HIPPOCAMPUS, VOL. 4, NO. 4, PAGES 410–421, AUGUST 1994

Spatial Information Content and Reliability of Hippocampal CA1 Neurons: Effects of Visual Input

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ABSTRACT

The effects of darkness on quantitative spatial firing characteristics of 235 hippocampal CA1 "complex spike" (CS) cells were studied in young and old Fischer-344 rats during food-motivated performance of a randomized, forced-choice task on an eight-arm radial maze. The room lights were turned on or off on alternate blocks of all eight arms.

In the dark, a lower proportion of CS cells had "place fields," and the fields were less specific and less reliable than in the light. A small number of cells had place fields unique to the dark condition. Like CS cells, Theta cells showed a reduction in spatially related firing in the dark. The specificity and reliability of the place fields under both light and dark conditions were similar for both age groups. Increasing the salience of the environment, by increasing the light level and the number of visual cues in the light condition, did not affect the specificity or reliability of the place fields.

Even though all rats had substantial prior experience with the environment, and were placed on the maze center under normal illumination before the first dark trial, the correlation between the firing pattern in the light and dark increased after the rat first traversed the maze in the light. Thus, even after considerable experience with the environment over days, experiencing the illuminated environment from different locations on a given day was a significant factor affecting subsequent location and reliability of place fields in darkness.

While the task was simple and errors rare, rats that made fewer errors (i.e., re-entries into the previously visited arm) also had more reliable place cells, but no such correlation was found with place cell specificity. Thus, the reliability of spatial firing in the hippocampus may be more important for spatial navigation than the size of the place fields per se. Alternatively, both spatial memory and place field reliability may be modulated by a common variable, such as attention.

Key words: place cells, place fields, spatial orientation, aging

INTRODUCTION

An intact hippocampal formation is necessary for effective spatial learning in rodents. This has been shown in numerous lesion studies (e.g., see O'Keefe and Nadel, 1978; Sutherland et al., 1982; Barnes, 1988; Nadel, 1991; Jarrard, 1993).

Perhaps the strongest evidence for a link between hippocampal function and spatial encoding comes from single unit recording. O'Keefe and Dostrovsky (1971) first showed that, in freely moving rats, hippocampal cells exhibit a spatially selective activity pattern, firing only when the animal is in certain locations (place fields) in the environment. It has been shown that some place fields persist when part or all of the spatially polarizing objects in the environment are removed in the animal's presence (O'Keefe and Conway, 1978; O'Keefe and Speakman, 1987), or when the cells are recorded in the dark (O'Keefe, 1976; McNaughton et al., 1989b; Quirk et al., 1990). It is less clear whether there is any change in the characteristics of those place fields that persist. The problem with focusing on whether a place field persists is that one needs to set criteria for the existence of a field, which leads to a dichotomous approach. Thus the "loss of a place field" under certain conditions may reflect either a degradation in the place field or else a complete elimination of spatially selective firing. Similarly, a "persistent place field" may show changes that are undetected as long as the field remains within the set criteria. It is, however, important to determine whether a reduction in sensory information causes a parallel change in the quality of the place field or whether place fields are robust, showing no change with a partial loss of sen-

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sory information and disappearing completely once a critical amount of sensory information is lost.

In the present study a number of spatial firing characteristics were examined in alternating light and dark trials on a simple task in which the animals traversed an eight-arm maze. Rather than trying to quantify place fields with a single metric and inevitably omitting the quantification of some characteristics, several different and at least partially independent measures were used in combination to describe different place field properties. Place fields were examined in terms of how similar the overall spatial firing pattern of a cell remains over time (reliability); how well does the fact that we know the cell fired predict the location of the animal (spatial information content); given that we know the animal's location, how well does the fact that the cell fired predict the direction the animal was facing (direction information content); how spatially compact is the place field (sparsity); and finally, what is the signal-to-noise ratio, or how selective is the cell in its firing (selectivity).

In addition, because Barnes et al. (1983) had observed that place fields were less specific and less reliable in old than in young rats under lighted conditions, we compared young and old rats under the more mnemonically demanding situation of darkness. Some of these data have been reported in abstract form (Markus et al., 1992).

MATERIALS AND METHODS

Subjects and behavioral task

Five young and seven old (see Table 1) male Fischer-344 rats (Charles River Breeding Laboratories) were food deprived to 80% of their *ad libitum* weights and trained to perform a forced-choice task on an eight-arm radial maze for a chocolate milk reward (Barnes et al., 1983). A forced-choice trial consisted of one visit, in a random order, to each of the eight maze arms (58 × 5.7 cm). Once all arms had been visited, the rat would return to a small platform at the center of the maze and rest while the maze arms were re-baited. Access to the arms was controlled by motor-driven drawbridges, operated by the experimenter, so that at any given time only the arm the rat was currently on (obtaining its reward) and the next baited arm were accessible. On occasion, rats returned to the arm just visited, rather than proceeding down the next baited arm; this was counted as an error. All rats were continuously screened for signs of illness, and, if the animal exhibited serious health problems or showed behavioral disruptions on the maze, the data were not included in the analysis.

Surgery and recording

Two slots were drilled in the skull bilaterally over the dorsal hippocampus under Nembutal anesthesia (33 mg/kg in young, 21 mg/kg in old). A miniature microdrive (McNaughton et al., 1989a) containing two stereotrodes (McNaughton et al., 1983), was mounted on each side of the skull with dental acrylic. The stereotrodes were implanted approximately 1 mm into the cortex, with the anterior stereotrodes placed at 3.3 mm posterior and 2.2 mm lateral to Bregma, and the posterior stereotrodes at 4.6 mm posterior and 3.0 mm lateral to Bregma (Paxinos and Watson, 1982). The stereotrodes were comprised of two, twisted, lacquer-coated 20 μm tungsten wires, cemented together with Epoxylite. Each stereotrode was cemented into a 30 gauge guide cannula and cut with sharp scissors so that approximately 3 mm of wire protruded from the end of the cannula. Prior to implantation, the electrode tip impedance was 300–700 kΩ. During recording, the animal wore a five-channel FET source-follower headstage with two arrays of infrared light-emitting diodes mounted on it. One array was positioned above the rat's head, while the second, smaller array was mounted 14 cm apart from the first, so that, when the animal's head was in line with his body (facing forward), the back array was centered above his hindlimbs. This arrangement allowed a resolution of head direction of about 7°, although the subsequent data analysis used directional bins of 45°. The position coordinates of both diodes were sampled at 20 Hz with an overhead video tracking system, providing data both on the rat's location as well as his head direction. Neuronal electrical signals were amplified between 5,000 and 10,000 times, filtered between 600 Hz and 6 kHz, and sampled concurrently with position information via a Brainwave 80386 acquisition system.

Histology

After the last recording session, the rats were deeply anesthetized with Nembutal and perfused with a 10% formal-sa-

Table 1. Age of Rats During Recording Sessions, and Number of Theta and Complex Spikes Cells That Contributed to the Data for Each Individual Animal

Animal No.	Group	Theta cells	Complex spikes cells	Recording age (months)
3120	Young	0	23	13–15
3161	Young	15	44	10.5–12
3200	Young	0	4	10.5–11
3258	Young	9	35	10–13
3259	Young	3	2	11–11.5
3115	Old	4	10	26–28
3196	Old	4	8	25.5–26
3197	Old	8	10	25–25.5
3261	Old	5	18	23–24.5
3262	Old	9	32	23.5–25
3324	Old	1	14	24–24.5
3325	Old	3	35	24–25.5

line solution. The electrodes were withdrawn and the brain was removed from the skull and placed in the formal-saline solution for at least 24 hours, after which they were allowed to sink in a formal-saline-30% sucrose solution. Thick coronal sections (40 μm) were cut and stained with cresyl violet and the electrode tracks were identified.

