motion-related information indicated no such rotation. Interestingly, in this W+F ON FAST case, there were also a relatively large number of observations in the *R* category, suggesting that the constant relation of the stripes to the animal during the rotation actually worked against the influence of the vestibular input.

In general, an overview of the effects of the various combinations of visual motion and vestibular manipulations suggested (1) both these variables could play a role in causing fields to "shift" (fire in a rotated position in relation to the apparatus floor), and the probability of this was greatest when the vestibular and visual motion inputs were presented together, and in such a way that they both suggested the same direction and degree of rotation, and (2) both types of input could play a role in causing the fields to change their overall firing characteristics (*Ch*), and this was most likely to happen when vestibular and visual motion information were not compatible, in that they did not, between the two of them, correspond to the natural consequences of the same movement through space.

The LIGHTS OUT manipulation revealed that only a minority of fields (36%) were affected in some way by a brief period of darkness. This is similar to previous results (Quirk et al., 1990) although the percentage of affected cells seen here is somewhat higher. This could be due to the symmetrical environment used here, or to the fact that repeated experience with the full set of experimental rotations caused a generalized increase in the probability of field changes.

Results from the W 45 OFF manipulation demonstrated that the static locations of the black and white stripes on the cylinder wall also influenced these cells. Surprisingly, most of these cells rotated *in the same direction* as the cylinder wall. This was not predicted, since evidence from the other manipulations suggested that there was little influence of differences that may have inadvertently existed between the different individual black and white stripes. One possibility is that the W 45 OFF manipulation provided a more sensitive measure of the ability to discriminate between different stripes of the same color. Thus, the system had to choose between two equidistant locations for a field shift, with no other information to influence the choice.

One interpretational difficulty with the overall set of results is that the exact pattern of vestibular and visual motion inputs provided by the experimental manipulations were almost certainly quite different from those that occur naturally, as the animal generates its own movements. Thus, it is not clear how well these results can be generalized to the possible effects of these sensory modalities during natural locomotion. However, manipulation effects were often predictable, both in terms of distance and direction of field rotation, suggesting that the manipulations may have, in fact, tapped into mechanisms that are present more generally.

Another difficulty is that the behavioral situation employed here did not allow for precision in the delivery of the vestibular and visual motion inputs. For example, the animal's locational eccentricity in the cylinder during a manipulation must have influenced the relative amounts of translational and rotational movement resulting from floor rotations. Also, compensatory movements made by the animals, such as optokinetic and optocollic responses, may have altered the intended effects of the manipulations on the animal's sensory apparati. This lack of control places limits on the detail with which inferences can be made about how the sensory inputs studied here influence the place cells, for example, whether the influences from the vestibular system were due mainly to activation of the semicircular canals, or the static labyrinth.

Effects of repeated experience. Repeated experience with the manipulations caused an increased probability of observations in the *Ch* category over the sessions conducted for any one animal, accompanied by a corresponding decrease in other categories. This suggests that there were long-term changes in the hippocampal circuitry (or its afferents) that were somehow responsible for this trend.

Interestingly, cells that had changed their fields could be reinstated to their original firing pattern by picking up the animal, and replacing it into the apparatus from the standard starting position. This demonstrated that the changes were not artifactually generated by changes in the recording preparation, and also, that the changes were not permanent. Rather, it seems that the hippocampus was capable of demonstrating two completely different representations of the same exact environment, and that it was possible to switch between these two representations by presenting a transient sensory event (either an experimental manipulation or handling and replacement into the cylinder).

This phenomenon has been observed in other contexts as well (e.g., McNaughton et al., 1989; Quirk et al., 1990; Sharp et al. 1990) and is compatible with theoretical suggestions that the hippocampal formation may be involved in working memory (e.g., Olton et al., 1980), since it consists a trial-specific firing pattern that is both initiated and terminated by a transient environmental event(s). Whether or not these different spatial representations actually have any relation to behaviorally observed working memory functions remains to be determined.

