Hippocampal Place Units in the Freely Moving Rat: Why They Fire Where They Fire

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Summary. Place units in the dorsal hippocampus of the freely-moving rat signal the animal's position in an environment (place field). In the present experiments, thirty four place units were recorded in two different environments: one, a small platform where the rat had received neither training nor reward; the other, an elevated T-maze inside a set of black curtains where the rat had been trained on a place discrimination. The places within the curtained enclosure were specified by four cues (a light, a card, a fan, and a buzzer) in addition to the food. Other cues were eliminated by rotating the maze and the four controlled cues relative to the external world from trial-to-trial.

Some units had place fields in both environments while others only had a place field in one. No relationship could be seen between the place fields of units with fields in both environments.

All twelve units tested extensively in the controlled enclosure had place fields related to the controlled cues. Probe experiments in which only some of the controlled cues were available showed that some of these units were being excited by one or two cues while others were influenced in a more complex way. The fields of these latter units were maintained by any two of the 4 cues and were due to inhibitory influences which suppressed the unit firing over the rest of the maze.

Key words: Hippocampus – Place units – Unit recording – Sensory cues – Rat

In previous papers, we reported that cells in the dorsal hippocampus of the freely-moving rat fire when the animal is located in one part of an environment but not in other parts (O'Keefe, 1976; O'Keefe and Dostrovsky, 1971). Ranck

(1973) also noticed the spatial aspects of some hippocampal cells although he did not emphasize them. Recent abstracts from his laboratory (Best and Ranck, 1975) and others (Branch et al., 1976; Hill, 1976) have confirmed the existence of these cells. We have called the region within which these place units fire maximally their 'place field' and have suggested that linked together in the appropriate fashion they provide a cognitive map (O'Keefe and Nadel, 1977). From a theoretical point of view, it is important to determine the factors responsible for this place field. Is it due to something the rat does in the place field or to some environmental factor? If the latter, is the cell responding to a stimulus, or is it signalling more abstract information such as the place itself, as we have previously suggested? How does the cell identify the place? Does it do so on the basis of a special set of cues or will any cue do?

Another set of questions about these cells relates to the way in which different environments are mapped within the hippocampus. Does each cell represent a place within only one particular environment or does it participate in the mapping of many environments? If the latter, is there any relationship between the places in two different environments which a particular place cell represents?

In an attempt to answer these questions, we have recorded hippocampal place cells from rats while they were making a place discrimination on a T-maze inside an environment where we controlled all of the spatial cues. This micro-environment was isolated from the external world by rotating the maze together with the spatial cues between trials. We reasoned that if place cells could be recorded in such an environment, it would rule out certain possible determinants of the place field such as fixed distant stimuli or geomagnetism. Furthermore once the place field of a cell had been located in such an environment, we might be able to assess the contribution of each of the spatial stimuli by removing one or more of them on probe trials. If the place field was determined by only one of the stimuli, then removal of that stimulus would abolish the field but removal of the other stimuli would not affect it.

In an attempt to answer the question about the role of each place cell in different environments, we have recorded them in a second environment in addition to the controlled environment. If the same cell has a place field in more than one environment it would indicate that many environments can be mapped onto the same hippocampal neurons. Furthermore a comparison of the place fields in the two environments might reveal some of the rules which prescribe where a place unit will fire in an environment.

Methods

The Small Platform, the Cue-Controlled Environment, and Pretraining

Figure 1 shows a ground plan of the experimental room. Pretraining and recording took place on both the small platform outside the curtains and the T-shaped maze inside the curtains.

The small platform was made of wood and measured 40 x 40 cm with a 5 cm lip around the perimeter to prevent the rat from slipping off. It was situated directly in front of the equipment rack with its surface 75 cm above the floor level. The area containing the T-maze was curtained off from
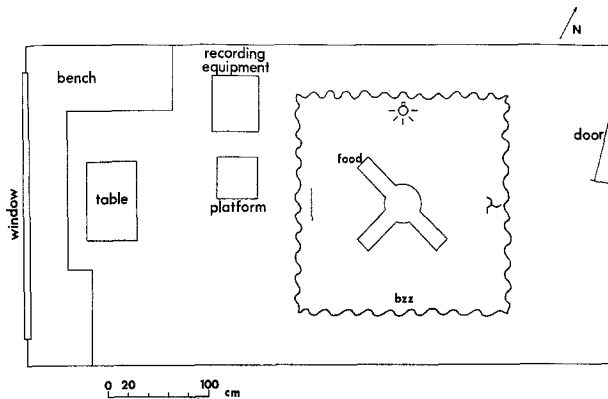
Fig. 1. Plan of the experimental room. The cue-controlled enclosure to the right of the centre contains four cues: $-\overset{\circ}{\underset{\shortmid}{\diamond}}-$ = low wattage light; $\not{\succ}$ = fan; Bzz = buzzer; | = large white card

the rest of the room. We henceforth refer to this curtained area as the cue-controlled enclosure. It consisted of a black false ceiling set 200 cm above floor level and 70 cm below the level of the ceiling of the room, and a set of four black curtains, 215 cm on a side, which hung from the false ceiling to the floor. Access to the cue-controlled enclosure was via the separations between the curtains at the four corners.

Within this enclosure, there were four sensory cues which the rat could use to locate itself: a low-wattage D.C. light which was the only source of illumination and which generated between 0.3 and 0.6 lux at different points in the enclosure; a white card (64 cm high x 50 cm wide), a fan, and a buzzer made from a mains relay. These cues will be referred to collectively as the *wall cues*. Together with the reward they constitute the five *controlled cues*. Each wall cue was located in the centre of a different wall of the enclosure. The fan and the buzzer were 110 cm above the floor, the centre of the card 140 cm above the floor and the light on the false ceiling. During the experiment these cues always maintained the same spatial relationship to each other and to the goal arm of the T-maze but varied relative to the world external to the cue-controlled enclosure and to any other cues within the enclosure.

There were four different configurations of the controlled spatial cues, each rotated 90° relative to the previous one. In the first configuration, the light was located in the North West, the fan in the North East, the buzzer in the South East and the card in the South West (see e.g. Figs. 1 and 6B, F). For the second configuration, the light was in the North East, the fan South East, the buzzer South West and the card North West (e.g. Fig. 6C, G). Further rotations of the cues by 90° and 180° gave the third and fourth configurations respectively (see Fig. 6D, H and A, E). Only the buzzer and card were physically moved from one configuration to the next. A light and a fan were permanently mounted in the centre of each wall and the appropriate ones switched on for each configuration.