Procedure

Prior to surgery the animals were trained to run ten consecutive, forced-choice trials on the eight-arm radial maze in a training room. Following surgery the rats were retrained to run ten trials on an identical maze in the recording room and the electrodes were advanced to CA1. During the process of advancing the electrodes and isolating single units, the rat was placed on a small, raised platform (25 × 5.5 cm) outside the recording room. Within the hippocampus, the electrode was advanced in 10–20 μm steps and, after a recording session was completed, the electrode was usually advanced 10 μm before returning the rats to the colony room overnight. Once discrete units were located and allowed to stabilize, they were recorded while the rat completed eight to ten forced-choice trials on the maze. The maze was centered in a sound-attenuating, light-proof room (389 × 397 cm) in which the only light sources were spotlights illuminating three objects (a large white sphere and two cue cards). The objects were 2–3 m distant from the maze edge and covered about 10–30° of visual angle depending on the rat's location on the maze. At the start of the recording session, the rat was confined to the center of the maze for a few minutes with the lights on before the first trial. Subsequently, alternate trials were conducted in light or darkness, with the order of light condition on the first trial balanced across sessions. During the dark trials the room was completely dark except for a small red light worn by the experimenter. The light's wavelength was over 600 nm with an intensity of under 0.35 lux, which is beyond the rat's spectral sensitivity (Silver, 1967). To dissociate the recording room from the animal's global spatial reference framework, the rat was disoriented during transport into and out of the room in a closed box which was rotated while in transit. In addition, the experimenter carried a noise generator throughout the recording session, masking auditory stimuli that could provide orientation information, and the maze was rotated 180° on half of the recording days to reduce the use of local cues.

Data analysis

The reliability of the spatial firing pattern (place field) was assessed by dividing each of the eight maze arms into four radial bins, each of which was further divided into an inward and outward bin depending on the animal's head orientation. For each trial, the firing rate of the cell in each bin was calculated and then standardized with a Z-transformation based on the firing rates in the corresponding bins at the same radial distance and head orientation on all maze arms for that trial. The use of standardized rates enabled those effects that were common to all arms to be factored out (e.g., changes in velocity, stopping to drink), leaving only the firing that was unique to a specific maze arm (i.e., location). The correlation coefficient between the standardized firing rates for each pair of trials was measured, and an overall mean correlation coefficient was calculated between all the "light" trials alone, all the "dark" trials alone, and between the light and dark trials. It should be noted that these correlations reflect the consistency in firing from trial to trial and not from session to session. The correlations are thus expected to be considerably smaller than similar measures based on average firing distributions across multiple entries into each location.

The second approach to quantifying place fields was to measure the specificity of the field in terms of the information content of cell discharge (Skaggs et al., 1993). For this analysis, an 8 × 8 square grid was superimposed onto the eight-arm maze, resulting in a similar number of bins per maze arm as the reliability index, but without regard to directionality. The specificity index examines the amount of information (in bits) that a single spike conveys about the animal's location (i.e., how well cell firing predicts the animal's location). The spatial information content of cell discharge was calculated using the formula:

$$\textit{information content} = \Sigma P_i(R_i/R)\log_2(R_i/R)$$

where i is the bin number, P_i is the probability for occupancy of bin i, R_i is the mean firing rate for bin i, and R is the overall mean firing rate.

In order to account for the effects of low firing rates (with fewer spikes there is a tendency toward higher information content) or random bursts of firing, the spike firing time-series was randomly offset in time from the rat location time-series, and the information content was calculated. A distribution of the information content based on 100 such random shifts was obtained and was used to compute a standardized score (Z-score) of information content for that cell. While the distribution is not composed of independent samples, it was nominally normally distributed, and a Z value of 2.29 was chosen as a cut-off for significance (the equivalent of a one-tailed t-test with $P = 0.01$ under a normal distribution). A similar measure was computed for the amount of information a single spike conveys about the animal's head orientation given the location information. The sparsity of the spatial firing distribution was measured, indicating the relative proportion of the maze on which the cell fired, using the formula:

$$\textit{sparsity} = \Sigma(P_i{*}R_i^2)/R^2$$

where P_i is the probability of occupancy of bin i, R_i is the mean firing rate in bin i, and R is the overall mean firing rate. (For example, a sparsity score of 0.10 indicates that the cell fired on 10% of the maze surface.)

The selectivity of firing in the field (ratio of maximum signal to noise) was calculated by dividing the firing rate of the cell in the bin with the maximum average rate by its mean firing rate across the maze.

In addition to the above spatially related measures, the correlation between firing rate and animal velocity was calculated with a corresponding Z value (based on 100 random time-offsets, see above). The mean spike amplitude, width (peak to valley), and firing rates of the cells were also obtained.

RESULTS

Only cells from CA1 were analyzed, based on the histology and/or physiological recording landmarks. (Histological analysis was not available for one animal.) A total of 235 complex

spike and 61 theta cells were analyzed from 12 animals (see Table 1). An effort was made to reduce duplicate recordings of the same cell in more than one session, by advancing the electrodes after each recording session by at least 10 μm and not including in the analysis recording sessions in which the cells and their place fields seemed similar to the previous recording. It should be noted that the criterion for recording cells was based purely on their signal-to-noise ratio (>2:1); thus a proportion of "units" may include two or more cells with similar wave-forms (a problem that is alleviated but not eliminated by the use of a stereotrode), or cells without place fields on the maze.

Two general types of cells were recorded, low-rate cells with a long spike duration and short-duration high-rate cells (see Fig. 1). The two cell populations correspond to Ranck's (1973) complex spike and theta cells. Complex spike cells were defined as having a spike duration (measured from maximum to minimum voltage) of at least 350 μs, and a mean firing rate of 0.03–2.5 Hz (the lowest rate cell with a significant field fired at 0.03 Hz, and this was used as the minimum firing rate for complex spike cells). Theta cells were defined by spike durations of less than 350 μs and mean firing rates greater than 2.5 Hz. Thirty-two cells whose firing characteristics did not fit these criteria for complex spike or theta cells were not included in the analysis (see Fig. 1).

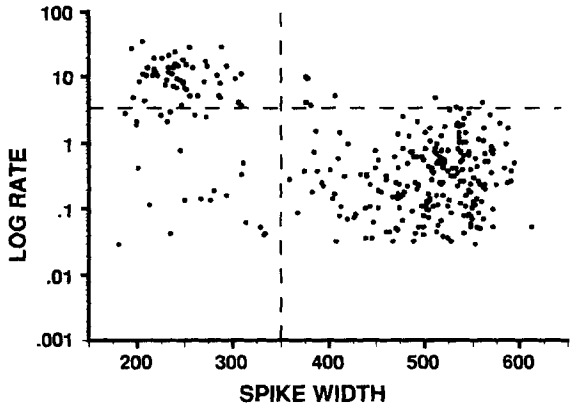

Fig. 1. Scatterplot of cell firing rate versus cell spike width (μs). Note that there are two clusters of cells, narrow high-rate theta cells, and wide lower-rate complex spike cells. Dashed lines denote the acceptance criteria for the two categories.

