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Special Report

A TEST OF THE TIME ESTIMATION HYPOTHESIS OF PLACE CELL GOAL-RELATED ACTIVITY

VINCENT HOK

Laboratory of Neurobiology and Cognition
Aix-Marseille Université — CNRS, Marseille, France
vhok@up.univ-mrs.fr

PIERRE-PASCAL LENCK-SANTINI

Neuroscience Center at Dartmouth, Lebanon, NH, USA
pierre_pascal.j.lenck-santini@dartmouth.edu

ETIENNE SAVE

Laboratory of Neurobiology and Cognition
Aix-Marseille Université — CNRS, Marseille, France
etienne.save@up.univ-mrs.fr

PHILIPPE GAUSSIER

Equipe ETIS, Université de Cergy-Pontoise, France
gaussier@ensea.fr

JEAN-PAUL BANQUET

Neurosciences et Modélisation
Université Pierre et Marie Curie, Paris, France
banquet@ccr.jussieu.fr

BRUNO POUCET*

Laboratory of Neurobiology and Cognition
Aix-Marseille Université — CNRS, Marseille, France
bruno.poucet@up.univ-mrs.fr

Place cells are hippocampal pyramidal neurons that discharge strongly in relation to the rat's location in the environment. We recently reported that many place cells recorded from rats performing place or cue navigation tasks also discharged when they were at the goal location rather than in the primary firing field. Furthermore, subtle differences in discharge timing were found in the two navigation tasks, with activity occurring later in the place task compared to the cue task. Here we tested the possibility that such delayed

*Corresponding author.

firing in the place task may reflect the differential involvement of time estimation, which would allow the rat to predict forthcoming reward delivery. More specifically, we reasoned that failure to obtain a reward after a fixed 2 s delay in the place task reliably reflected the rat's misplacement relative to the correct location, thus making time a valuable cue to help the rat perform the task. To test this hypothesis, well-trained rats were run on a partial extinction procedure in place and cue navigation tasks so that no feed-back signal was provided about their actual accuracy during extinction periods. Although the time estimation hypothesis predicts that only in the place task will the rat make correction movements at the end of goal periods during extinction, we found that such movements occurred in all rats, indicating correct time estimation in both place and cue tasks. We briefly discuss the results in the light of current computational theories of hippocampal function.

1. Introduction

Although hippocampal place cells mainly code for a rat's location in the current environment [15, 18, 19, 22, 23], we recently reported that they also discharge in specific ways as the animal stands at its goal location [9]. Place cells were recorded while rats performed a place navigation task in which they had to enter an unmarked circular goal zone in a cylindrical environment to release a food pellet from an overhead dispenser [13]. Task solution required the rat to locate the goal zone on the basis of its location relative to a distal landmark and stay there for two seconds to release a food pellet from an overhead food dispenser equipped with several release tubes. Since the released pellet could land and bounce anywhere in the cylinder, the rat had to leave the goal area to find the pellet and eat it before performing another navigation trial to the goal zone.

A detailed examination of place cell activity during task performance [9] revealed that, under these circumstances, many well identified hippocampal place cells discharged a few spikes when the rat was at the goal location rather than in the primary firing field [11, 25]. Interestingly, increased discharge at the goal was also found in other rats that had to solve a cued navigation task in which the goal location was consistently signaled by a salient cue on the apparatus floor. However, while cell activity increased slightly before the rat entered the cued goal zone in the cue task, the increase was offset by about one second in the place task. In other words, discharge increased later when goal location had to be calculated. We proposed that this goal-related discharge could serve as a signature of the rat's awareness of its correct placement in the goal zone. We further proposed that the delayed occurrence of goal-related firing in the place task results from uncertainty of position which would require the rat to perform some extra computations to calculate its position [9].