Set in the centre of this cue-controlled enclosure was an elevated black perspex T-maze which rested on a circular table. The surface of the maze was 30 cm above the table and 80 cm above the floor. The maze consisted of a central disc (35 cm dia.) and three detachable arms (38 x 15 cm). The three edges of each arm not facing the centre disc were lined with a 0.5 cm lip. There were eight possible T-maze configurations relative to the external world, two for each configuration of the spatial cues.

The T-maze was always orientated so that the goal and non-goal arms maintained the same spatial relationship to the wall cues. For three of the four rats this meant that the goal always pointed between the light and the card, and the non-goal between the fan and the buzzer. The start arm on the other hand, varied from one side to the other. On some trials it pointed between the light and fan while on others it was rotated 180° to point between the buzzer and card (see Fig. 6A–H). For the fourth rat, the goal pointed between the card and the buzzer and all other relations were similarly rotated by 90° anticlockwise with respect to the wall stimuli. In consequence for all rats on

approximately one-half of the trials the animal had to make a right turn to reach the goal while on the other half it had to go left.

The use of intramaze cues (other than the reward itself) to locate the goal was eliminated by interchanging the physical arms (labelled x, y and z) from trial to trial. For example, arm x might serve as the start arm on one trial, and as the goal arm on the next.

Training on the place discrimination proceeded as follows. Four male hooded rats weighing between 275 and 400 gm were placed on a food deprivation schedule which reduced their weights to 85% of normal. After about a week on this schedule, they were placed on both the platform and the maze and allowed to explore. The exploratory phase on the T-maze varied from animal to animal. The minimum was 15 min on 1 day and the maximum was 1 hour per day on two consecutive days. Following this exploratory phase, the rats were trained to go to the goal arm of the T-maze when placed down in the start arm. The animal was always placed down on the start arm from the corner directly behind it. There was a small bakelite lid (6 cm diam x 1 cm high) at the end of both the goal and non-goal arm, but only the one in the goal arm contained reward. The reward consisted of 4 x 45 mgm pellets of rat food for two animals and a few drops of sweet condensed milk for the other two. The sides of the cups were sufficiently high so that the rat could not see the reward until it reached the end of the goal arm. The two cups were randomly interchanged from trial to trial. If the rat made an incorrect choice on a trial it was not allowed to correct and was removed from the maze. Ten trials a day were given with an average inter-trial interval of 15 min. During the interval the rat waited on the platform. It should be emphasized that no rewards or punishments were ever delivered to the animal on this platform.

Each rat was trained to go to the goal arm as defined by its spatial relationship to the four wall cues. For three rats the goal was the arm pointing between the light and the card; for the fourth rat, it was the arm between the card and the buzzer. From trial to trial, the spatial relationship between the goal arm and the wall cues remained constant but varied randomly relative to the rest of the room, the physical arms serving as start, goal, and non-goal were randomly interchanged and the position of the stem or start arm of the T was randomly rotated 180° relative to the cross piece. Thus if the rat learned to find the food it must have done so on the basis of one or more of five cues (the wall cues plus the food). The four animals learned the task to a criterion of 9 correct out of 10 consecutive trials in 0, 11, 14 and 20 trials.

After an animal reached criterion it was given further training. It continued to receive a reward for initially going to the goal. Now, however, it could earn an additional reward in the start arm, if, after finishing the food in the goal arm it ran first to the non-goal and then to the start arm. This ensured that the animal visited the whole maze on each trial, giving each unit an equal opportunity to fire on all parts of the maze. Furthermore, it provided reward in more than one arm so that we could distinguish between responses which were due to approach to food and those which were due to the animal's position on the maze.

Microdrive Implantation

The rats were anaesthetized with ether and switched to halothane (Fluothane, ICI) and nitrous oxide/oxygen. The anaesthetized rat was fixed in a stereotactic frame with its skull flat between bregma and lambda. The skull was exposed and a 2 mm diameter hole trephined above the dorsal hippocampus with its centre 4 mm posterior to bregma and 2 mm lateral to the midline. Three large stainless steel screws (6 BA, $1/_4$") were threaded into tapped holes to serve as anchors for the dental cement. One of these screws also connected the rat to the system earth during recording.

A small lightweight perspex microdrive carrrying four independently movable microelectrodes was permanently fastened to the skull via the anchor screws (see Ainsworth and O'Keefe, 1977 for a description of the microdrive). The microelectrodes protruded from the base of the microdrive which was held in the micromanipulator of the stereotactic instrument. The microelectrodes were slowly driven through the intact dura and left to rest in the upper layers of the neocortex above the dorsal hippocampus. The exposed area around the microelectrodes between the base of the microdrive and the dura was flooded with flowable silicone rubber and covered with dental acrylic. The acrylic also permanently attached the base of the microdrive to the anchor screws. The microelectrodes had previously been lightly greased to prevent the silicone rubber from sticking to them. A single injection of Intramycetin (Parke-Davis) was given at the end of the operation.

Unit Recording and Data Collection

Electrical activity from two microelectrodes was led into a dual field-effect transistor amplifier, the first stage of which was directly mounted on the rat's head. After the signal was amplified and filtered to remove the slow wave EEG, it was fed into a window discriminator which was set to pick out the unit of interest. In order to display the relationship between the firing of a unit and the rat's position in the cue-controlled environment, we used a variation of a technique first developed by Marey (1894) and most recently used by Czopf, Karmos, Bauer and Grastyan (1964) to depict movement. In the Marey technique, the position of a bright spot or line fixed to a moving object such as a limb is periodically photographed on the same film. The resultant sequence of dots or lines represents the successive positions of the object over time. In our modification, we took the pulse from the window discriminator and fed it to a light-emitting diode on the rat's head. The spike-driven flashes were photographed on Polaroid film (Polaroid, Type 52) by a 4 x 5 camera mounted on the ceiling of the cue-controlled room. The camera shutter was left open throughout the trial. After each trial, a mask of the outline of the T-maze was placed on top of the maze and the film briefly exposed again. The resulting picture gave an accurate representation of the places on the maze where the unit fired[1]. Here we took advantage of the fact that the place units have little or no 'spontaneous' firing when the animal walks or runs outside the place field. It should be noted that it was necessary to use a relatively long voltage pulse (typically 25 msec) to produce a bright enough flash of the L.E.D. so that unit firings which occurred with an interspike interval of 25 msec or less were counted as one spike. In practice, this means that complex spikes are represented as one spike and occasionally the number of spikes shown in the place field is fewer than actually occurred.