Behavior

Even though the behavioral task was fairly simple, the rats would nevertheless, on occasion, go down the wrong arm (i.e., return to the arm just visited). These occurrences were rare, usually occurring only once or twice per recording session. Both age groups of rats made more errors when performing the task in the dark ($P < 0.01$), and while not significant, there was a tendency for old rats to make more errors than the young (Fig. 2A). Both age groups ran faster in the dark (paired t-test, $P < 0.01$), and the young rats ran faster than old rats ($P < 0.01$; Fig. 2B). No relationship was found between mean velocity and any measures of reliability or spatial characteristics of the place fields (light condition regression on velocity all $P > 0.10$).

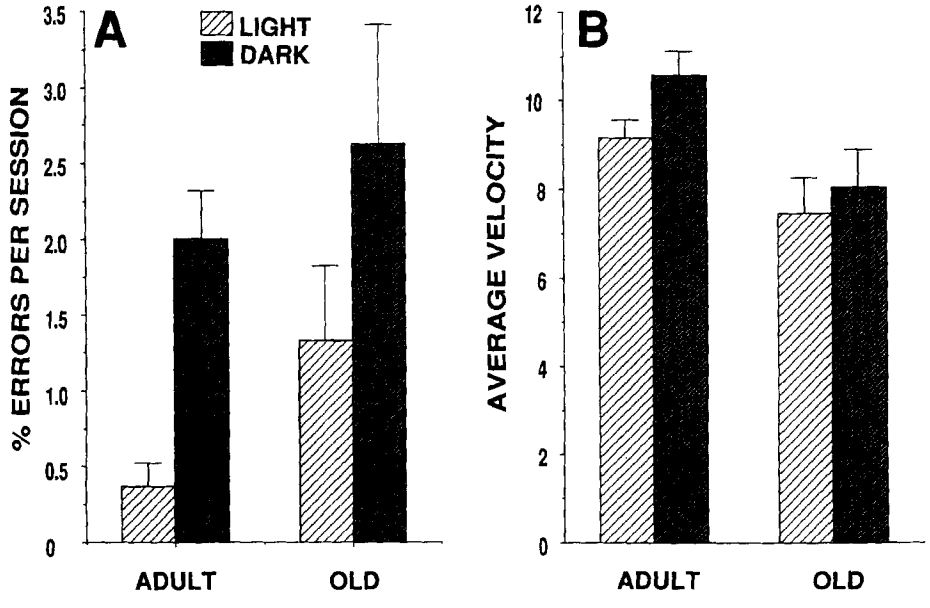

Fig. 2. **A:** The mean (and SEM) percent of errors made in the light and dark conditions by the young and old rats. An error was defined as returning to the arm that the animal had just visited. Both age groups made more errors in the dark than in the light condition ($P < 0.01$). **B:** The mean (and SEM) linear velocity of the rats by age and light condition. Both age groups ran faster in the dark (paired t-test, $P < 0.01$) and the young ran faster than the old rats ($P < 0.01$).

Complex spike cells (place cells)

A comparison, by animal, of the young and old rats showed no effect of age on the amplitude, width, or firing rate of CS cells. Similarly no age differences were found in the reliability and spatial characteristics of the place fields, or in the effects of the dark on these measures (Table 2). Consequently the data from both age groups were pooled, and the firing characteristics of each cell were compared in the light and dark conditions.

Cells were considered to have place fields if their spatial information content was significant ($Z > 2.29$, see Materials and Methods). A comparison of place fields in the light and dark showed that some fields were unchanged, others existed only in one of the two lighting conditions, and other place fields became coarser in the dark (see Fig. 3). A higher proportion of the CS cells had place fields in the light than in the dark ($P < 0.01$; Fig. 4A,B), and the firing pattern was more reliable in the light ($P < 0.01$; Fig. 4C,D). Of the 87 cells with significant place fields, 29 cells had fields in both light and dark, 45 cells had fields only in the light, and 13 cells had fields only in the dark.

Those cells with place fields in both the light and dark were examined further. Because the same cells were recorded in the light and in the dark, the difference in place field characteristics between these two conditions could be examined (Fig. 5). While there was a correlation between the quality of the fields in the light and dark, a two-tailed paired *t*-test showed significant reductions on measures of CS cell specificity and reliability in the dark (information for place, $P < .03$; sparsity, $P < .01$; selectivity, $P < .01$; reliability $P < .01$). There was no significant effect of the dark on place field directionality or on mean firing rate ($P > .10$).

The relationship between the location of the place fields in the light and dark was examined further by comparing the correlation of the firing pattern across trials of the same type to the correlation between the light and dark trials. All cells with significant place fields in the light were examined for their spatial firing pattern in the dark. Approximately one-third of the cells had a similar firing pattern in the dark, one-third showed no correlation between light and dark, and the remainder showed a partial correlation between the light and dark conditions (Fig. 6A). Conversely, almost all cells with significant fields in the dark had similar fields in the light (Fig. 6B).

Darkness is an extreme example of a reduction in the salience of the visual environment. Two young and four old rats were additionally tested in a "very salient visual environment," in order to assess the effects of increasing the salience of the environment. In these very salient visual environment sessions, the procedure was the same (alternating dark-light

Table 2. Mean and Standard Error of the Mean for Complex Spike and Theta Cell Waveform Characteristics, and Place Field Indices for Young and Old Rats in the "Light" and "Dark" Conditions

	Complex spike cells		Theta cells	
	Young (N = 5)	Old (N = 7)	Young (N = 3)	Old (N = 7)
Amplitude (mV)	108 (2.8)	106 (3.4)	91.7 (6.2)	85.2 (5.9)
Width (ms)	521 (14)	501 (10)	246 (1.2)	238 (7.2)
Firing rate (Hz)				
Light	.56 (.07)	.50 (.08)	12.5 (2.5)	12.0 (5.2)
Dark	.56 (.10)	.50 (.07)	12.9 (2.4)	11.4 (1.5)
% Cells with fields				
Light	34 (17)	42 (7)		
Dark	20 (7)	23 (5)		
Correlation of firing rate to velocity				
Light			.11 (.02)	.10 (.02)
Dark			.10 (.02)	.10 (.01)
Cells with fields				
Place information[a]				
Light	1.09 (.07)	1.54 (.32)		
Dark	1.24 (.09)	1.38 (.15)		
Reliability[b]				
Light	.215 (.04)	.191 (.03)	.15 (.06)	.10 (.03)
Dark	.112 (.05)	.190 (.04)	.04 (.02)	.10 (.01)
Light-dark	.093 (.03)	.066 (.02)	.00 (.01)	.08 (.07)
Directionality[c]				
Light	.324 (.05)	.373 (.05)		
Dark	.256 (.08)	.375 (.09)		
Selectivity[d]				
Light	10.7 (1.8)	15.1 (3.7)		
Dark	10.5 (3.1)	10.7 (2.2)		

[a]Information (in bits) a single spike conveys about animal's location.
[b]Mean correlation coefficient of standardized firing patterns between pairs of trials.
[c]Additional information (in bits) a single spike conveys about animal's head orientation given the place information.
[d]Maximal firing rate/mean firing rate.