Summary. These results provide evidence that the vestibular and visual consequences of an animal's movements through space combine with static environmental cues to enable the hippocampal system to accurately calculate the animal's momentary position in space. It is not clear how the movement-related information is utilized by hippocampal circuitry to cause the observed effects, although some theoretical progress has been made (e.g., McNaughton, 1989). It is also not clear how this information reaches the hippocampus, although movement-related activity has been observed in the parietal cortex (McNaughton et al., 1989) and also in the medial septum (Ranck,

1976), both of which are anatomically related to the hippocampus. Further work is needed to determine how the hippocampal circuitry utilizes movement-related information to accurately calculate the momentary locational signal.

References

Best PJ, Thompson LT (1989) Persistence, reticence, and opportunism of place-field activity in hippocampal neurons. Psychobiology 17: 236–246.

Foster TC, Castro CA, McNaughton BL (1989) Spatial selectivity of rat hippocampal neurons: dependence on preparedness for movement. Science 244:1580–1582.

Hill AJ, Best P (1981) Effects of deafness and blindness on the spatial correlates of hippocampal unit activity in the rat. Exp Neurol 74: 204–217.

Kubie JL, Muller RU, Bostock E (1990) Spatial firing properties of hippocampal theta cells. J Neurosci 10:1110–1123.

McNaughton BL (1989) Neural mechanisms for spatial computation and information storage. In: Neural connections and mental computations (Nadel LA, Cooper P, Culicover P, Harnish R, eds), pp 285–349. Cambridge, MA: MIT Press.

McNaughton BL, Leonard B, Chen L (1989) Cortical–hippocampal interactions and cognitive mapping: a hypothesis based on reintegration of the parietal and inferotemporal pathways for visual processing. Psychobiology 17:230–235.

Muller RU, Kubie JL (1987) The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells. J Neurosci 7:1951–1968.

Muller RU, Kubie JL, Ranck JB Jr (1987) Spatial firing patterns of hippocampal complex-spike cells in a fixed environment. J Neurosci 7:1935–1950.

O'Keefe J (1976) Place units in the hippocampus of the freely-moving rat. Exp Neurol 51:78–109.

O'Keefe J, Conway DH (1978) Hippocampal place units in the freely-moving rat: why they fire where they fire. Exp Brain Res 31:573–590.

O'Keefe J, Dostrovsky J (1971) The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely moving rat. Brain Res 34:171–175.

O'Keefe J, Nadel L (1978) The hippocampus as a cognitive map. New York: Oxford UP.

O'Keefe J, Speakman A (1987). Single unit activity in the rat hippocampus during a spatial memory task. Exp Brain Res 68:1–27.

Olton DS, Becker JT, Handelmann GE (1980) Hippocampal function: working memory or cognitive mapping? Physiol Psychol 8:239–246.

Quirk GJ, Muller RU, Kubie JL (1990) The firing of hippocampal place cells in the dark depends on the rat's experience. J Neurosci 10: 2008–2017.

Ranck JB Jr (1973) Studies on single neurons in dorsal hippocampal formation and septum in unrestrained rats. Exp Neurol 41:461–555.

Ranck JB Jr (1976) Behavioral correlates and firing repertoires of neurons in the septal nuclei in unrestrained rats. In: The septal nuclei (DeFrance, ed). New York: Plenum.

Sharp PE, Green C (1994) Spatial correlates of firing patterns of Single cells in the subiculum of the freely-moving rat. J Neurosci 14:2339–2356.

Sharp PE, Muller RU, Kubie JL (1990) Firing properties of hippocampal neurons in a visually-symmetrical stimulus environment: contributions of multiple sensory cues and mnemonic processes. J Neurosci 10:3093–3105.

Zipser D (1985) A computational model of hippocampal place fields. Behav Neurosci 99:1006–1018.