The recording session had two stages. In the first, we located units in the dorsal hippocampus and searched for place fields in both environments. In the second stage we selected some of these units for further detailed study in the cue-controlled enclosure.

The first stage of a typical recording session proceeded as follows: while the rat sat quietly on the platform, a microelectrode was advanced into the CA1 field of the hippocampus in search of units with complex spike wave forms (see Ranck, 1973). Once a stable complex spike unit was isolated, the rat was coaxed or pushed around the platform and any changes in firing rate noted. Areas within which there was a large and consistent increase in firing were called the place field. As we noted above, the rat had not received any training on this platform nor had it ever been rewarded there. Therefore, if place fields were related to these factors, there should not be any on the platform or at the very least, there should be fewer on the platform than on the T-maze where the rat had been rewarded.

During the first stage, the rat was also given several trials on the place discrimination inside the cue-controlled enclosure. In some cases, the maze and environment were rotated at least once during these preliminary trials but often they were not. From the thirty-four units which were studied in this preliminary stage we selected twelve units with place fields inside the cue-controlled enclosure for further study in stage two.

In this second stage, we systematically tested whether the place field was related to the controlled-cues or to some other variable by running the animal on four 'ground' trials with the maze and controlled cues in four different positions relative to the external world. For units with place fields in the start arm of the T-maze at least two more trials were run. In these the start arm was rotated 180° relative to the crossbar of the T in order to test whether the place was related to the arm in which the animal was started or to a position in the environment.

After these ground trials were completed, additional probe trials were given, where possible, to analyze the role of each of the five cues within the cue-controlled enclosure (reward and wall cues). Three sets of probe trials were run: a. *no-food* trials in which the reward was removed, b. *no-wall-cues* trials in which all the wall cues were absent and the animal was rewarded for choosing either arm and c. *some-wall-cues* trials in which two or three of the four wall cues were present. Different cues were present on different trials so that over a series of 2 to 4 trials each cue had been omitted at least once. Because the light was the only source of illumination, instead of

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[1]   More recently we have combined this technique with the original Marey technique for depicting motion with good results. Two different coloured diodes are placed on the rat's head, one flashing at a fixed rate and representing the rat's position and the other showing the unit firing. Coloured Polaroid film is used instead of the black and white film described here
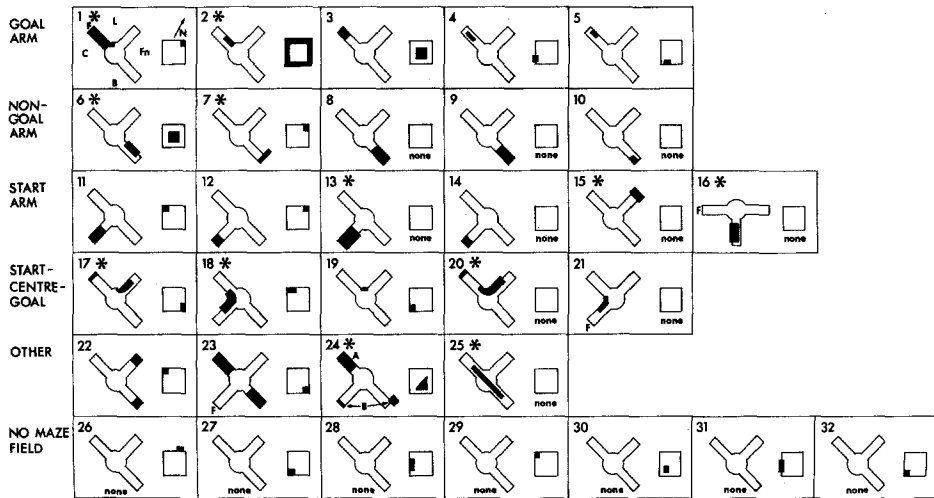
Fig. 2. Place fields on the T-maze inside the cue-controlled enclosure and on the small platform, grouped according to the maze fields. Each T-maze is oriented with respect to the controlled-cues, as shown for unit 1; each platform is oriented with respect to the external world. L = light; C = card; Fn = fan; B = buzzer; F = food; N = north. *units studied in stage two. Unit 24, **A** initial field, **B** additional fields which appeared during probe trials

turning it off when no light cue was intended, all four lights were turned on. For two units, there was some sign of a place field on the no-wall-cues probe, with food present. For these plus a third we ran the animal on an additional no-wall-cues probe without food.

On the basis of these probes we hoped to identify the cues responsible for generating the place field and in particular to find out whether single or multiple cues were responsible. Should more than one cue be involved, we might be able to isolate the contribution of each individual cue. Finally, with reduced cues the place fields might fractionate in ways which would provide clues to the underlying neural mechanisms.

Usually we gave only one probe of each type. There were several reasons for this. First, given a limited amount of time to study each unit, we wanted to examine all its properties rather than to be absolutely certain of any particular one of them. We hoped a) that the results of each individual probe would be unambiguous b) that the results of the different probes for a given unit would be consistent and c) that the results for the same probe given to different units would also be consistent, so that we could draw conclusions from the pattern as a whole.

A second reason for limiting the number of times each probe was given was the potential disturbance to the unit behaviour and to the animal's behaviour. In previous experiments (O'Keefe, 1976) we noted that although the place field of a place unit remained stable over repeated trials under invariant conditions, gross environmental changes such as rotating the maze relative to the environment or turning off the lights and changing the arms of the maze sometimes caused marked changes in the unit activity. In the present experiment, it was hoped that several different probes would prove less disruptive than several repeats of the same probe. In order to assess the disruption caused by each probe and to return the behaviour to normal if necessary, one or more trials with all cues present were given after each probe. In general, the potentially more disruptive probes such as the no-wall-cues probe were given later than the less disruptive ones. Only one unit showed any permanent changes as a result of the probes.

A unit was considered to have a place field in an arm if the number of spikes in that arm exceeded the number on the rest of the maze. For this computation the centre disc was divided into 4 quadrants and each quadrant assigned to the contiguous arm.