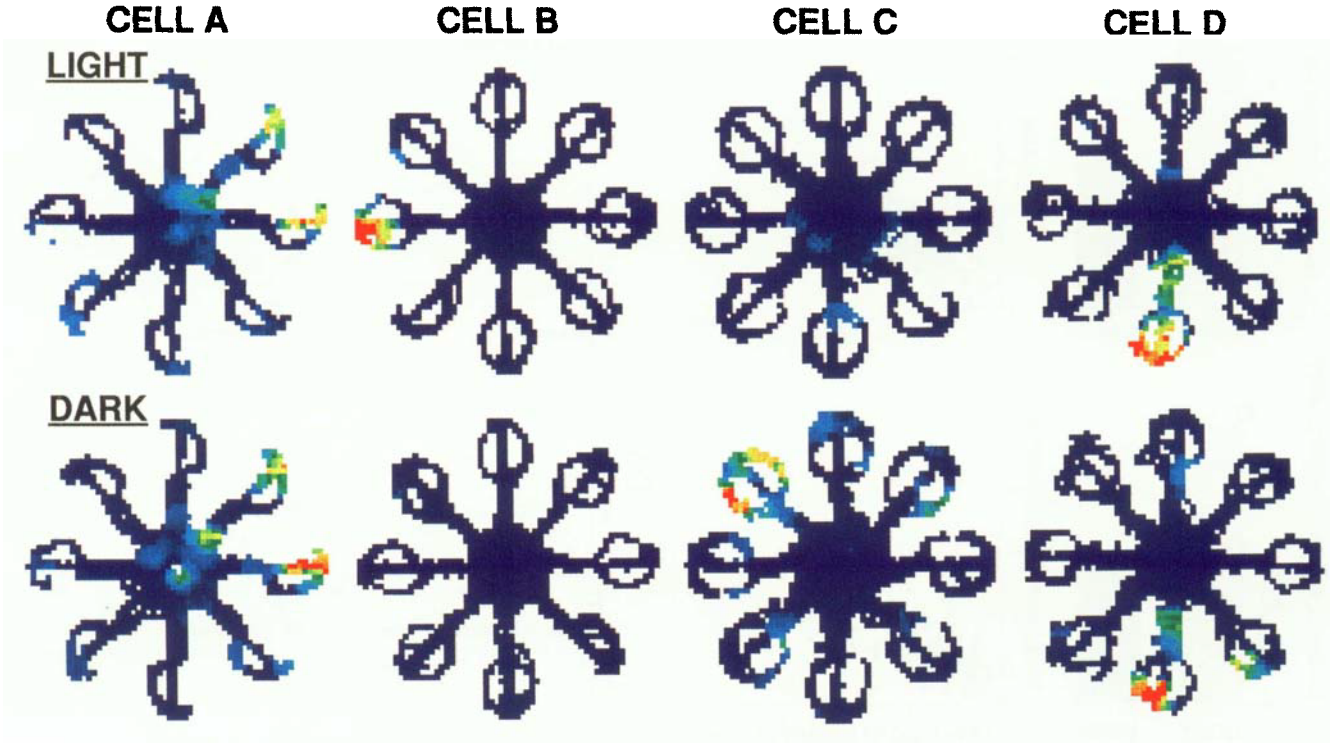

Fig. 3. Examples of firing rates of place cells in the light and dark. **A:** Place field maintained in both light and dark; red represents firing rate > 2.6 Hz. **B:** Place field only in the light; red represents firing rate > 7.9 Hz. **C:** Place field only in the dark; red represents firing rate > 3.2 Hz. **D:** A place field that became less specific in the dark; red represents firing rate > 7.1 Hz. The characteristics of the place fields are as follows:

Cell	Place Info.	Place Z-value	Sparsity	Selectivity	Reliability
(A) Light	1.99	5.15	0.16	12.1	0.28
Dark	2.10	5.36	0.15	12.8	0.31
(B) Light	3.20	6.54	0.08	19.3	0.15
Dark	1.74	0.76	0.18	18.7	0.05
(C) Light	2.60	0.64	0.12	24.5	−0.01
Dark	1.92	2.44	0.16	17.9	0.09
(D) Light	2.78	5.27	0.13	15.7	0.73
Dark	1.66	2.90	0.19	12.2	0.37

trials); however, a large white curtain that covered a 90° arc with a black vertical stripe (15° arc) was added to the room. In addition, an extra light source was used that increased the illumination of the room from 1.4 lux to 9.1 lux. A comparison (for each group n = 159 cells) was made of the data from the lighted trials of the very salient recording sessions, to the lighted trials on the standard room configuration in these same animals. There were no age or salience effects on firing rates, the proportion of cells with place fields, their specificity, reliability, selectivity, or directionality ($P > 0.10$).

Behavior and the quality of place fields

No relationship was found between the number of errors exhibited by an animal and the reliability or spatial characteristics of its place fields on a given trial. Similarly, for sessions in the dark, no correlation was found between the amount of time it took the rat to reach the location of the place field and the quality of the field. On the other hand, the mean number of errors for each animal was significantly correlated ($r^2 = 0.55$, $P < 0.01$) with the average reliability of its place fields (Fig. 7). This was true, even when age, mean running velocity, and mean firing rate were taken into account (multiple $r^2 = 0.65$, $P < 0.05$). The same trend was present in the light and dark data considered separately; however, it was not statistically significant in the dark, possibly because there were fewer cells. The mean number of errors for each animal was not correlated with the measures of place field spatial characteristics (place information, sparsity, and selectivity in multiple regression: $P > 0.10$).

Trial sequence and the quality of place fields

An examination of 19 cells with similar firing patterns in the light and dark (overall correlation between light and dark, r > 0.3), showed a difference in firing pattern between the first and second trial in the dark (Fig. 8). There were two types of sessions, "initial trial dark sessions" and "initial trial light sessions" (half the recording sessions started with a light trial and the other half with a dark trial). Consequently, the first dark trial of a recording session was either the first or second trial of the session. A *t*-test showed that the standardized correlation

