

# The Presence of a Second Rat Has Only Subtle Effects on the Location-Specific Firing of Hippocampal Place Cells

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**ABSTRACT:** We compared the spatial firing properties of hippocampal place cells as a hungry rat foraged for randomly scattered food pellets in a familiar environment while it was by itself and while it shared the arena with a second rat that also was trained in the same task. Our goal was to determine if the hippocampal mapping system remained functional in the presence of the second rat, despite a strong initial tendency of the two animals to stay close together and despite the increased complexity of the sensory surroundings. We found that almost all place cell firing fields were only marginally changed by introducing the second rat. In particular, there was no evidence of the remapping characteristic of place cells in a sufficiently different novel environment. Instead, firing fields became somewhat less well organized and slightly weaker in the presence of the second rat. These second order changes were found to be distance dependent; the degradation of firing properties was maximal when the two rats were near each other. We conclude that signals in the hippocampal mapping system are affected to a small enough extent that accurate navigational is still possible when the environment is enriched in this realistic fashion. © 2011 Wiley Periodicals, Inc.

**KEY WORDS:** cognitive map; hippocampal pyramidal cells; spatial navigation; social interaction; animal-to-animal distance

## INTRODUCTION

In general, recordings of place cells have been made in static circumstances even if the circumstances are changed between recording sessions to determine the nature of relevant stimuli (see O'Keefe, 2007). It is obvious, however, that many features of natural environments change rapidly in time. One especially important source of variation is the presence of other animals including prey, predators, and conspecifics whose key property is self-mobility. Self-mobility can be also a property of motorized objects whose movements are determined by chance or by a preselected program. Given this source of complexity in the environment, it is essential to ask if the putative hippocampal map formed by place cells remains substantially intact or is greatly disrupted by introducing self-mobile entities.

In the only previous study on the effects of self-mobile objects on place cell activity (Ho et al., 2008), rats were given rewarding brain

stimulation according to two different rules concerning their proximity to a motorized toy car. In the car-independent navigation condition (CIN), stimulation was given after the rat traveled 150 cm, regardless of its position relative to the car; in the car-dependent navigation condition (CDN), stimulation required the rat to come within 20 cm of the car but then to stay further away for 10 s before another stimulus was available. It was reported that compared to recordings in the absence of the car, spatial firing distributions were more persistent during CIN than CDN, where most cells remapped (Muller et al., 1991). It was also concluded that pyramidal cell firing did not come, in either condition, to signal the location of the car itself.

To investigate the robustness of the place cell representation when challenged by a mobile object, we used a second rat instead of a car. According to preliminary observation of two rats confined to a small arena, this choice has several strengths that include: (1) spontaneous interaction between the target and second rats without a need to reward proximity, (2) the execution of natural paths determined by the two rats rather than the angular paths selected by the person controlling the car, and (3) a closer approximation to species-specific conditions for recording. For each target rat in our study, we recorded as it chased food pellets while alone, then when the second rat was present, and finally, to ask if possible changes in place cell activity were reversible, again when the target rat was alone. The target rat and the second rat were both familiar with the pellet chasing task before the recordings were made.

We analyzed our data in two ways. First, we focused exclusively on the rats' behavior including running speed and the separation between them. We then looked for possible changes in place cell discharge induced by the second rat and whether such changes depended on mutual proximity. In brief, we found that the second rat had detectable but second order effects on place cell activity; there was virtually no hint of remapping although there were reductions in the organization of firing fields (measured by a nearest-neighbor autocorrelation measure called "spatial coherence"). For only one pyramidal cell did we see a strong indication that its discharge depended on

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Accepted for publication 9 August 2011

DOI 10.1002/hipo.20977

Published online 13 October 2011 in Wiley Online Library (wileyonlinelibrary.com).

distance from the second rat as well as the location of the target rat in the arena.

## METHODS

### Subjects

The subjects were 17 male Long Evans rats (Harlan, UK). Each rat was fitted with a 4-tetrode implant used to make single cell recordings. A pair of closely spaced red light-emitting diodes (LEDs) was attached to the implant so that the rat's position could be tracked. Recordings were made from each rat under two circumstances, namely, by itself and in the presence of a second rat. During two-rat sessions, the position of the second animal was tracked with a pair of blue LEDs.

At all times during the experiment, rats had ad libitum access to water in the home cage and were held in an environmentally controlled animal facility on a 12 h light/dark cycle with lights on at 7.00 A.M. Before surgery, the rats were housed in groups of four with ad libitum access to food. Animal treatment complied with Home Office Animals (Scientific procedures) Act, 1986 UK guidelines.

### Surgery

Rats were implanted with 4-tetrode microdrives in the right dorsolateral hippocampus. Each tetrode was spun from H-ML insulated 25  $\mu\text{m}$  90% platinum-10% iridium wire (California Fine Wire, CA). Microdrive implantation was performed under sterile conditions using isoflurane anesthesia. A 0.07 ml injection (23  $\mu\text{g}$ ) of the analgesic Temgesic (buprenorphine hydrochloride) and a 0.1 ml injection (10 mg) of the antibiotic Baytril (enrofloxacin) were administered before surgery. The tetrode tips were aimed at stereotaxic coordinates AP  $-3.8$  mm, ML 2.8 mm, DV  $-1.5$  mm relative to bregma above CA1 so they could be gradually advanced into the pyramidal cell layer after surgery. The drive was attached to the skull with gentamicin-impregnated Palacos<sup>®</sup> R-40 cement and five stainless steel skull screws, one of which served as a ground connection for recording. After surgery, rats were individually housed with continued ad libitum access to food. After a 7 day recovery period, rats were put on a controlled diet to reduce them to 85% of preoperative weight and then allowed to gain 5 g per week.

### Behavioral Apparatus

Screening and recording were done in a 90-cm-diameter, 51-cm-high cylinder whose center was at the middle of a 2.9 by 2.8 m room. The cylinder was put on top of a sheet of gray photographic backdrop paper that was replaced after each recording session to minimize olfactory cues. A 35-cm-wide ( $45^\circ$  of circumference) white card and an equal sized black card provided polarizing cues. The card centers were separated by  $135^\circ$  such that the midpoint between the cards was at 3:00 when

viewed from overhead. During all recording sessions, the cylinder was surrounded with a 120-cm-diameter circular black curtain to visually isolate it from the room. Eight regularly spaced incandescent bulbs were hung overhead to dimly illuminate the curtained area. An overhead dispenser randomly scattered 25 mg food pellets onto the cylinder floor at  $\sim 4$  per minute, encouraging the hungry rats to forage everywhere in the cylinder.

### Recording Setup

For recordings, a cable plus amplifier (gain of 1 for each channel) headstage was attached to the implant. The cable went to an overhead commutator that turned to prevent kinking. Each channel was led to a preamplifier (20,000 to 50,000 gain), band-pass filtered (300 to 6,000 Hz), and digitized (12 bit resolution, 30,000 Hz). The digitized signals were monitored with a spike-capture algorithm (Neuralynx, AZ) such that 1.5 ms of data from all four channels of a tetrode were stored on disk whenever the voltage on any channel exceeded a threshold set between 55 and 75  $\mu\text{V}$ .