Another extra computation however could involve temporal estimation of the time spent in the goal zone [21]. In this view, the place and cue tasks would require time estimation to a different extent. More specifically, in the cue task the rat can

confirm its spatial accuracy in a very direct way because the goal zone is clearly signaled by the landmark on the floor. In contrast, the main feedback signal available to the rat in the place task is provided by the cues related to the food dispenser activation, which occur after two seconds spent in the goal zone. Thus, if no change has occurred in the reward schedule, the absence of reward-related cues for a well-trained rat reflects its misplacement relative to the correct location, thus requiring spatial correction. In other words, while a significant aspect of the rat's performance in the place task would involve an estimation of time spent in the putative goal zone, such estimation would be less necessary in the cue task. To test this hypothesis, well-trained rats were run on a partial extinction procedure in which the food dispenser was occasionally switched off during the initial four minutes of task performance. In these conditions, no feed-back extra-signal was provided to the animal about its actual accuracy. If only rats trained in the place task use time as an additional cue helping task performance, then they should behave differently during these extinction trials compared to rats trained in the cue task. More specifically, the time estimation hypothesis predicts that only in the place task will the rat make correction movements at the end of the 2 s period. Contrary to this expectation, however, our results revealed that all rats moved at the end of the 2 s period, suggesting that rats estimated time in both tasks.

2. Methods

The general methods are similar to those used in our previous studies [9, 12, 13] and will be very briefly described to rather focus on procedures specific to this study.

2.1. *Behavioral procedures*

Food deprived rats were trained in either the place task or the cue task before electrode implantation. The apparatus was a gray cylinder at the center of an area surrounded by opaque circular curtains. A white card covering 100° of internal arc was attached to the wall of the cylinder. When activated, a food dispenser 2 m above the cylinder dropped 20 mg food pellets on the apparatus floor. The dispenser was equipped with several release tubes that allowed varying the general area in which the pellet could land. Rats were taught the task in several steps [9, 10, 24]. In both place and cue tasks, the rats were required to enter a circumscribed zone and stay there for at least 2 s at which time the overhead dispenser was triggered to release a single food pellet (Fig. 1). The occurrence of a correct trial was recorded as the timestamp of the event. Since the released pellet bounced when landing on the apparatus floor and therefore could go anywhere in the cylinder, the rat had to forage over the entire area to find it. To trigger the release of another pellet, the rat had to spend at least 3 s outside the goal zone before it could be reentered. In the place task, the goal zone was unmarked and its location had to be calculated based on the location of the cue card. In contrast, in the cue task it was directly indicated
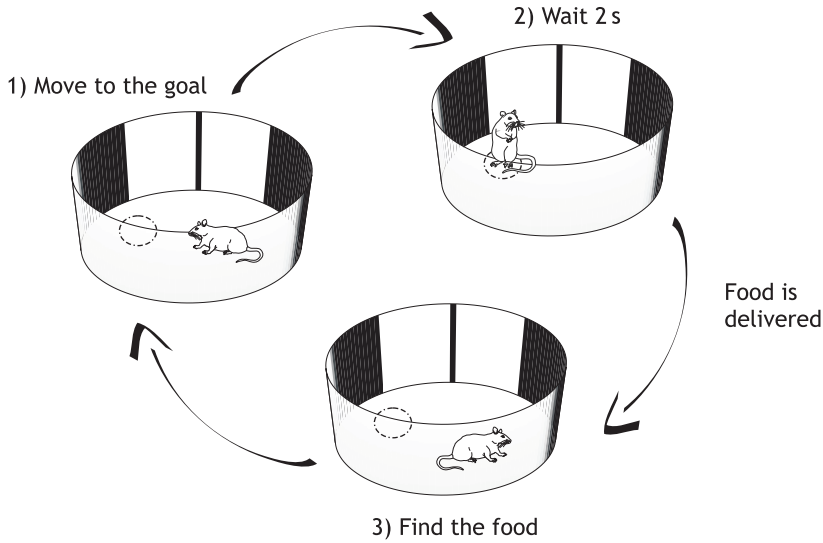
Fig. 1.   Schematic representation of the place navigation task. In the place task, the only visual landmark was a cue-card attached to the wall of the cylinder apparatus. The spatial relationship between the goal area and the wall card was constant. The apparatus used for the cue task is the same with the exception that the goal location was directly indicated by a black metal disk put on the cylinder floor. The disk was moved in a pseudo-random fashion between sessions so that it neither stayed in its previous position nor it stayed in register with the wall card.

by a black cue put on the cylinder floor, which was moved to a different location between sessions [13]. In the last step of training, rats were also familiarized with a partial extinction procedure: the pellet feeder was turned off at the beginning of each session for 4 min so that the rats had to perform without feedback provided by food delivery. The feeder was then switched on for the remaining 12 min of each session. At the completion of training, a well-trained rat made approximately two correct responses per min on average, including during the 4 min when the feeder was switched off.