After recording was finished for the day, the electrode was usually retracted 175–350 μm above the pyramidal cell layer. On the final day of recording with a particular electrode it was left at its
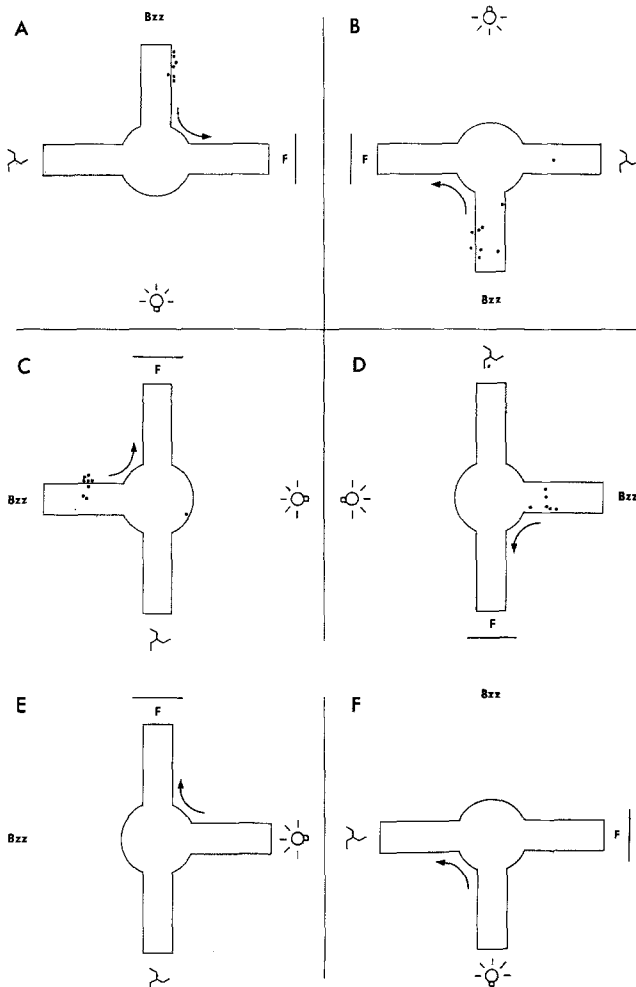
Fig. 3. Place unit (no. 16). Firing of unit on the T-maze inside the cue-controlled enclosure. Each dot represents one action potential. **A–D** show four ground trials in which T-maze and the cues on the wall have 4 different orientations relative to the external world. Unless otherwise stated, in this and all subsequent figures, all pictures have the same relationship to the external world. The arrows next to the maze show the direction the rat ran when it left the start arm. Note that this particular unit fires when the rat is in the start arm when it is on the side close to the buzzer regardless of the orientation relative to the external world. **E** and **F** show two ground trials with the start arm rotated 180° so that it is on the side close to the light. There is no unit firing in the start arm in spite of the fact that in F the rat makes the same body turn (incorrectly) as it had in **A–D**

deepest penetration. The resulting gliosis around the tip made the location of its position in the histological material relatively easy. During recording, the vertical position of the electrode was estimated from the spatial distribution of units, from the types of units recorded and from the various non-unit waveforms which are unique to the hippocampus (see O'Keefe, 1976).

On the day after the last recording session, the rat was killed by ether and perfused with 10% formalin in saline. The brain was frozen and cut into 50 μm sections which were stained with cresyl violet. All electrodes but one were found to be in the CA1 field of the dorsal hippocampus. The one exception was located at the border between CA1 and CA3.
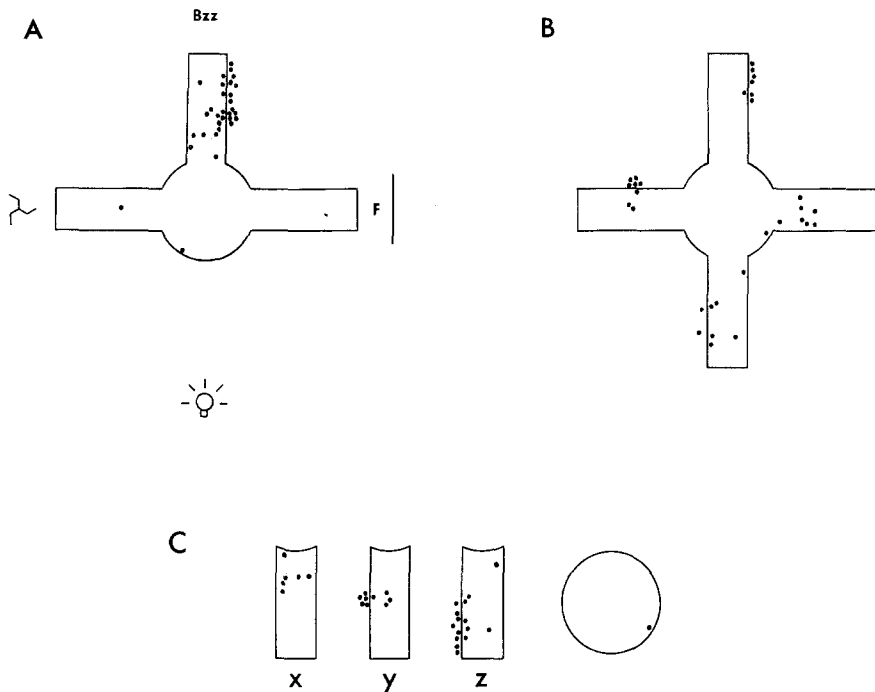
Fig. 4. Same unit as in Figure 3. Figure 3A–D superimposed in different ways. **A** pictures aligned with the same orientation to the controlled-cues on the wall. **B** pictures aligned with the same orientation to the external world. **C** physical components of the maze separated and superimposed. Note that the Z arm was used as the start arm *twice* while the other arms were used only *once* each

Results

Thirty-four units with low spontaneous firing rates and complex spike shape were recorded from the dorsal hippocampus in four rats. Seventeen units were recorded from one animal, 11, 4 and 2 from each of the others. Figure 2 shows the fields of these units on the T-maze inside the cue-controlled enclosure and on the platform outside. Note that in each case the crossbar of the T-maze has been oriented the same way with respect to the wall cues inside the cue controlled environment whereas the platform is oriented relative to the external environment.

Fifteen units had place fields on both the T-maze and the platform, 10 had a field on the T-maze alone, 7 on the platform alone, and 2 units did not have a field in either place.