Tracking was done with a video camera (Sony Handycam, Japan) mounted 2.2 m overhead with the center of the field of view aimed at the cylinder center. The video signal (25 Hz) was digitized by a frame grabber that allowed the blue and red LEDs to be detected in a  $256 \times 256$  grid of  $0.94 \times 0.94$   $\text{cm}^2$  pixels. This initial resolution was used to determine the distance between the rats. To visualize spatial firing patterns, the spatial resolution was reduced to a  $64 \times 64$  grid of  $3.75 \times 3.75$   $\text{cm}^2$  pixels. The locomotor speed of the recorded rat was determined by measuring the distance traveled every 0.40 s. If the rat moved  $<1.0$  cm in this time, its speed was set to 0.0 cm/s and the interval was classified as "still time." The total still time was accumulated for the entire 16 min session. Based on this estimate of still time, we calculated "overall speed" as the total distance traveled divided by the session duration and moving speed as the distance traveled divided by the session duration minus still time.

### Data Analysis

Spikes were categorized into clusters with Offline Sorter software (Plexon, Dallas, TX); each cluster is taken to represent the activity of a single cell. The same criteria were used to cluster spikes for all sessions from a given rat. To enhance the likelihood that accepted data are from single cells, clusters were eliminated if the interspike interval distribution contained values  $<1.5$  ms. Spikes were further classified as being generated by either pyramidal cells or interneurons. To be called a pyramidal cell, the duration of the positive portion of the filtered waveform had to be  $>250$   $\mu\text{s}$ , interspike intervals  $>0.25$  s had to occur and the presence of complex spikes had to be detected. Only cells classified as pyramids were further considered.

The spike time series for each cluster was combined with the position time series to produce spatial firing rate distributions that could be visualized with color coded maps and further

used to extract numerical properties that included: (1) Firing fraction: the ratio of the number of pixels with a rate  $>0$  to the total number of visited pixels in the apparatus. (2) Number of firing fields: a firing field is an area of at least nine pixels each of which has a rate  $>0$  and shares a side with another pixel in the field. (3) Field size: the number of pixels in the field. (4) Field rate (spikes/s): the number of spikes in a field divided by the total time spent in the field. (5) Field peak rate (spikes/s): the maximum average rate of any 3 by 3 block of pixels in the field. (6) Overall rate (spikes/s): the number of spikes fired during the session divided by the session duration. (7) Spatial coherence: the product-moment correlation between the rate in a pixel and the average rate in its nearest neighbors is computed. Coherence (Coh) is the z-transform of the correlation. (8) Spatial information content (bits/pixel): the value reported here is the average pixel-wise spatial information content (using a 140 ms time window) for pixels lying within the largest place field of the cell (Olypher et al., 2003). This spatial information (info) content then is the average amount the uncertainty of the spike count is reduced when the rat is somewhere within the primary field. This number tends to compare favorably with our subjective judgment of location specificity. Paired *t*-tests were used to determine if mean values of the stated parameters changed between sessions. A pyramidal cell was considered a place cell and included in the final sample if *coh*  $>0.35$  in the first control session.

### Experimental Protocol

Once rats were at 85% of ad libitum weight, they were pre-trained by allowing them to find food pellets dropped on the floor of a 90-cm-diameter gray cylinder. Subsequently, each tetrode was screened twice a day for place cell activity. If less than two well discriminated place cells were seen, the electrodes were advanced by 30–60  $\mu\text{m}$ . Otherwise recordings were started.

The first and all subsequent odd number sessions during an experimental day were “standard sessions.” In a standard session, the midpoint between the cue cards was at 3:00 and only the recorded rat was in the cylinder. After each trial, the rat was removed from the cylinder without disconnecting the cable and put in its home cage outside the curtains for 3–5 min during which time it had access to water. During each intertrial interval, the floor paper was changed and other manipulations scheduled for the next session were made.

In the key experimental manipulation, a two-rat session with a second rat in the cylinder was interposed between a pair of standard sessions. As the second rat was from a separate experiment, it also had an implant, which allowed us to connect a blue battery-powered (LED) to independently track its position. The second rat was seen only once for each subject rat.

### Histology

Rats were given an overdose of anesthetic and perfused trans-cardially with saline followed by 10% formalin. Brains were removed, stored in 4% PFA, and transferred to 30% su-

crose solution for  $\sim 48$  h after which 30  $\mu\text{m}$  sections were taken on a microtome and stained with Cresyl Violet. Histological assessment confirmed that the tetrodes were in the CA1 pyramidal cell layer in all rats.

## RESULTS

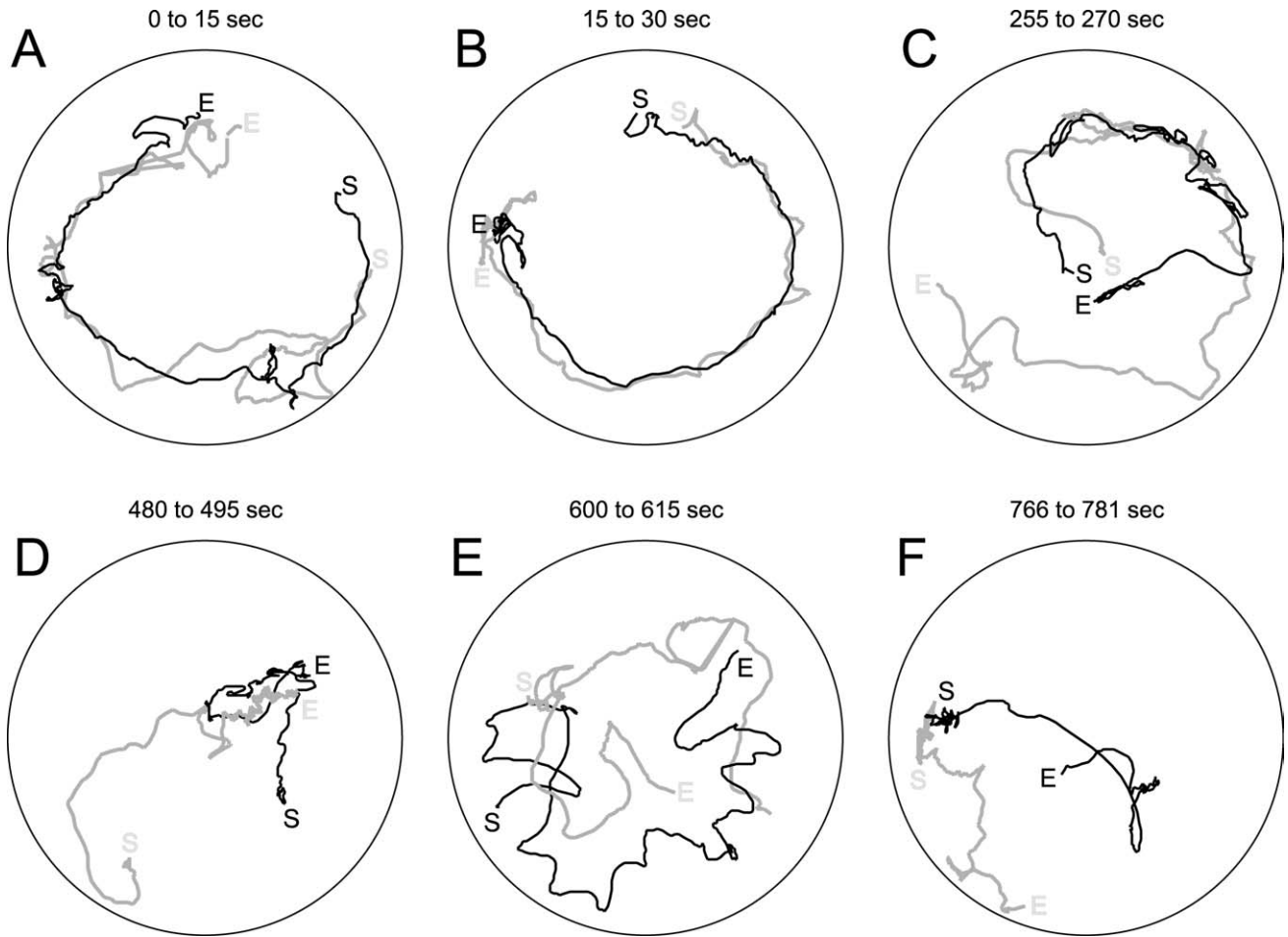
### Behavioral Effects of Introducing the Second Rat: Qualitative Description