2.2. *Electrode implantation and recording methods*

At the end of training, electrodes were surgically implanted in the CA1 area of the dorsal hippocampus so as to record unit activity. Light emitting diodes (LEDs) were attached to the rat's head to track the head position. The signals from each electrode wire were amplified, band-pass filtered and stored by a Datawave Discovery system. All details about the recording setup and procedures can be found in [9].

2.3. *Data analyses*

Color-coded firing rate maps were created to visualize firing rate spatial distributions [16]. Furthermore, for each place cell, comparisons were made of discharge relative to correct responses by building peri-event time histogram (PETH) in which firing

activity for the 2 s before the rat entered the goal zone ("before"), the required 2 s in the goal zone that triggered pellet release ("during") and the 2 s after pellet release ("after") was accumulated across all trials of the recording session. To obtain the estimates of activity changes at population levels, the PETH for each cell was then normalized by dividing its rate in each bin by the grand average rate. The normalized rate in each bin was then averaged across all simultaneously recorded cells. Finally, for each session, the mean speed was calculated over 500 ms intervals starting 2 s before and ending 2 s after activation of the food dispenser (i.e., 6 s later). Because our focus was on time estimation as reflected by the rat's movement in the absence of any potentially corrective information from the pellet feeder, speed was plotted only for the first 4 min of each session, i.e., during the partial extinction procedure.

3. Results

Data were collected from four rats (32 recording sessions) in the place task and five rats (27 recording sessions) in the cue task. On average, rats performed reliably on each task and made more than two correct responses per minutes during 16 min sessions. More importantly, they also performed very reliably during the initial 4 min period of each session, during which the feeder was switched off. Their overall response rate during the extinction period was 2.33 ± 0.19 entries per min and 2.32 ± 0.16 entries per min in the place and cue tasks, respectively. The consistency with which rats attempted to solve the task during partial extinction allowed us to examine their speed profiles during the goal period in the absence of any sensory feedback provided by food delivery.

Consistent with our previous findings [9], cell activity recorded from hippocampal CA1 showed a distinctive increase during goal periods. We analyzed the activity of 39 place cells recorded as rats solved the place task and 49 cells in the cue task. All analyzed place cells had well delimited firing fields away from the goal zone. Yet, most of them also fired in excess in the vicinity of the goal location in addition to their characteristic firing field (see Fig. 2a for example firing rate maps). We made a cumulative peri-event time histograms (PETHs) for all trials from all cells across all subjects after normalizing the activity of each cell separately (Fig. 2b; see methods). In these PETHs which were built separately for the partial extinction period (1–4 min) and for the remainder of the recording sessions (5–16 min), the time-course of ensemble cell activity was plotted with $t = 0$ s when the rat entered the goal zone and $t = 2$ s when the timestamp associated with the end of the goal period was automatically recorded by the computer (triggering feeder activation when necessary). The average patterns were different in the two tasks and this was true whether or not the rat was rewarded at the end of the 2 s goal period, i.e., in both extinction trials (1–4 min) and regular test trials (5–16 min). While there was a gradual activity increase peaking about 1 s after goal zone entry in the place task, peak activity occurred slightly before the rat reached the goal zone in the cue task,

(a)