Of the 25 units with fields on the T-maze, 16 had fields restricted to one arm or less. Another three (units 17, 20, 21) fired when the animal ran from the start to the goal. The fields are relatively evenly distributed amongst the centre, start arm and non-goal arm but the goal arm is over represented.

On the platform the fields are preferentially situated around the edge with only 4 of 21 units having fields in the centre. All four sides of the platform are well represented.

A no wall cues, no food   B no wall cues, no food

C no light, no fan   D no light, no card
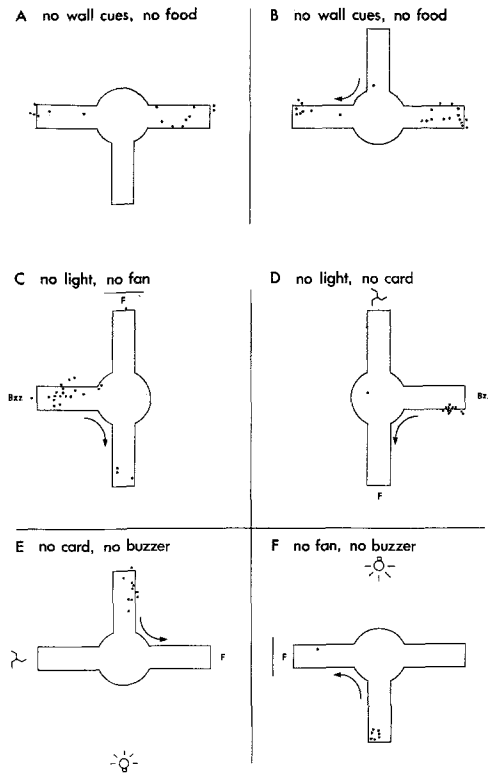
Fig. 5. Same unit as Figures 3 and 4. **A** and **B** two no-wall-cues probe trials in which both the wall cues and the food are absent. **C–F** four some-wall-cues probe trials in which only two of the four wall cues are present. **C** no light, no fan. **D** no light, no card. **E** no card, no buzzer. **F** no fan, no buzzer. Note that the place field is maintained in all these some-wall-cue trials but there is a marked increase in total spikes in **C**. The animal ran incorrectly to the right in **C**. The information about the animal's first choice in **A** is not available

E no card, no buzzer   F no fan, no buzzer

Fifteen units had place fields on both the T-maze and the platform. We could find no obvious topographic or size relationship between these place fields regardless of whether we considered the T-maze fields with respect to the wall cues or with respect to the external environment. For example, units with fields in the goal arm of the T-maze (Fig. 2, top row) might have a small field in one corner of the platform (units 1, 4, 5), a larger one in the middle (unit 3) or fire anywhere around the perimeter (unit 2). Conversely units with platform fields in one corner (e.g. north) might have fields in any arm of the T-maze (unit 1 goal arm; unit 7 non-goal arm; unit 12 start arm) or might have no field at all (unit 26).

Factors which Generate the Place Field

In the second stage of the experiment, twelve units with fields on the T-maze were selected for further study. Rotation of the maze and the controlled-cues between the trials would show whether the fields were related to these cues or to some uncontrolled feature of the environment; probe trials during which different cues and/or the food were omitted would explore their relative roles in generating the place field. The results of these trials will be illustrated by three
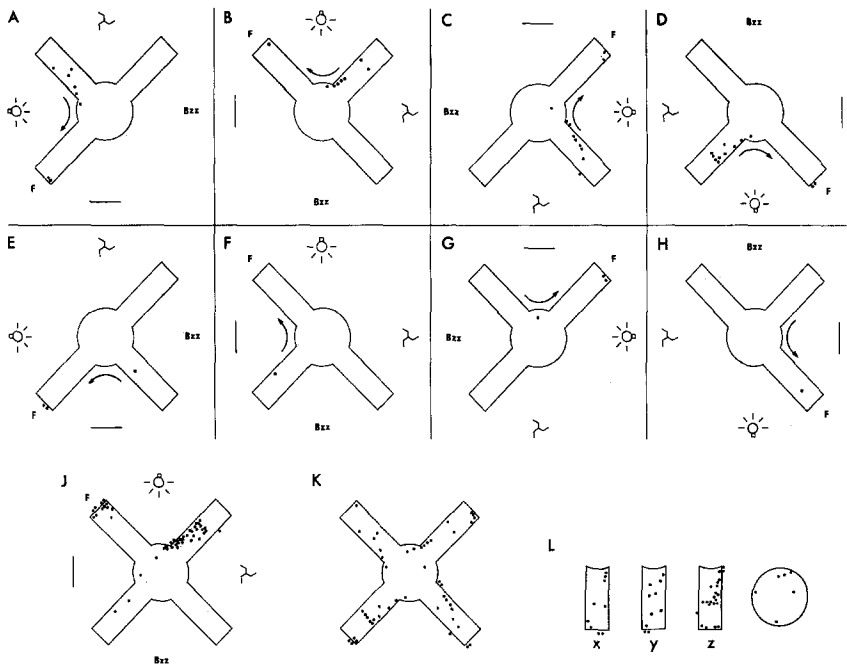
Fig. 6. Unit no. 17. Eight ground trials inside cue-controlled environment. A–D four ground trials in which start arm points between light and fan. Unit fires as rat runs from start to goal. E–H four ground trials in which start arm points between buzzer and card. Negligible unit firing. J A–H superimposed with respect to wall cues. K A–H superimposed with respect to external world. L physical components of the maze separated and superimposed. Arm Z used as start arm four times, arms x and y used twice each