Place cells were recorded during three 16 min sessions carried out in rapid succession so that the total elapsed time was  $<1.5$  h. In the first session, the recorded “target” rat foraged for dropped food while alone. In the second session, the only change was to put a second rat into the cylinder. The other rat was familiar with the foraging task because it had participated in a different study. The two-rat session was the first time the two rats were in each others presence. In the third session, the target rat was again alone. Direct observation revealed that the two animals stayed closer to each other at the beginning of the two-rat session than later on. In no case did we see aggressive activity on the part of either rat, even when their separation was relatively small and even though they were competing for pellets.

To characterize behavior during the two-rat session, we show examples of the paths taken by a pair of rats during six 15 s time slices (Fig. 1). At the session start (Figs. 1A,B), the two paths were very similar, indicating that the animals tracked each other. About 4 min into the session (Fig. 1C), the rats were near each other for several seconds after which the recorded rat nearly halted while the secondary rat continued to walk. In Figure 1D, the two rats were initially separated but later approached each other at the end of the 15 s interval. In Figure 1E, the rats seemed to act independently for the entire 15 interval; they were never at the same place at the same time although their paths crossed. Figure 1F illustrates that even late in the session the rats sometimes approached each other; they spent several seconds together at the start of the slice but then went their separate ways.

### Behavioral Effects of Introducing the Second Rat: Quantitative Description

We numerically analyzed trajectory data in three ways. First, we compared measures of locomotion in control sessions and two-rat sessions. Specifically, we computed average speed, still time, and average speed for moving time (intervals during which the rat was not still) for the bracketing control sessions and the two-rat session. Since it is our experience that running speed decreases over time, we tested the values from the two-rat session against the averages from the preceding and following control sessions. The means of the three locomotor parameters are summarized in Table 1. According to paired *t*-tests, none of the variables differed between the control and two-rat



**FIGURE 1.** Examples of two rat paths for 15 s intervals taken at the start (A and B), from the middle (C, D and E), and near the end (E) of a 960 s (16 min) recording session. At the beginning of the session (A and B), the rats stay near each other for most of the 15 s time slice. In sample B, the rats begin near each other but then separate. In D, they start out far apart and then later approach each other. In sample E, their paths cross each

other but they are never at the same place at the same time so that the separation distance is always quite substantial. In sample F which begins a 12.8 min into the session, the rats are nevertheless near each other for several seconds at the start of the interval but then separate. The impression that the two animals tend to be closer to each other at the start of the two rat session is borne out by a quantitative analysis for all animals summarized in Figure 2.

sessions, showing that the presence of a second rat did not alter gross locomotor behavior.

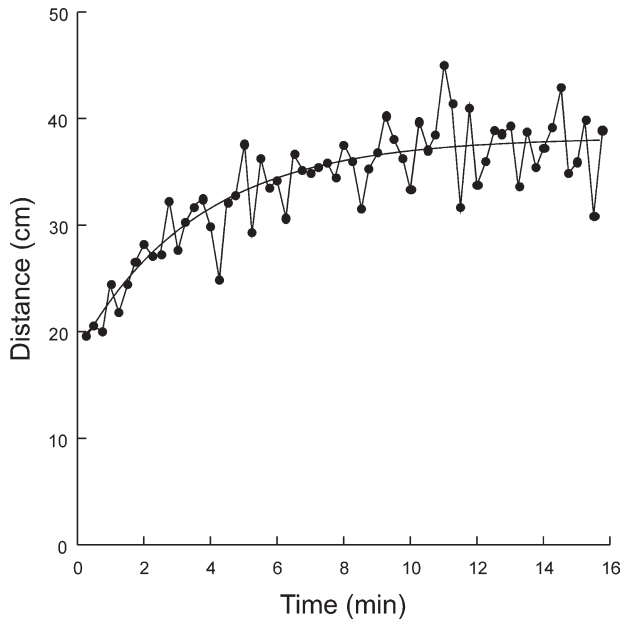
In a second behavioral analysis, we looked for changes in the distance between the two rats as a function of time in the two-rat session. The average separation of 12 rat pairs for successive 15 s intervals is plotted in Figure 2. The relationship corroborates the examples of Figure 1 in that the rats spent more time near each other at the beginning of the session than later on. The relation between distance ( $d$ ) and time ( $t$ ) is well fit by  $d = d_{\infty} - (d_{\infty} - d_i)e^{-t/\tau}$  where  $d_{\infty} = 39.6$  cm is the asymptotic distance at long times,  $d_i = 17.7$  cm is the extrapolated distance at  $t = 0$  and  $\tau = 3.54$  min is the time constant; the correlation between the data and the exponential function is 0.876 ( $r^2 = 0.77$ ). The same effect is seen for each rat pair. Thus, the average distance during the first two minutes is smaller than the average for the last 2 min for all pairs (bino-

**TABLE 1.**

*Comparisons of Locomotor Measures in Control and in Two-Rat Sessions*

Session type	Overall speed (cm/sec)	Still time (%)	Moving speed (cm/sec)
Control 1+2 mean	16.47 ± 1.00	10.83 ± 2.3	18.23 ± 0.77
Two-rat	16.01 ± 0.72	8.47 ± 1.1	17.43 ± 0.63
Paired $t$ (df = 10)	0.487	1.167	1.095
$p(t)$	0.63	0.27	0.30

Mean locomotor parameters and  $t$ -tests for 12 recorded rats in the averaged control sessions and the two-rat session. The parameters were averaged for the bracketing control sessions because of a systematic tendency for rats to run more slowly with increasing time in the recording arena.



**FIGURE 2.** The average distance between the two rats is smallest at the beginning of two-rats sessions and increases to an asymptote over a first order time course with a time constant,  $\tau$  of 3.54 min. The averaged distance as a function of time is shown at each 15 s interval. The smooth curve is the function  $d = d_{\infty} - (d_{\infty} - d_i)e^{-t/\tau}$  where  $d$  is the separation distance,  $d_{\infty}$  is the asymptotic distance,  $d_i$  is the initial distance, and  $t$  is the time since the start of the session.

mial  $P = 0.00024$ ); a  $t$ -test comparing the mean separation for the first 2 min and the last 2 min of the session yields a similar result ( $t_{11} = 5.38$ ;  $P = 0.00023$ ).