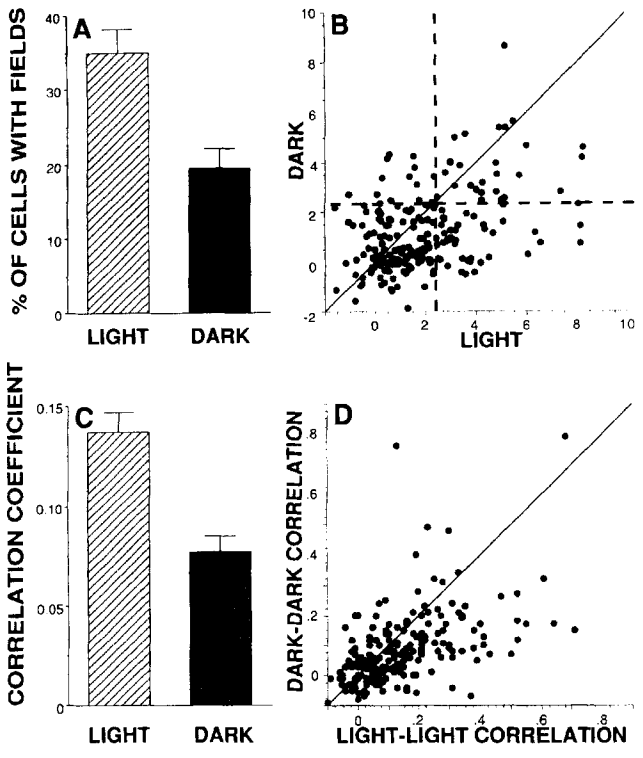

Fig. 4. Spatial firing of all complex spike cells. **A:** Proportion of cells with place fields by light condition. A Z-value of over 2.29 (dashed lines in **B**) was considered significant spatial information (i.e., a place field). A higher proportion of cells had significant place fields in the light than in the dark ($P < .01$). **B:** Scatterplot of place information Z-scores in light and dark. Most cells had a higher Z-score in the light condition than in the dark condition: 45 cells had significant fields only in the light, 13 only in the dark, and 29 in both conditions. **C:** Mean correlation coefficients (reliability) across light and across dark trials. Place fields were more reliable in the light than in the dark ($P < 0.01$) **D:** A scatterplot of correlation coefficients across dark trials versus light trials. Note the bias toward higher correlations across light trials.

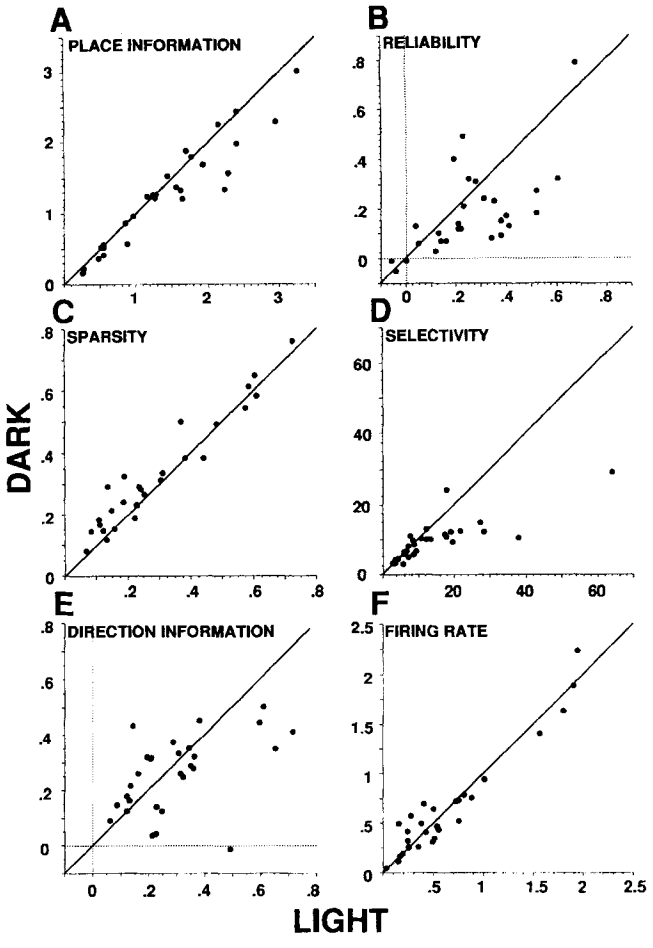

Fig. 5. Comparison of spatial firing parameters for cells with significant place information content in both light and dark. **A:** Spatial information content is higher in the light than in the dark (paired t-test, $P < 0.05$). **B:** Reliability across light trials was greater than across dark trials (paired t-test, $P < 0.01$). **C:** Sparsity of firing (proportion of the maze over which the cell fired) was lower in the light than in the dark (paired t-test, $P < .01$). **D:** Selectivity of firing (rate in location of maximum/mean firing rate) was higher in the light (paired t-test, $P < 0.01$). **E:** direction information content and **(F)** mean firing rates were similar under both lighting conditions ($P > 0.10$). Note the tendency for the fields with higher information content and selectivity and lower sparsity to be more affected by the dark.

between the firing pattern of the first dark trial to the mean firing pattern in the light was significantly higher on initial trial light sessions ($P < 0.01$; also see Fig. 9B). Thus, when the first trial of a recording session was in the dark, place fields were poorly correlated with the following light condition trials. The correlation of the place fields on dark condition trials to the light condition trials improved once the animal completed a light condition trial. No such relationship was found for the first light trial. In other words, there was no effect of whether the first trial of the session was in the dark or light on the standardized correlation between the firing pattern of the first light trial to the overall firing pattern in the light.

This finding prompted us to examine the effect of trial order on the standardized correlation of single trials to the overall firing pattern in the light (the mean of all five light trials). There was a main effect of trial order with no effect of session type on the standardized correlation of light trials, within a given recording session. As the trials progressed, the firing pattern of the cells became more reliable (Fig. 9A). No such general progression was found for trials in the dark; however, there was a significant interaction for trials in the dark between the trial order and the type of session (Fig. 9B).

Theta cells

Theta cells had similar waveform characteristics in the young and old rats (Table 2), and there were no age or light effects on the mean firing rates ($P > 0.10$). The firing pattern of theta cells was more reliable across light trials than across the dark trials (see Fig. 10).

As with CS cells, the reliability of the firing pattern was more consistent across trials of the same type than between

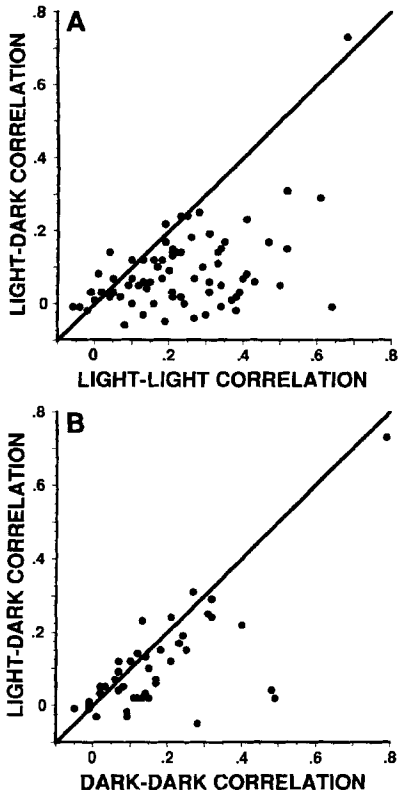

Fig. 6. A scatterplot depicting the correlation in spatial firing patterns across light and dark trials in relation to the correlation of firing patterns across trials of the same type. **A:** All cells with significant place fields in the light. Note that for most cells the correlation within the light condition is substantially higher than the correlation between light and dark conditions. **B:** All cells with significant place fields in the dark. Note that with few exceptions, when there was a significant place field in the dark it was equally correlated with the cell's place field in the light (on the 45° line).

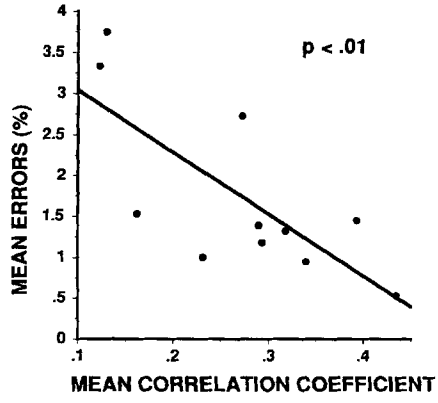

Fig. 7. Comparison of the tendency of an animal to make errors on the behavioral task with the mean reliability of its place cells ($r^2 = 0.55$, $P < 0.01$).

trials of different types (Light-Light r = 0.10, Dark-Dark r = 0.04, Dark-Light r = 0.02; $P < 0.01$).