units which have been selected as paradigm examples of the three major types of unit found in the CA1 dorsal hippocampus of the rat: a) Unit 16 (Fig. 2 and Figs. 3, 4, 5) is a simple place unit as described by O'Keefe (1976). This unit fired whenever the rat was in the end of the start arm when it was on the side close to the buzzer, both when the rat was first put down in the arm and when it returned there after running to the other arms. This is the only unit which we recorded with the maze arms square rather than on the diagonal. There was no place field with the arms in the usual diagonal position. b) The second unit (Fig. 2 unit 17, and Figs. 6 and 7) is a good example of Ranck's (1973) approach consummate units. It fired as the rat ran from the start to the goal and also during eating in the goal arm. O'Keefe (1976) suggested that these might also be place units. c) The third unit illustrated (Fig. 2 unit 1, and Fig. 8) is a misplace unit of O'Keefe (1976) and an approach-consummate-mismatch unit of Ranck (1973). It also fired as the rat ran from the start to the goal but occasionally fired when the animal ran across the place field on its way from the goal to the non-goal. On trials where there is no food in the goal arm there is an increase of firing as the animal sniffs around the goal area (see Fig. 8, F).
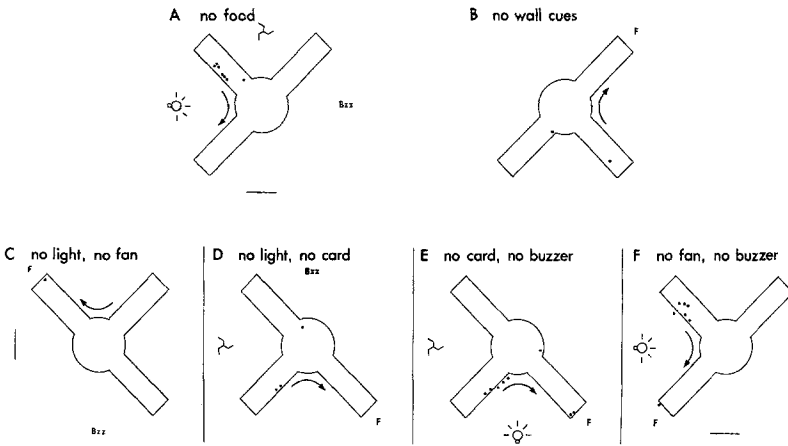
Fig. 7. Same unit as Figure 6. **A** No-food probe trial: unit fires in start arm. **B** no-wall-cues probe trial, food present: no place field. **C–F** four some-wall-cues probe trials, different pairs of cues have been removed on each trial. Note absence of place field in **B** and **C** and reduced firing in **D** despite the initial right turn choice on these trials

Place Fields are Related to the Controlled-Cues

The results of the ground trials show that for all twelve units tested the place fields were related to the controlled-cues inside the enclosure and not to other factors. Figure 3A–D illustrates four trials for the place unit, each with a different orientation relative to the external world. The constant relationship between the unit firing and the controlled spatial cues is made clearer in Figure 4 where the four pictures of Figure 3A–D are superimposed according to different rules. In Figure 4A they are all aligned with the same orientation to the controlled-cues and the external world is disregarded. The spikes superimpose in the start arm. In contrast, alignment of the four pictures with the same orientation to the external world and without regard to the controlled-cues (4B) shows no consistent relationship. Finally, superimposing the physical components of the maze from the four trials shows that there is no consistent firing in relation to the intra-maze olfactory or tactile cues (4C). A similar relationship to the controlled-cues can be seen in the other two units illustrated (Fig. 6A–D, J–L and Fig. 8A–E).

There are several different possible explanations for the fact that a unit fires in one part of the maze: I. it might be signalling the animal's position on the maze relative to one or more of the controlled spatial cues (wall cues and food) (place correlate); II. it might be identifying an arm of the maze as a component of the T configuration (e.g. the stem) regardless of its relationship to the controlled spatial cues (maze configuration correlate); III. it could be registering the fact that the rat has been placed down on the maze or that a certain time has passed since that event (event correlate); or finally IV. it might be signalling some motor aspect of the animal's behaviour such as the fact that it has made, is
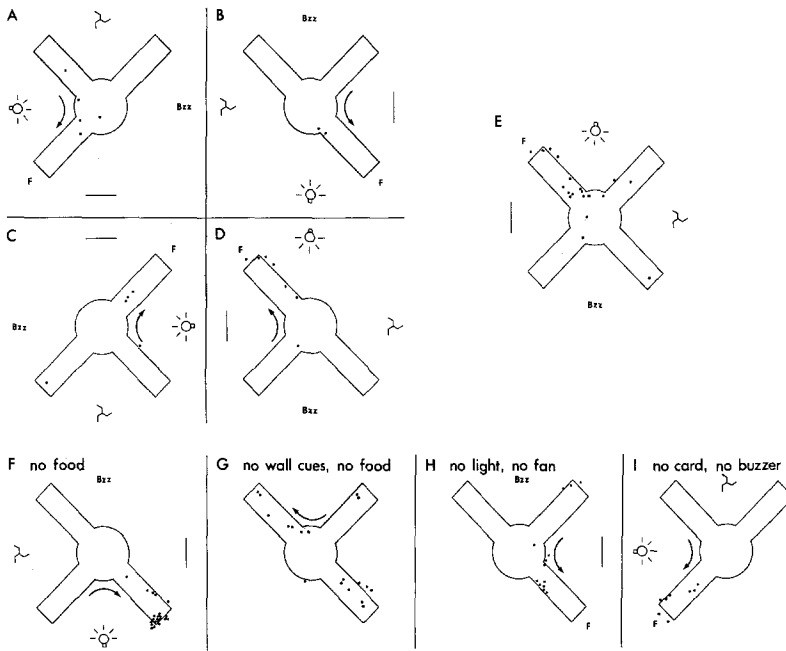
Fig. 8. Misplace unit (no. 1) **A–D** four ground trials, unit fires in goal arm. **E** A–D superimposed with respect to the wall cues. **F** no-food probe trial: unit maintains place field but increases firing around reward area. **G** no-wall-cues probe, food absent. **H** some-wall-cues probe trial: no light, no fan. Place field maintained but increased firing. **I** some-wall-cue probe trial: no card, no buzzer; place field maintained

making, or is about to make a left turn (motor behaviour correlate). The ground trials eliminate all of these possibilities except the first for the following reasons.

The important units for distinguishing between the place correlate on the one hand, and the maze configurations and event correlates on the other, are those with fields in the start arm or in the start and goal arm (units 13, 15, 16, 17, 18, 20). If such a unit is signalling the animal's position relative to the spatial cues, it should not fire on those trials in which the start arm is rotated 180° relative to the cross bar of the T and the controlled spatial cues. On the other hand, if the unit is signalling that the rat is in the stem of the T-maze regardless of its orientation relative to the spatial cues, or if the unit is responding to the arm in which the rat is put down at the beginning of the trial, then the unit should also fire in the start arm in its rotated position. None of the six start arm units tested in the second phase of the experiment fired significantly during those trials with the start arm rotated, averaging less than 1 spike per trial (18 trials) as compared with an average of 8 spikes per trial (24 trials) when the start arm was on the preferred side. Figures 3E, F and 6E–H show this absence of a place field in the rotated start arm. One unit which was not tested in the second phase of the experiment (no. 23) had a place field in the start arm on either side of the cross bar but since this unit was not tested with the maze and controlled-cues rotated,

we cannot rule out the possibility that it was responding to cues in the uncontrolled external environment. One can conclude then that for units with start arm fields tested in the second stage of the experiment, the unit firing in the start arm is not determined by a particular event such as being placed on the maze, or by the animal's position relative to the maze configuration.