Finally, we compared the asymptotic separation distance of 39.6 cm in two-rat sessions to the separation distance expected if two rats moved independently. The independent separation was calculated from the distance between position pairs taken at equal times along the target rat’s path during the two control sessions. The mean random separation distance ( $n = 12$ ) was 45.0 cm, the cylinder radius. (A Monte Carlo simulation confirms that the radius is the mean separation for two random points in a circle.) The random separation was significantly greater than the mean separation of 38.5 cm for the last 2 min in two-rat sessions ( $t_{10} = 4.97$ ;  $P = 0.00042$ ). We conclude that even late in the two-rat session, the rats spent more time nearer each other than expected if their behavior were truly independent.

**The Presence of a Second Rat Leaves Place Cell Discharge Largely Unchanged**

Inspection of firing rate maps suggests that putting a second rat into the environment does not drastically alter the spatial firing patterns of most place cells. Examples of this constancy are shown in the rate maps for six simultaneously recorded place cells in Figure 3. For each cell, only details of the overall spatial firing distribution appear to change across the first control session, the two-rat session, and the second control session.

These examples indicate that the presence of another rat does not silence place cells, cause their spatial discharge to become random or induce a complete remapping (Muller et al., 1996) so that numerical analysis is required to detect any effects of the second rat.

Averaged measures of the spatial firing patterns for the cells in Figure 3 in control session 1, the two-rat session, and control session 2 are given in Table 2. The first two columns are estimates of place cell firing rates, the next two describe the organization of the spatial firing patterns, and the last two are based on the size of the region in which the cell discharges. Using a Bonferroni correction for significance level of  $0.05/7 = 0.00833$ , coherence is the only measure for which there is a reliable effect of the second rat in this particular set of six cells; coherence in the two-rat session is lower than in either of the control sessions.

Table 3 is organized in the same way as Table 2 except that the averages are for the entire place cell sample ( $n = 56$ ). Each of the six numerical estimates was tested for reliability by an analysis of variance (ANOVA) with correlated values for each cell in the three recording sessions. The firing area measures still do not distinguish the two-rat session from the controls although their larger values suggest that discharge tends to be more dispersed. With the full sample, however, the main field peak firing rate measure is reliably decreased in the two-rat session. In addition, spatial information content in the largest field shows a strong trend towards decreasing; the probability value from the ANOVA is only slightly greater than  $\alpha = 0.05$ . Finally, coherence is strongly reduced in the two-rat session. Thus, putting another rat into the environment decreases both the intensity and precision of spatial firing.

A further indication that spatial firing patterns are altered by the second rat comes from calculating pixel-by-pixel correlations (“similarities”) between pair of sessions for each cell, as shown in Figure 4. The mean similarity is  $0.497 \pm 0.048$  for the control sessions,  $0.414 \pm 0.035$  between control session 1 and the two-rat session, and  $0.410 \pm 0.038$  between control session 2 and the two-rat session. The mean similarity between the pair of control sessions is reliably higher than the mean similarity between either the two-rat session and control session 1 (paired- $t_{55} = 2.53$ ;  $P = 0.011$ ) or between the two-rat session and the second one rat session (paired- $t_{55} = 2.87$ ;  $P = 0.006$ ). In keeping with the previous conclusion that the second rat has only moderate effects on spatial firing patterns, the mean similarity between each cell in the two-rat session and a randomly selected cell from control session 1 is only  $-0.0093$ . This is much lower than the mean similarity between the two-rat session and control session 1 (paired- $t_{55} = 10.1$ ;  $P = 3.5E-14$ ), implying that one-rat firing patterns are largely preserved in the two-rat session.

**Place Cell Discharge Depends on the Separation Between the Rats**

Place cell firing might be degraded simply by the presence of the second rat regardless of their separation. Alternatively, the

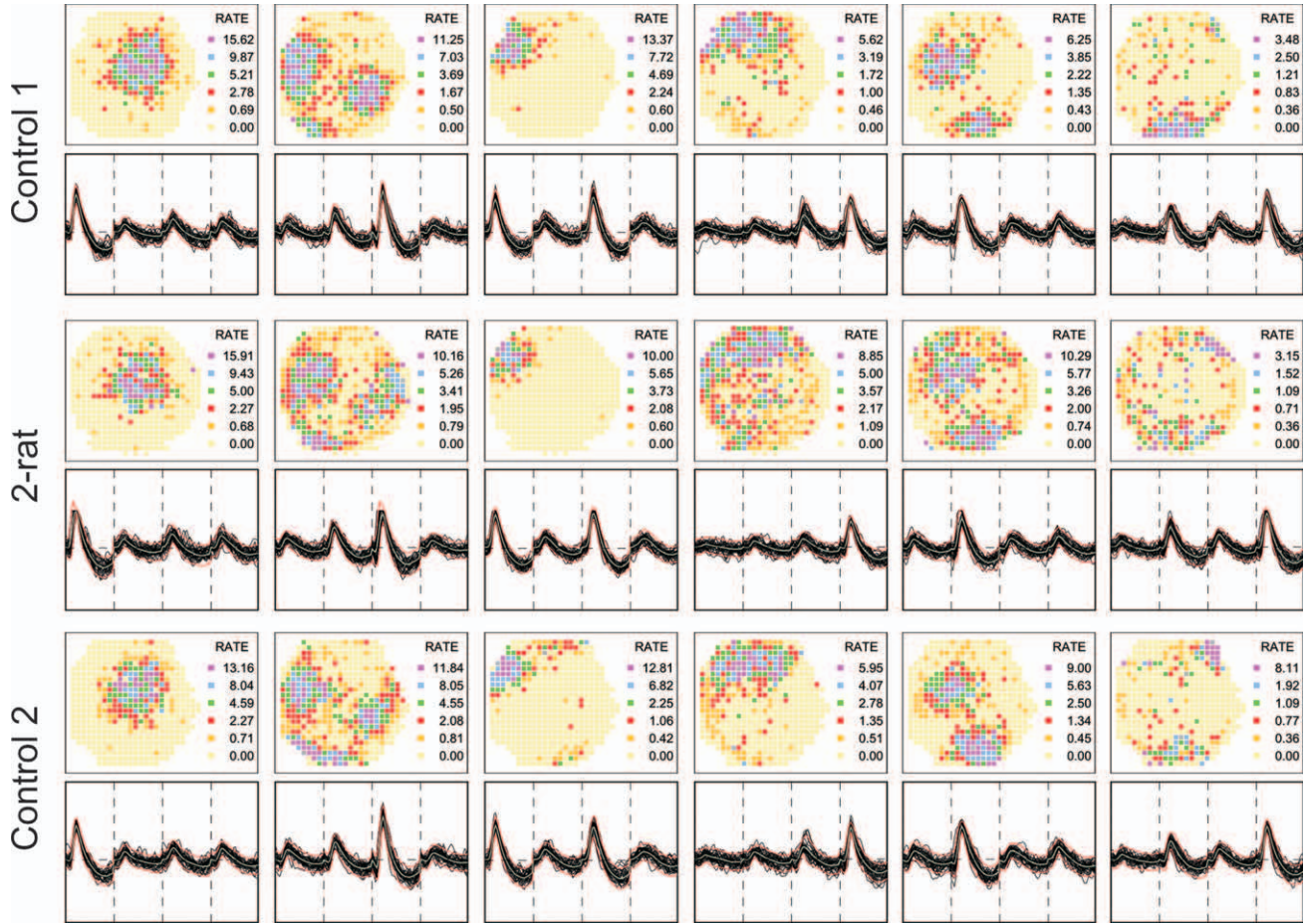


FIGURE 3. Paired illustrations of firing rate maps and tetraode waveforms for six place cells recorded simultaneously during an initial control session (top row pairs), a two-rat session (middle row pairs), and a final control session (bottom row pairs). For each cell, the waveform is constant across the three sessions. In addition, there are no major changes in the spatial firing patterns of any of the cells although by inspection the discharge is somewhat more scattered for the two cells on the right side of the Figure. The firing rate in the median pixel for each color category is shown in the key to the right of each map in the top row.