While there was a relationship between the animal's velocity and the firing rates of theta cells (mean r = 0.09, mean Z = 7.66), this relationship was not affected by the light condition ($P > 0.10$).

DISCUSSION

Different spatial representations in light and dark

In the present study, there was a decrease in the specificity and reliability of spatial information expressed by complex spike cells and in the reliability of theta cell spatial firing patterns in the dark. This was the result of some place fields being lost in the dark, and most of the remainder, while having similar fields in both light and dark, conveying less spatial information in the dark. The fact that place fields differed in the degree to which they were retained in the dark is consistent with the possibility that place cells may vary in the type of sensory information they integrate, although other explanations are also possible.

Place cells have mnemonic properties, in the sense that they can continue to fire normally even when some of the inputs that, when present, control their firing are reduced or corrupted (e.g., O'Keefe and Conway, 1978; O'Keefe and Speakman, 1987; Mizumori et al., 1989). This suggests that the hippocampus can recall stored representations by a "pattern completion" operation (Marr, 1971; McNaughton and Morris, 1987). Such pattern completion would predict little or no effect on place fields when a small amount of sensory information is removed; however once a critical amount of information is missing there might be a notable effect on the place field. This might provide part of the explanation for the greater degradation of place fields in dark conditions in the present experiment than in previous studies in which the primary visual cues were removed, but the room remained illuminated. Under the latter conditions, numerous visuo-spatial cues, such as distance from the enclosing curtains, remained, and could have helped to stabilize the representations. The present findings of a graded loss of place field specificity and reliability in the dark (see Fig. 6A) may be taken as evidence that hippocampal pattern completion is not complete and that place cells are more stimulus bound than previously thought. Conversely, the apparent graded degradation in the dark may be the result of combining the data across trials. For example, if the place field was robust on four of the five dark trials, but was completely gone on the fifth, our computation would show on average an overall small degradation of the field in the dark, even though the real effect was all or none.

Quirk et al. (1990), recording from rats in a lighted cylinder for 8 minutes followed by 8 minutes of darkness, found that 22 of the 24 cells recorded had place fields in both the light and the dark, and that the overall decrease in reliability score in the dark was not significant. There are a number of variables that may have contributed to the different pattern of results obtained in the present study, including task demands, methods of measuring fields, apparatus size and configuration, control of auditory cues, disorientation of the animal during introduction to the test apparatus, and age, sex, and strain of rats. An important difference is that Quirk et al. (1990) con-

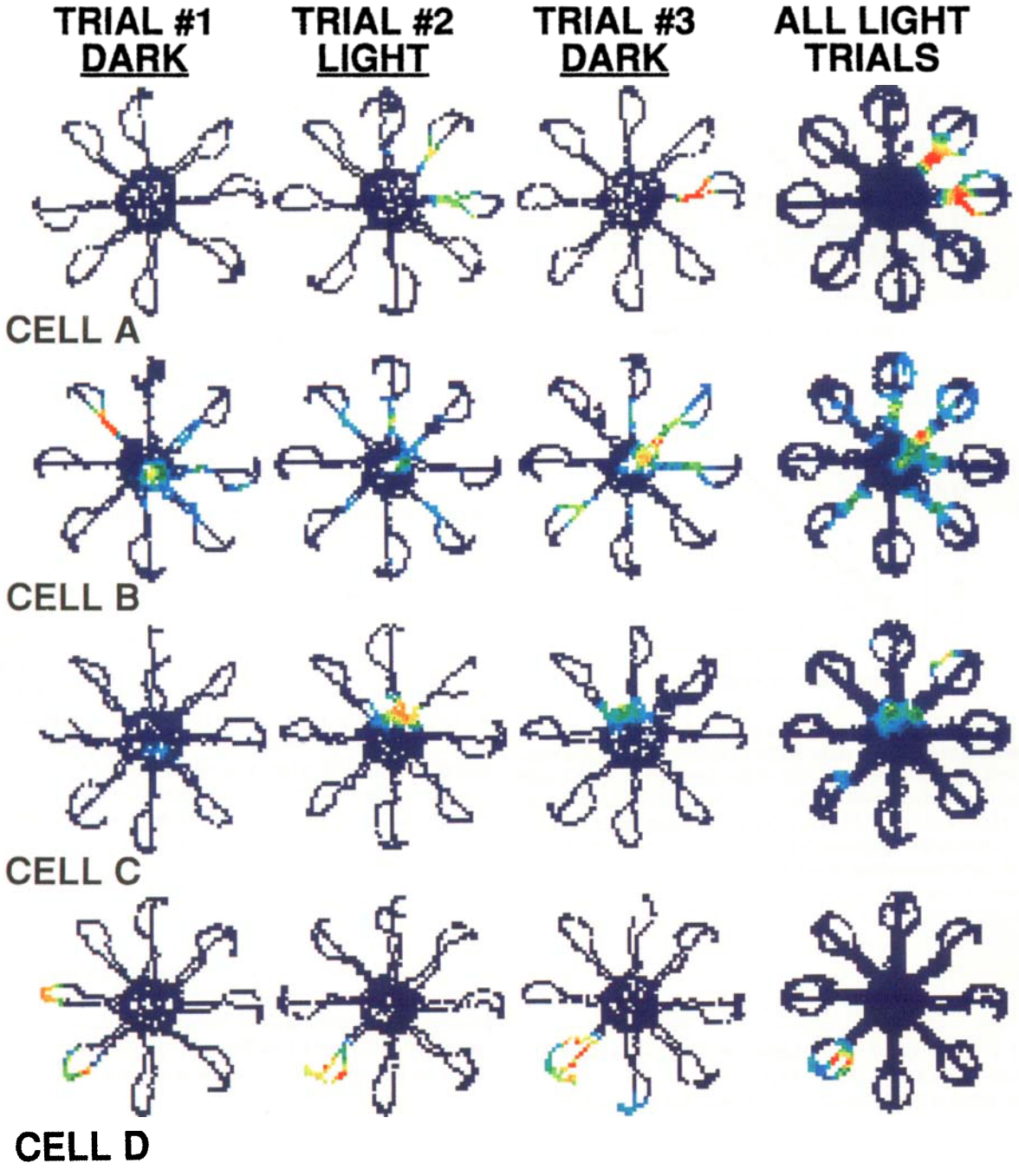

Fig. 8. The firing pattern of four cells on the first three trials of a session (dark, light, dark), and the average firing pattern in the light for that session. Note how the firing pattern in the dark becomes more similar to the firing pattern in the light once the rat has traversed the maze in the light trial. Scale: Red represents firing rate above 12 Hz, cells **A** & **D;** 7 Hz, cells **B** & **C** (cells recorded were from different rats and sessions).

ducted the dark trials following an extended session of navigation in the light. According to the present finding of an effect of the light condition on the first trial, this would be expected to improve the correlation between light and dark trials substantially. In addition, during the dark session, the rat visited each location in their apparatus many times, whereas in the present study the maze was traversed only once on each trial.