The fourth alternative, that the unit firing is related to some aspect of the animal's motor behaviour, cannot be rejected on the basis of the absence of firing in the rotated start arm since the animal uses a different turn to reach the goal. If some such gross motor behaviour were responsible for the field one might expect these start arm units to fire in the non-goal arm or between the non-goal and start since the animal makes the same turn there as it originally did to get from the start to the goal. No such firing was seen in the six start arm units studied in the second stage. Furthermore on a small percentage of ground trials (3 of 40) the animal made a mistake, running to the non-goal arm on its first choice. Figure 3F shows an example of this. Here it is clear that the unit firing is appropriate to the place in the enclosure and not to the body turn which the rat makes. The same was found on the other two trials. Finally as we shall see in subsequent sections the pattern of unit activity during the probe trials also goes against the motor behaviour explanation.

Similar arguments can be used to reject the maze configuration and motor behaviour explanations for the firing patterns of units with fields in the goal or non-goal arms (Fig. 2: units 1, 2, 6, 7). For example, a goal arm unit such as no. 1 (Fig. 8) could not be responding to the configuration of the T-maze since the goal arm is on the left side of the crossbar on some trials and on the right on others. Similarly the goal is reached by a right turn on some trials and by a left on others, ruling out an explanation in terms of body turns. Unfortunately the possibility that the firing of these non-start arm units is correlated with an event such as approach to food cannot be rejected on the basis of the ground trials alone. However, as we will see in the next section, the evidence from the probe trials does enable us to rule out this explanation.

It might be argued that under motor behaviour alternative we have considered only gross aspects of the animal's motor behaviour such as right turns and that the unit firing might be correlated with other more subtle motor movements such as its speed of running, turning its eyes to the left to watch the light, or twitching its right ear. Correlations of this sort were looked for but not seen in the present (and previous) experiments. Nevertheless they cannot be completely ruled out.

No-Food Probes

For eight units, we tested the effect of a non-reward trial on the place field. During this trial, the rats ran to the goal arm and, finding it empty, sniffed around in it before going to the non-goal arm. None of the units tested lost their ability to discriminate the place field from the rest of the maze. Nor was there any appreciable change in the average number of spikes in the arm containing the place field: 11 spikes (average of 8 no-food trials) vs. 8 spikes (average of 24

ground trials). A closer look at the data however suggests that while four units showed no change, one (no. 2) decreased while three (nos. 1, 24, 25) increased firing in the place field. It is interesting that these latter three units had fields wholly or in part in the goal arm. Two of them (nos. 1, 24) showed increases during the myostatial sniffing which occurred in the goal arm in response to non-reward. Figure 8F illustrates this increase. These units are clearly identical to the misplace units of our previous paper and to Ranck's approach-consummate-mismatch units. As we shall see in the next section, they also increase firing during the removal of other controlled-cues.

No-Wall-Cues Probes

Eight units were recorded during trials in which no cues were present on the walls and the animal was rewarded for whichever arm it chose. For three of these units additional no wall-cues trials were run in which no food was given. Of the eight units tested with the food present, six lost the ability to discriminate the place field from the rest of the maze while two maintained their fields. Of these latter two, one (no. 15) lost its place field when the rat was tested without the food. Thus the place fields of seven of 8 units tested were shown to be dependent on the wall cues and/or the reward.

The final unit (no. 25) was not tested with both the wall cues and the food removed and it is possible that its firing patterns on the no-wall-cues probe may have been due to the presence of the food. Since it also maintained its field on the no-food probe, the food could not have been the only factor.

It is of considerable theoretical interest to examine how these units change their rates of firing during the no-wall-cues trials. Taken as a group, they show no significant change in the number of spikes in the arm containing the place field (8 spikes average of 32 ground trials vs. 7 spikes average of 9 no-wall-cues trials) nor in the number of spikes over the whole maze (12 spikes average of 32 ground trials vs. 15 spikes average of 9 no-wall-cues trials). As with the no-food probe trial results, a closer examination suggests that this average masks the fact that while five units showed no change or even decreased in overall number of spikes, three (nos. 1, 16, 25) actually doubled or more than doubled their total firing on the maze. Two of these units (nos. 1, 25) also increased their firing during the no-food trial suggesting that for some units, an increased firing is a consistent reaction to the removal of significant cues.

Figure 5A and B show the increase in firing in unit no. 16 on two no-wall-cues probe trials while Figure 8G shows the no-wall-cues probe for unit no. 1. An example of a unit which decreased its overall firing during this probe (no. 17) is shown in Figure 7B.

Some-Wall-Cues Probes

For eight units, we gave enough probe trials in which some of the cues were removed to be able to draw some conclusions about their relative importance in

determining the place field. For seven units, two stimuli at a time were removed while for the last unit, one stimulus was removed on each of 4 trials. The results show that for five of the 8 units, the place field remained intact in spite of the removal of any two cues. It should be noted that for one of these (no. 25), the field appeared to be maintained in the absence of any wall cues so it is not surprising that it should survive removal of any one of these.

For two other units (no. 2 and no. 17) the place field appeared to depend on one or two of the four cues. Unit no. 2 maintained its field if either the light or card was available but not if both were removed simultaneously. The firing in the no light, no fan probe was reduced suggesting that the card was not as strong a cue as the light and the fan might also be making a contribution. The influences on unit 17 are (Fig. 7C–F) also uncertain. It maintained a normal field on both probe trials where the light was one of the two remaining stimuli and it is clear that this cue is a major determinant of the unit firing. There may also be a small contribution from the fan (see Fig. 7D) but this is uncertain.

For the final unit (no. 24) the role of the wall cues is difficult to assess, since the field changed during testing.