TABLE 2.

Average Firing Properties and ANOVAs for the six Example Cells in Figure 3

Property	Overall rate	Main field peak rate	Coherence	Spatial info content	Firing fraction	Main field size
(A) Mean value $\pm$ SEM for six properties of the six example place cells shown in Figure 3. Each cell was recorded in a first control session, a two-rat session, and a second control session						
Control 1	1.11 $\pm$ 0.22	9.42 $\pm$ 2.1	1.14 $\pm$ 0.08	0.27 $\pm$ 0.06	0.37 $\pm$ 0.05	137.3 $\pm$ 30.4
Two rat	1.14 $\pm$ 0.35	8.50 $\pm$ 1.8	0.69 $\pm$ 0.11	0.23 $\pm$ 0.07	0.43 $\pm$ 0.09	182.3 $\pm$ 49.2
Control 2	1.19 $\pm$ 0.32	9.34 $\pm$ 1.9	1.17 $\pm$ 0.06	0.25 $\pm$ 0.04	0.38 $\pm$ 0.06	151.2 $\pm$ 37.1
(B) ANOVAs for six place cell properties found in a control session, a two-rat session, and a second control session for six example cells						
F value	0.02	0.13	18.76	0.37	0.85	0.68
Probability	0.98	0.88	4.13E-4	0.70	0.47	0.53

Definitions of firing field properties are given in Methods.

The *F* values are for 2 and 10 df. The only significant effect was for coherence. Post-hoc Tukey HSD tests show that coherence for the two-rat session differed significantly from each control session (*P* < 0.01) although the control sessions did not differ reliably from each other.

TABLE 3.

Average Firing Properties and ANOVAs for the Entire Place Cell Sample

Property	Overall rate	Main field peak rate	Coherence	Spatial info content	Firing fraction	Main field size
(A) Mean value $\pm$ SEM for six firing properties of 56 place cells in a first one-rat session, a two-rat session, and a second one-rat session						
Control 1	0.75 $\pm$ 0.09	7.10 $\pm$ 74	0.88 $\pm$ 0.05	0.20 $\pm$ 0.02	0.31 $\pm$ 0.02	93.6 $\pm$ 9.4
Two rat	0.73 $\pm$ 0.09	5.28 $\pm$ 55	0.63 $\pm$ 0.04	0.17 $\pm$ 0.01	0.33 $\pm$ 0.03	104.6 $\pm$ 9.3
Control 2	0.73 $\pm$ 0.08	6.91 $\pm$ 61	0.86 $\pm$ 0.05	0.19 $\pm$ 0.01	0.30 $\pm$ 0.02	91.4 $\pm$ 8.0
(B) ANOVAs for six properties of place cell firing in a two rat session and bracketing one rat sessions for the entire cell sample						
F value	0.09	8.06	21.4	2.81	0.87	1.03
Probability	0.91	0.00054	1.05E-8	0.065	0.42	0.36

Definitions of firing field properties are given in Methods.

The degrees of freedom for each ANOVA are 2 and 108. The *F* ratio was significant for coherence and main field peak rate. For these properties, post-hoc Tukey HSD tests showed that the two-rat session was different from each control session ( $P < 0.01$ ) although the control sessions did not differ from each other.

degree of disruption might depend on the distance between the rats. To distinguish between these possibilities, we partitioned the activity of each cell into three conditions, one in which the separation between the head lights was less than a near distance  $D_n$ , a second in which the separation was greater than a far distance  $D_f$ , and a third “excluded “ range of distances  $D_x$  such that  $D_n < D_x < D_f$ . Firing rate maps for partitioning with  $D_n = 15$  cm and  $D_f = 30$  cm are shown along with overall rate maps for six example cells in Figure 5. (Note that the near and far maps are binary; yellow indicates pixels where no spikes were fired, whereas nonyellow indicates pixels where at least one spike occurred. The same binary encoding is used in Fig. 7) For all six cells, the coherence was lower for the near partition; for four of the cells, the rate was also lower for the near partition but was higher for the remaining two cells; these are the two most common outcomes of partitioning, as shown below. The near and far maps were matched by randomly eliminating individual 20 ms samples from whichever partition began with the larger number of spikes until the number of spikes in the two partitions was equal; the same procedure was used for numerical comparisons.

The cells in Figure 5 suggest that the spatial firing pattern in both the near and far partitions strongly resemble the overall spatial firing pattern although, as stated, the spatial coherence for the near partition is lower in each case. Thus, the tendency of spatial firing to be somewhat less organized in the presence of the second rat appears to reflect partial degradation that occurs when the rats are near each other.

We next numerically compared distance-dependent spatial firing properties with  $D_n = 15$  cm and  $D_f = 30$  cm by calculating coherence for the near ( $C_n$ ) and far ( $C_f$ ) partitions and firing rate for the near ( $R_n$ ) and far ( $R_f$ ) partitions. With these partitions,  $C_n = 0.297 \pm 0.023$ , significantly lower than  $C_f = 0.355 \pm 0.28$  (paired- $t_{64} = 2.49$ ;  $P = 0.015$ ) and  $R_n = 0.749 \pm 0.076$ , also significantly lower than  $R_f = 0.888 \pm 0.090$  (paired- $t_{64} = 2.42$ ;  $P = 0.019$ ). Thus, by two measures, it is the proximity and not the mere presence of the second rat that

disrupts place cell firing patterns. This same analysis was done for the similarity measure by computing pixel-wise correlations of each partition map with the control session maps, and the results were comparable to those for coherence (data not shown).