A small number of cells in the present study had unique fields in the dark, a phenomenon not seen by Quirk et al. (1990). While this may be due to their smaller sample size, it may also be the result of differences in training and task demands. Unlike the Quirk et al. (1990) task, in the present study the animals were required, at least minimally, to keep track of their location on the maze, in order to distinguish

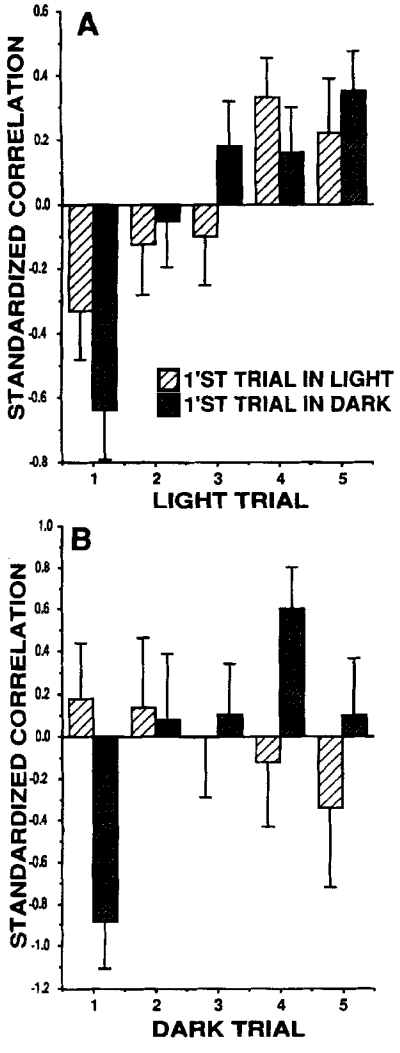

Fig. 9. Effect of light–dark trial order and session type, on the correlation between the light and dark trials. The correlation between the firing pattern on each single trial to the mean light condition firing pattern was calculated and standardized separately for dark and light trials, reflecting the relative degree of correlation to the light condition on the various trials of a given session. Because the sessions were counterbalanced (half the recording sessions starting with a light trial and the other half with a dark trial), they were separated into "initial trial dark" and "initial trial light" sessions. **A:** For trials in the light (n = 69 cells, with fields, and with data on all ten trials) there was a main effect of the trial order ($P < 0.01$), with the firing patterns progressively becoming more stable over the recording session. There was no effect of session type. **B:** For trials in the dark (n = 19 cells, with similar fields in light and dark), there was an interaction between the trial order and the type of session ($P < 0.05$). A t-test showed that the firing pattern on the first dark trial was better correlated with the firing pattern on the light trials when it was preceded by a light trial ($P < 0.01$).

between the arm just visited and the newly presented one. Furthermore, the rats in this study were overtrained in the performance of the task in both the light and the dark, and thus had experienced a large number of light-dark transitions. These considerations suggest the possibility that the animals in the present study had learned to represent the two conditions differently, possibly because of the different nature of the cues available for task solution in the two cases. For example, they may have made more use of kinesthetic and inertial cues in the darkness and more use of distal visual cues in the light. It is known from other studies that the hippocampus can develop different internal representations for the same apparatus if the animal is presented with a different dominant visual cue on alternate trials (Bostock et al., 1991), if the animal is brought into the apparatus in the dark (Quirk et al., 1990), or even if the task demands are altered within the same environment (Markus et al., 1994). Thus, two overall conclusions tend to emerge from these and previous results. First, when animals are subjected occasionally to conditions of darkness in a familiar environment, or to the removal of the dominant visual cues (O'Keefe and Speakman, 1987), the hippocampal spatial representation tends to be driven by memory of the relationships among the visual landmarks; however, with extended experience in the dark situation, a representation develops that is determined primarily by the relevant sensory cues available for orientation. Second, when the sensory cues used to represent the space transmit less information about location, the spatial representation is correspondingly poorer and less reliable. In the present case, the prevalent cues in darkness, which were primarily (but probably not exclusively) those local to the maze, including maze geometry itself, were much less rich and much less spatially polarized than those available in the light. This would account for the poorer reliability and specificity of the spatial representation in darkness in the present study.

Firing pattern, reliability, and behavior

The rats were very familiar with the recording room and well trained on the behavioral task, both in the light and dark. Nevertheless, when the first trial of a recording session was in the dark, place fields were poorly correlated with the following light condition trials. This was in spite of the fact that the lights were on and the animal could view the room before the beginning of the first trial. Similarly, repeated trials also improved the correlation of the light trials. Thus it seems that, even in a familiar environment, upon subsequent exposures, actively traversing the visible environment facilitates the reliability of the place fields. A similar effect was noted, but not quantified, by O'Keefe (1976). This finding supports and extends reports that place fields become more reliable as a rat experiences a novel environment (Wilson and McNaughton, 1993; Austin et al., 1993). It should be noted that, in the present experiments, in which the rats had received extensive previous experience on the apparatus, the effect of trial sequence was relatively small in relation to the overall effects of light versus dark on place field quality.

Rats with more reliable place fields on average also made fewer errors. No correlation was found between the spatial characteristics of the place fields (size, specificity, selectivity) and errors. This could be due to the fact that these measures are more closely dependent on the quality of unit isolation than reliability and thus inherently more variable; however, in a distributed code, the actual breadth of neuronal tuning on the relevant dimension may be less important than reliability;

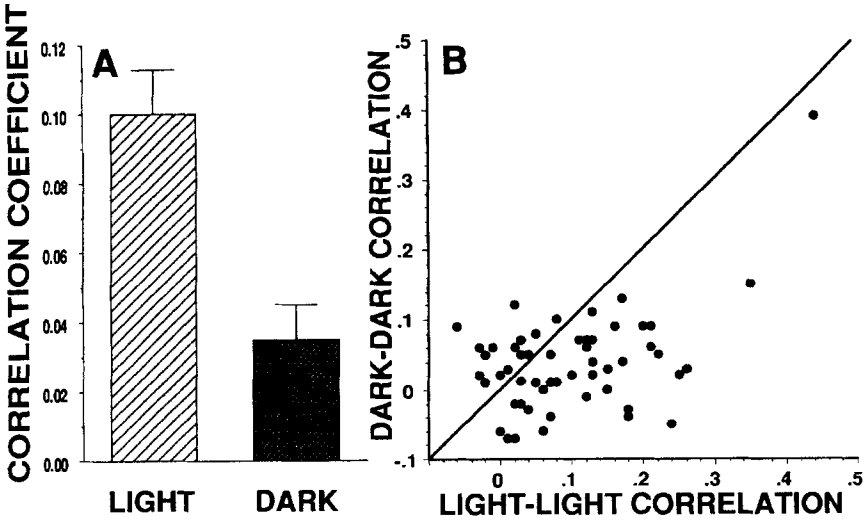

Fig. 10. The reliability of the spatial firing of theta cells in the light and dark. The reliability measure is based on standardized scores, factoring out any firing pattern that is uniform on all maze arms. **A:** Mean correlation coefficients (reliability) were higher across light than across dark trials ($P < .01$). **B:** A scatterplot of correlation coefficients across dark trials versus light trials. Note the bias toward higher correlations across light trials.

the population response can be extremely specific in spite of rather broad tuning of the individual units, if the tuning curves are reliable (e.g., see Georgopoulos et al., 1989; Wilson and McNaughton, 1993). A similar decrease in reliability, but not in specificity of hippocampal place fields, was found in rats spatially impaired by colchicine lesions of the dentate gyrus granule cells (McNaughton et al., 1989a). The current task was very easy (less than 2% errors overall), and could, in principle, have been accomplished without reference to spatial cues, using path integration, a skill that rodents have been shown to possess (Mittlestaedt, 1983). Nor was there a serious penalty for an error, in terms of the overall level of reinforcement. It remains to be determined, therefore, whether poorer place field reliability is a cause of the increased errors, or the result of some common third variable such as reduced attention. Increasing task difficulty and/or introducing a penalty for error might help resolve this issue.