Discussion

The results confirm our previous findings (O'Keefe and Dostrovsky, 1971; O'Keefe, 1976) that units in the dorsal hippocampus are active in some parts of an environment and not others. The present study asked two questions about these place units. First, does a particular place unit have a field in more than one environment and if so is there any relationship between its place fields in different environments? Second, what aspects of the environment are responsible for the unit firing in the place field?

As reported in our previous paper (O'Keefe, 1976) there was a reasonably even distribution of fields on the T-maze and on the edges of the small platform. The paucity of fields in the centre of the small platform might indicate that the edge of the platform was an important cue for the place units in this situation. In unpublished experiments on a similar platform, we have noticed that many of the place units with fields at the edge lose their field if the edge is covered with a hard flat surface.

The finding that fifteen of 34 units had place fields on both the small platform and on the T-maze suggests that a substantial percentage of place cells can participate in the representation of more than one environment. Another way of looking at this is that the map of each environment involves a large number of cells in CA1 (and perhaps other fields) of the hippocampus, many of which participate in the maps of other environments. This would account for the relatively large percentage of CA1 hippocampal units which are found to have a place field in any given environment.

In the present study, we found no obvious topographic relationship between the place fields in the two environments. In our previous experiment we reported that place units recorded next to each other in the hippocampus were as likely to have fields in different parts of the same environment as to have

contiguous fields. Taken together these findings suggest that the hippocampal map of an environment is not spatially isomorphic to that environment or to maps of other environments. We must look elsewhere for the rules by which maps are constructed. There are several possibilities. First, it might be that each place cell responds to a particular set of sensory cues but that these same cues identify different places in different environments. Since we could only investigate the cues controlling the place field in one of the two environments, the present study throws no light on this possibility. Another possibility is that the cues which determine the place fields are different in the two environments and that the determination of where a place unit will have its field depends on some other mechanism such as the order in which the animal first explores an environment. The last possibility is that the physical shape of an enclosure is a major determinant of the way in which the place fields are distributed. Recall that the fields on the spatially extended and differentiated T-maze were much more equitably distributed than on the platform where they were disproportionately concentrated around the edge. The answer to the relationships between the place fields in different environments must await a study in which they are recorded in two cue-controlled environments.

The second question asked was what determines the location of a place field in a particular environment, in this case in the cue-controlled environment? There are two possible anwers to this question. On the one hand, there might be some environmental influence, either a stimulus or stimuli to which the unit responds or, as we have previously suggested, cues which provide information that the unit can use to 'calculate' the rat's location. On the other hand, the preferential unit firing in a part of the environment might be due to something the animal does there. We have previously argued against this possibility since many units continue to fire in the place field regardless of what the rat does there (walk, run, rear, sniff etc.) while other units, which fire best when a rat performs a particular behaviour (e.g. myostatial sniffing) in its place field, do not fire when the identical behaviour is performed in a different part of the environment. In the present experiment, we attempted to control for the role of the more obvious aspects of the animal's behaviour by requiring it to make different turns to reach the goal on different trials and by making it repeat the same turn to approach reward at two different points of the maze. Every effort was made to get the animal to traverse the whole maze at the same speed to exclude this factor. For units with place fields outside the start arm, the body turns which the animal made during the trial had no influence on the place field. For units with place fields in the start arm, the animal usually makes the same turn to reach the goal during the trials when the unit fires. There are several observations which would tend to rule out a role for motor behaviours in the firing of these units. First, the animal makes the same turn to get from the non-goal arm back to the start arm as it did to get from the start arm to the goal arm and none of the units with place fields in the start arm fired during this second turn. Second, on a small number of trials there was a dissociation between the spatial cues in the start arm and the body turn which the animal subsequently made. For example, on three of 40 ground trials with all cues present, the rat made a mistake and went first to the non-goal arm. On these

three trials the unit response was appropriate to the place as defined by the spatial cues and not to the body turn. Conversely if the rat made the usual body turn in the absence of the spatial cues, the unit did not show the usual firing. On three of 10 no-wall-cues trials (where either turn is "correct"), the animal made the same turn as it usually did from the start arm in the preferred position. Now, however, there was no selective firing in the start arm. Figure 7B shows a good example of this.

We can conclude then that it is unlikely that the body turn which an animal makes on the maze is an important factor in the unit firing on the place field.

What environmental cues might be determining, directly or indirectly, the place field of these hippocampal units? The finding that all twelve units tested had place fields which maintained a constant spatial relationship to the wall cues and not to the external environment enables us to make the positive statement that the place fields can be determined by cues such as lights, sounds, and feels, and are not necessarily dependent on distal cues fixed to the earth's axis such as geomagnetism. Of course, we cannot rule out these latter influences since they may have been overshadowed by the wall cues in our cue-controlled situation.

In attempting to analyse which of the cues within the cue-controlled environment were responsible for the place fields, we choose three units to illustrate the different types of response to the probes: a simple place unit (no.16), a place unit or approach-consummate unit of Ranck (1973) which fired when the animal ran from the start to the goal (no. 7), and a misplace unit or approach-consummate mismatch unit of Ranck (1973) which fired when the animal ran to the goal arm and increased its rate during the exploratory sniffing which occurs when reward is omitted (no. 1). The present results suggest that there are two fundamentally different classes of CA1 complex spike unit. The pattern of unit firing of both classes is dependent on the controlled-cues inside the curtained enclosure. One class two of 7 units including unit no. 7) was found to depend on only one or two of the wall cues and in general to decrease firing when these were removed. These units appear to have relatively limited excitatory environmental influences upon them.

The place unit (no. 16) and the misplace unit (no. 1) exemplified a different type of reaction to the removal of cues. Unlike the units in the first class, these units maintained their place fields intact when any two of the 4 cues were present. There was a discernable tendency to increase firing during some of these some-wall-cues trials. When all the controlled-cues were removed, these units actually doubled the number of spikes on the maze, firing in previously silent areas. The place fields of this second class of place unit appeared to be the residual excitatory patches which escaped inhibitory environmental influences.

Two of the units in this second class also increased firing during the exploratory sniffing associated with the omission of expected reward. Both had place fields which included the reward area. This suggests that the misplace units are actually units of the second class whose place field includes the reward area. From this perspective, the reward functions in the same way as any other cue.

We have previously suggested that the increased firing of misplace units might act as a signal for exploration and for changing the map of that environment. The present results indicate that in addition to the absence of

expected reward or the presence of a novel object in the place field, the removal of a distal cue can also result in this increased firing.

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