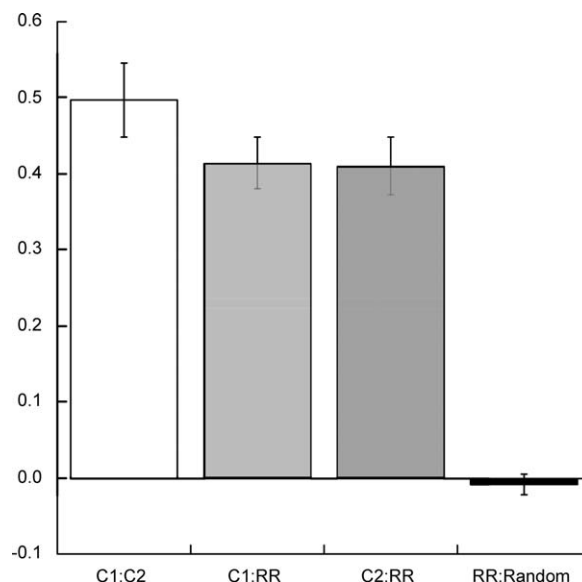
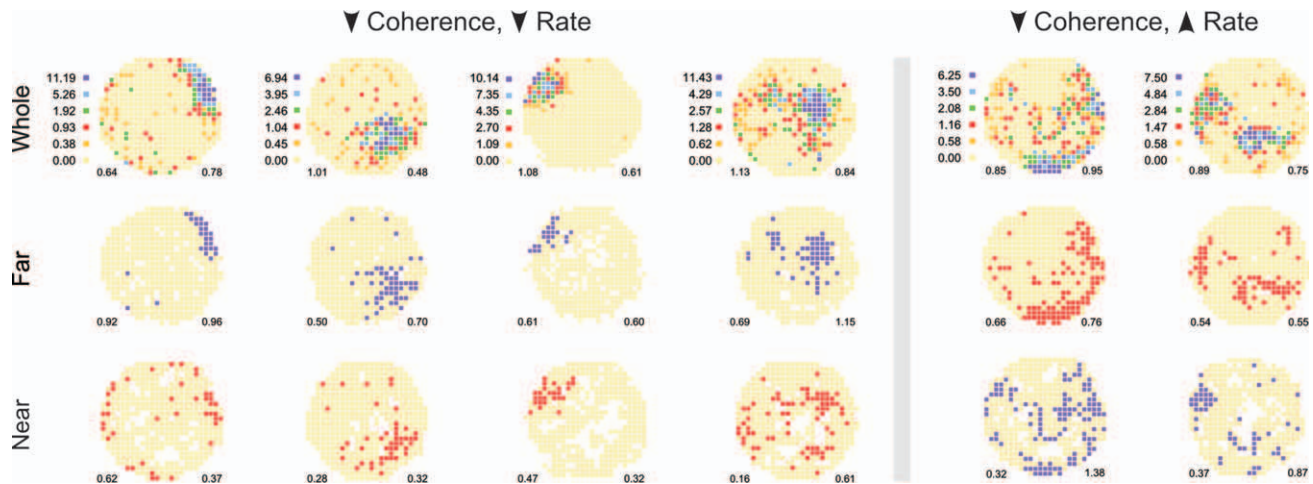


FIGURE 4. Preservation of spatial firing patterns in the presence of a second rat measured by similarity. The mean similarity for the pair of control sessions for 56 place cells is just below 0.5, whereas the mean similarity of the two-rat session and either control session is slightly more than 0.4. Paired *t*-tests reveal that the mean for the control session pair is reliably greater than the mean similarity of the two-rat session compared to either control session. Nevertheless, the mean similarities for the two-rat session vs. the control sessions are both much greater than zero or than the mean similarity of the two-rat session for each cell compared to a randomly chosen (without replacement) rate map for a second cell. Thus, introduction of the second rat reduces firing pattern similarity but not nearly to the extent that the pattern becomes unrecognizable.



**FIGURE 5.** Each of the six columns shows three maps for a single place cell. The top map in each column is a color coded firing rate map for an entire two-rat session; the median firing rate in each color category is shown in the key to the left of each whole-session map. Coherence for the whole session, for the far partition and the near partition is indicated below and to left of the appropriate map; the overall average firing rate (spikes/s) is indicated below and to the right of the appropriate map. The four cells on the left were selected because both the coherence and the rate were lower for the near partition than the far partition. In

contrast, the two cells on the right were included because rate was higher for the near partition although the coherence was lower. These two patterns are the most common combinations of alterations seen when time was partitioned into near (< 15 cm) and far (> 30 cm) ranges; numerical values and distributions for near vs. far separation are summarized in Figure 6. Note that the far and near maps use a binary encoding; pixels in which the rate was exactly zero are colored yellow, whereas pixels in which the rate was greater than zero are coded nonyellow (blue for the far map, red for the near map).

Although both mean coherence and mean firing rate decrease with proximity, the effect of reduced separation on the two measures differs widely from cell to cell. We summarized the coherence and rate values for each cell by calculating the quantities  $\log(C_n/C_f)$  and  $\log(R_n/R_f)$  and then displayed them as a scattergram in Figure 6. Examples of near and far rates differing by a factor of two or greater in either direction were not uncommon, but statistical analyses of those changes were unconvincing because the data lack independence. The overall tendency for both measures to decrease when the rats are near each other is shown by the preponderance of  $[\log(C_n/C_f), \log(R_n/R_f)]$  points in the third quadrant. As expected, a chi-square analysis rejects the hypothesis that there are equal numbers of points in each quadrant (chi-square (3 df) = 21.2;  $P < 0.001$ ). Nevertheless, the correlation between the two variables is only 0.096 and is not significant ( $t_{63} = 0.766$ ;  $P = 0.45$ ). Thus, the parallel proximity effect on coherence and firing rate is not simply a result of a tight relationship between the two measures.

### Can Pyramidal Cells Signal the Proximity of the Second Rat?

For most cells, the presence of another rat weakens the precision and intensity of location-specific firing, but it is also possible that some pyramidal cells fire preferentially when the rats are near each other. We looked for such cells by inspecting several representations of their spatial firing distribution including: (1) overall rate maps; (2) rate maps for near and far distance

partitions; and (3) plots of rate and coherence as a function of the distance between the rats.

The parts of Figure 7A correspond to each of these representations for a place cell that was chosen because its overall rate map has two fields like that of the cell of Figure 7B, the only one whose activity was judged to clearly signal proximity to the other rat. As expected for the comparison cell, the far (Fig. 7A middle map) and near rate maps (Fig. 7A lower map) strongly resemble the overall map (Fig. 7A top map) except for being somewhat noisier. On the other hand, the far (Fig. 7B middle map) and near (Fig. 7B bottom map) rate maps for the candidate proximity cell are distinct from each other. Thus, pixels with spike activity in the far map generally reproduce the outlines of the most intense firing regions in the overall map (Fig. 7B top map), whereas pixels with spike activity in the near map are quite scattered over the entire cylinder surface.

Differences between the comparison place cell (Fig. 7A graph) and the proximity-signal cell (Fig. 7B graph) are evident when firing rate and coherence are plotted as a function of the separation distance. For the place cell, both measures are nearly independent of distance. In contrast, the proximity cell fires much more rapidly when the rats are near each other whereas coherence increases as a function of separation. The growth of coherence with distance is particularly striking as this measure tends to decline when place cell activity is less intense. When the number of recorded spikes is small, the rate map is less likely to vary smoothly, so coherence tends to be lower.

Our sample, therefore, leads us to think that the extent of proximity encoding by hippocampal pyramidal cells is limited