Age effects

As reported previously, waveforms and firing rates of theta cells recorded in stratum pyramidale (Mizumori et al., 1992) and CS cells (Barnes et al., 1983) were not different between age groups; however, in contrast to the latter study, there were also no age effects on the spatial firing characteristics in the present data. Barnes et al. (1983) found that place cells in aged rats exhibited a decrease in their spatial selectivity and reliability scores. In that study, however, the comparison was done by cells, rather than based on the mean per animal as in the present study. The drawback of the by-cell analysis is that, if there is a disproportionate sampling of cells across animals and the animals differ greatly in the quality of their place fields (or recording quality of their electrodes), then the differences observed may not reflect group differences. Thus, it is not clear if, in the Barnes et al. (1983) study, an age-related effect would have been found had the analysis been done by animal using the present measures of reliability and information content.

Two other important differences between the Barnes et al. (1983) and the present study should also be noted, as they may contribute to the apparent discrepancy. First, the rats in the earlier study were much less overtrained on the task and had less overall experience with the environment. To the extent that these factors interact with place field specificity, this may partly account for the different results. For example, old rats could take longer to establish a consistent mnemonic representation of the environment. Alternatively, young rats may simply attend better to the spatial cues early in training, but not later. The second difference between the studies is that, in the Barnes et al. (1983) study, the aged rats examined were approximately 2 months older than the old rats in the present study. Recently it has been shown (Barnes et al., 1992) that there is an accelerated age-related change occurring between 24 and 27 months in the sensitivity of CA1 pyramidal cells to iontophoretically applied AMPA (glutamate receptor agonist), and in the magnitude of synaptic responses. Thus, it may be expected that, during this period, changes may occur in the temporally and spatially related firing characteristics of these old pyramidal cells.

In conclusion, the present results suggest that hippocampal place cells differ in the degree to which they integrate information from different modalities, and that, with repeated exposures to the same environment in conditions of light and darkness, the hippocampus may develop new representations for the two conditions. The representation in the dark condition was less precise and less reliable than the one for the light condition, paralleling the reduction in available sensory information. This was reflected in an increase in errors made by the rats in the darkness. Even in the light condition, however, error probability was well correlated with reliability of place

fields, suggesting the possibility that the two are either causally related, or affected by a third variable, such as attention.

ACKNOWLEDGMENTS

We are grateful for the technical assistance provided by V. Bohbot and T.W. Abel and for the helpful comments by L. Nadel and R.U. Muller. This work was supported by AG03376, MH00897, and NS20331.

REFERENCES

Austin KB, White LH, Shapiro ML (1993) Short- and long-term effects of experience on hippocampal place fields. Soc Neurosci Abst 19:797.

Barnes CA (1988) Spatial learning and memory processes: the search for their neurobiological mechanisms in the rat. TINS 11:163–169.

Barnes CA, McNaughton BL, O'Keefe J (1983) Loss of place specificity in hippocampal complex spike cells of senescent rat. Neurobiol Aging 4:113–119.

Barnes CA, Rao G, Foster TC, McNaughton BL (1992) Region-specific age effects on AMPA sensitivity: electrophysiological evidence for loss of synaptic contacts in hippocampal field CA1. Hippocampus 2:457–468.

Bostock E, Muller RU, Kubie JL (1991) Experience-dependent modifications of hippocampal place cell firing. Hippocampus 2:193–206.

Georgopoulos AP, Lurito JT, Petrides M, Schwartz AB, and Massey JT (1989) Mental rotation of the neuronal population vector. Science 243:234–236.

Jarrard LE (1993) On the role of the hippocampus in learning and memory in the rat. Behav Neural Biol 60:9–26.

Markus EJ, Barnes CA, McNaughton BL, Gladden V, Abel TW, Skaggs WE (1992) Decrease in the information content of hippocampal CA1 cell spatial firing patterns in the dark. Soc Neurosci Abst 18:1216.

Markus EJ, Qin Y, McNaughton BL, Barnes CA (1994). Place fields are affected by behavioral and spatial constraints. Cognitive Neurosci Soc Abst 1:91.

Marr D (1971) Simple memory: a theory for archicortex. Philos Trans R Soc Lond [Bio] 262:23–81.

McNaughton BL, O'Keefe J, Barnes CA (1983) The stereotrode: a new technique for simultaneous isolation of several single units in the central nervous system from multiple unit records. J Neurosci Methods 8:391–397.

McNaughton BL, and Morris RGM (1987) Hippocampal synaptic enhancement and information storage within a distributed memory system. TINS 10:408–415.

McNaughton BL, Barnes CA, Meltzer J, Sutherland RJ (1989a) Hippocampal granule cells are necessary for spatial learning but not for spatially-selective pyramidal cell discharge. Exp Brain Res 76:485–496.

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

Mittlestaedt H (1983) The role of multimodal convergence in homing by path integration. Fortschr Zool 28:197–212.

Mizumori SJY, McNaughton BL, Barnes CA, Fox KB (1989) Preserved spatial coding in hippocampal CA1 pyramidal cells during reversible suppression of CA3c output: evidence for pattern completion in hippocampus. J Neurosci 9:3915–3928.

Mizumori SJY, Barnes CA, McNaughton BL (1992) Differential effects of age on subpopulations of hippocampal theta cells. Neurobiol Aging 13:673–679.

Nadel L (1991) The hippocampus and space revisited. Hippocampus 1:221–229.

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

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, Conway DH (1978) Hippocampal place cells in the freely moving rat: why they fire where they fire. Exp Brain Res 31:573–590.

O'Keefe J, Nadel L (1978) *The Hippocampus as a Cognitive Map*. London: Clarendon.

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

Paxinos G, Watson C (1982) *The Rat Brain in Stereotaxic Coordinates*. Sydney: Academic Press.

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

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

Silver PH (1967) Spectral sensitivity of the white rat by a training method. Vision Res 7:377–383.

Skaggs WE, McNaughton BL, Gothard KM, Markus EJ (1993) An information-theoretic approach to deciphering the hippocampal code. In: *Advances in neural information processing 5* (Hanson SJ, Cowan JD, Giles CL, eds), pp 1030–1037. San Mateo, CA: Morgan Kaufmann Pub.

Sutherland RJ, Kolb B, Wishaw IQ (1982) Spatial mapping: definitive disruption by hippocampal or medial frontal cortical damage in the rat. Neurosci Lett 31:271–276.

Wilson MA, McNaughton BL (1993) Dynamics of the hippocampal ensemble code for space. Science 261:1055–1058.