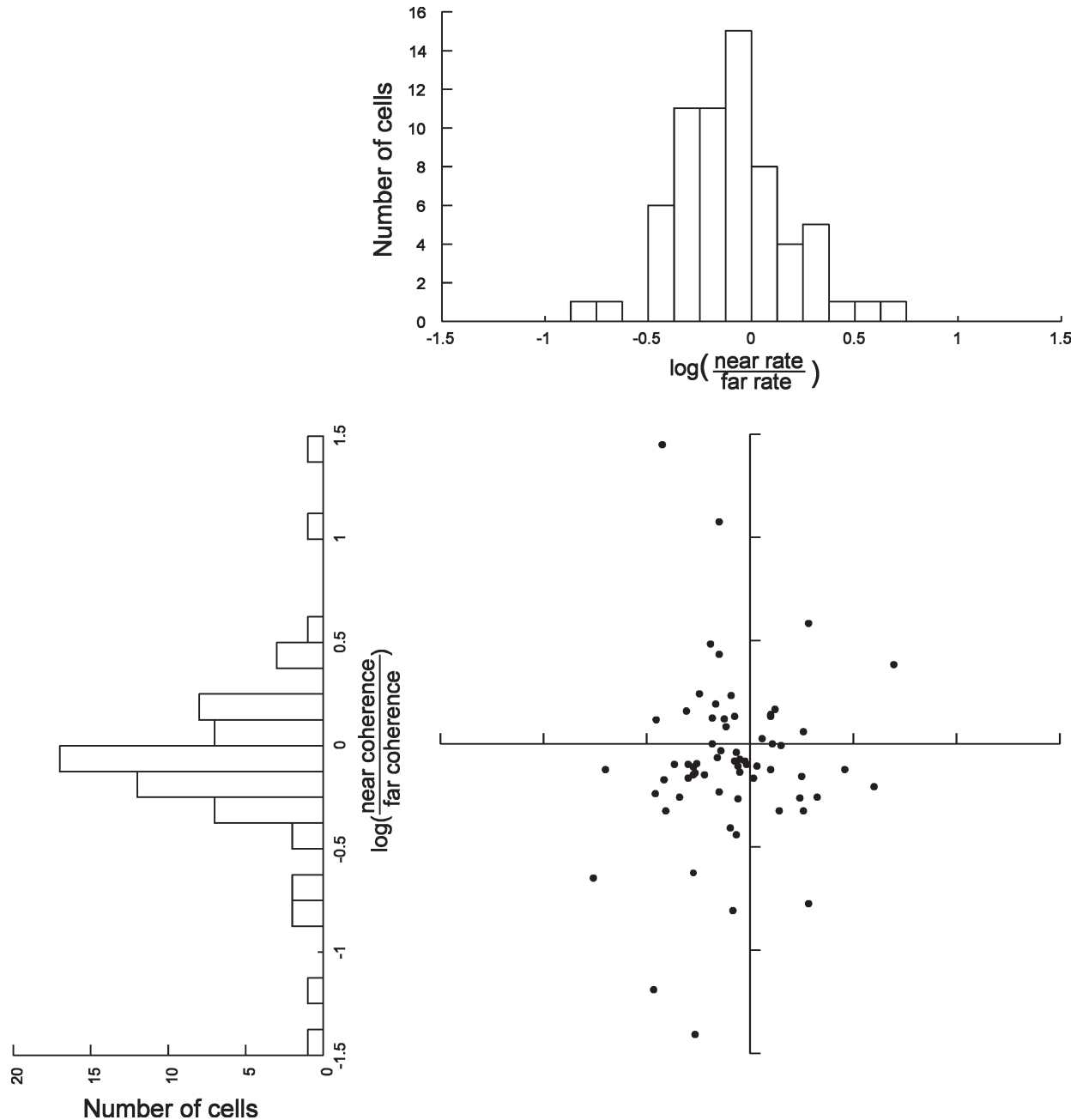


FIGURE 6. The histograms at the top and left of the Figure show the distributions of  $\log(\text{near\_rate}/\text{far\_rate})$  and  $\log(\text{near\_coherence}/\text{far\_coherence})$  for 56 place cells. The scattergram in the middle of the figure shows the joint distribution of the two quantities. Note that both independent distributions are shaded toward negative val-

ues, indicating that on average both rate and coherence are higher in the far than the near separation partition. Also note that most joint values are in the 3rd quadrant with the next most common concentration in the 4th quadrant; examples showing rate maps in Figure 5 were chosen from these regions of the joint distribution.

in two senses. First, despite a concerted effort to uncover such encoding, we could find only the single clearly significant example presented here. Second, even in this case, the putative proximity encoding is superimposed on what seems to be rather ordinary place cell activity. This finding is in agreement with the earlier work of Ho et al. (2008) who saw no special encoding of the proximity of target rats to a self-mobile object.

## DISCUSSION

In the only previous study of how introducing a self-mobile entity into the environment affects the discharge of hippocampal place cells in a target rat, Ho et al. (2008) distinguished between two cases. In one, reward delivery (reinforcing intracranial stimulation) was independent of the location and movements of the added object, a remote controlled toy car. In the

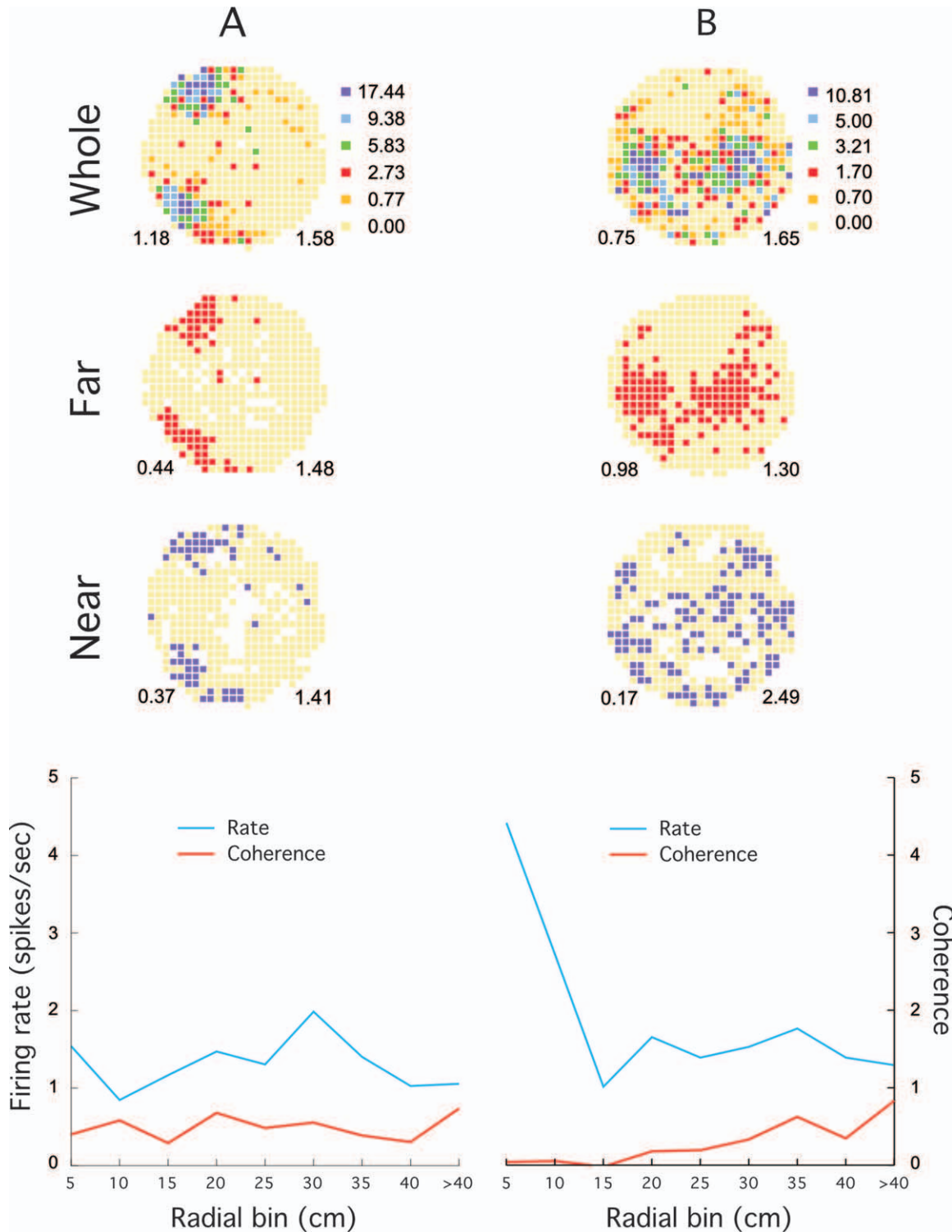


FIGURE 7. Comparisons of properties of an ordinary place cell (column A) and an exceptional cell whose activity apparently signaled both location and proximity to the second rat. The three rate maps are similar to those in Figure 5; the top map is for the entire session, the middle shows discharge when the rats were  $>30$  cm apart, and the bottom when the rats were  $<15$  cm apart. Color keys for the entire session maps are shown to their right. Below each map is shown the coherence (to the left) and the time-averaged rate (to the right). The far and near rate maps encodings are binary, as in Figure 5. The maps for the comparison cell in A resemble those for the usual place cells exemplified in Figure 5; discharge is mainly in the firing fields regardless of distance between the rats. The same resemblance holds between the whole-session and far rate maps for the exceptional cell. In contrast, the

exceptional cell discharges everywhere in the cylinder when the rats are close together. It is possible that this represents random discharge that happened to occur only when the target rat was near the second rat, but a more parsimonious explanation is that this discharge signals the second rat's proximity. The difference in firing characteristics between the two cells is underscored by the plots of rate and coherence as a function of separation distance at the bottom of Figure 7. For the place cell, both rate and coherence are approximately constant at all distances. The other cell shows a distinct elevation of discharge rate for the smallest separations and an opposite trend for coherence which is very low when the rats are near each other and increases appreciably when the rats are far apart.

second, reward was given only if the distance between the rat and the car was  $<20$  cm, so long as the distance was  $>20$  cm for at least 10 s since the previous reward.

When reward was independent of car motions, place cell firing fields were mainly undisturbed. In contrast, when reward depended on the rat-to-car distance, the firing fields of many place cells remapped, in a fashion similar to the outcome of changing a rat's task, as in Marcus et al. (1995). Specifically, the remapping was ascribed to the association of reward with the car. Alternatively, remapping might have been due to the requirement for the rat to attend to the car to trigger a reward. In any case, no evidence was found that the locus of discharge moved along with the car, as would be the case if pyramidal cells came to signal the rat's location relative to the car rather than the rat's location within the environment.

In our study, the self-mobile entity was a second rat whose intrinsic properties were sufficient to result in proximity of the target rat without any need for contingent reward. We saw that the inter-rat distance was time-variant; it was smallest at the start of the 16 min session and increased with a first-order time course with a time constant of about 3.54 min. Interestingly, this is quite similar to the time constant of the exponential course that characterizes how rats explore a new environment or spend additional time near familiar objects displaced within an environment (Save et al., 1992; Parron et al., 2006). It suggests a general species-specific behavior pattern for rats confronted by novelty in their surroundings, regardless of the nature of the novelty.

Despite the obvious attraction of the rats for each other at the start of two-rat sessions, we saw no remapping relative to the preceding or following control sessions. Moreover, despite the reliable trend for the rats' movements to gradually become independent of each other during the two-rat session, place cell firing fields were time-invariant; there were no striking alterations of spatial firing distributions as the inter-rat separation increased during the session. Thus, possible motivational changes that may underlie time-dependent distance shifts were unaccompanied by any sign of remapping. In the same vein, the added stimulus complexity and moment-to-moment sensory variability associated with the second rat had no striking effects on place cell activity. We conclude that location-specific firing is, to a first approximation, unperturbed by any consequences of a second rat's presence. Thus, navigational computations are fundamentally intact in a more realistic environment.

Even if signaling in the navigational system is largely intact when a second rat is present, detectable changes are nevertheless seen. On average, place cell discharge is slower and less organized during two-rat sessions. These changes are reflected by the fact that the similarity of the spatial firing patterns between the two-rat sessions and the control sessions are lower than between the two control sessions. Importantly, these relatively subtle degradations of spatial firing patterns are not due to the mere presence of the second rat. To the contrary, the effects of the second rat increases as the two rats get closer to each other; both coherence and firing rate tend to go down as the proximity increases. Presumably, this distance dependence reflects

increasing dominance by the second rat of the total cue constellation experienced by the target rat. This proximity effect may be a direct consequence of the increased visual size of the second rat, the increased intensity of olfactory cues or of auditory signals from the second rat; even physical contact is a possible contributor. Alternatively, the proximity effect may be due to distance-dependent, directed attention by the target rat. Our results do not allow us to distinguish among these possibilities although, as noted above, direct observation indicates that the two rats are actively attracted to each other, regardless of whether the attraction itself plays a role in the marginal degradation of spatial firing patterns.

In addition to the observed modulatory effect of the second rat, it is also possible that its proximity is explicitly signaled by a subset of hippocampal pyramidal cells. In line with the work of Ho et al. (2008), we looked for such cells by asking if the discharge was more condensed if rate is plotted as a function of the distance between the two rats. In agreement with Ho et al. (2008), we saw little indication that firing represents the inter-rat distance although we found one cell whose activity seemed to combine such a signal "multiplexed" with ordinary location-specific activity. For this cell, discharge was mainly confined to two firing fields when the rats were far apart but was scattered over the whole cylinder when the rats were near each other. It is important to recognize that it would not take a large number of proximity-specific cells to add this source of information to others known to be operating including location relative to fixed landmarks (O'Keefe and Conway, 1978; O'Keefe and Speakman, 1987; Muller et al., 1987), activity confined to the vicinity of extended barriers (Rivard et al., 2004) and the apparatus boundary (Muller et al., 1987; Solstad et al., 2008). Nevertheless, it is our belief that the second rat modifies the hippocampal representation but is not manifested as a separate entity. We imagine that the rules for interacting with other animals may not utilize the geometric representation proposed to exist in the hippocampal formation.

A study showing that bilateral hippocampal inactivation impairs avoidance of a moving object suggests that the rapidly changing strategies required to stay away from it require hippocampus-based spatial cognition to keep track of its position (Telensky et al., 2011). Those behavioral results would be consistent with either the results of this study or the Ho et al. (2008) study. Either the distance-related changes in rate across the place cell population that are shown here or the more dramatic remapping of Ho et al. could underlie such behavioral results. Recordings made in the behavioral avoidance paradigm are required to distinguish between the two possibilities.

In addition to showing that a second rat leaves the operation of the hippocampal mapping system fundamentally intact, our results may provide normative data against which established animals models of behavioral disorders can be compared. Our behavioral methods are by no means as sophisticated as many developed to assay models of autism and its impairment of social activities (Silverman et al., 2010) or schizophrenia (Young et al., 2010). Nevertheless, given a consensus that a model has passed appropriate validity tests, it should be very

fruitful to extend research into the realm of single neuron recording. It would be exciting to characterize behavioral correlates of single cell discharge for model animals in both isolated and social situations. Obviously, selecting the recording target region(s) is a difficult task, but with models of schizophrenia, for example, there are reasons to believe that the hippocampus would yield interesting results. Social interactions have been investigated in another rodent species and the behaviors normally differ depending on the familiarity of the other animal and the environment, but after hippocampal lesions those differences in behavior are lost (Uekita and Okanoya, 2011). In the case of models of autism, there may be better areas in which to start than hippocampus, but it is not at all implausible that place cells in an autistic-like mouse or rat would show a different kind or degree of interference than the modest changes shown here.

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