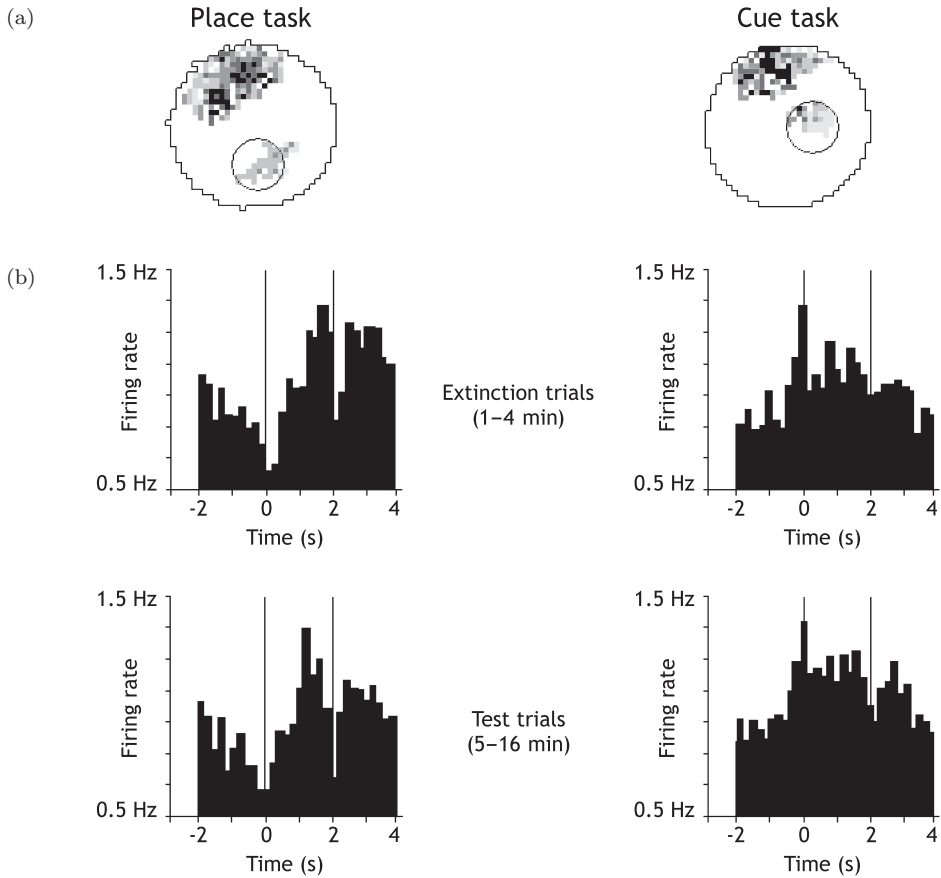Place task   Cue task

(b)

Fig. 2.   Spatial and temporal analysis of place cell firing. (a) Firing rate maps for example place cells with excess discharge in the goal zone in the place and cue tasks. Each firing rate map was generated using data from the entire recording session. In both maps, white indicates no firing and black indicates maximum firing (gray shades indicate intermediate firing rates from low to high). The goal zone is shown as a small circle within the arena explored by the rat. (b) Cumulative PETHs for all place cells recorded from rats tested in the place and cue tasks. The 2 s goal period (0–2 seconds) is bracketed by vertical lines (200 ms bins). Upper histograms are for the partial extinction period only (first four min). Lower histograms are for the remaining duration (12 min on average) of the recording sessions when the food dispenser was switched back on and the rat could get a reward on each correct response. The activity of each cell was normalized before summation was done over the sample. Note that increase in activity is delayed in the place task compared to the cue task.

thus replicating our previous findings. As said in the introduction, this effect could be explained by the differential involvement of time estimation in the two tasks, and is therefore at the origin of the current analysis of speed profiles during the 4 min extinction period.

The rats' speed profiles in the first four minutes of each session are shown in Fig. 3 for the two tasks. For each animal, the mean speed was calculated over 500 ms intervals starting 2 s before and ending 4 s after the rat reached the goal zone, i.e.,
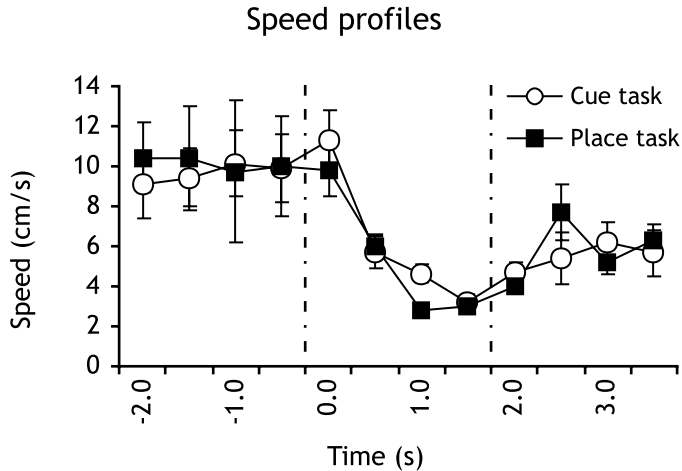
Speed profiles

Fig. 3.   Comparison of speed profiles (mean and standard error) in the place and cue task during partial extinction periods (1–4 min of recording sessions). Goal zone entry is at $t = 0$ s. Several behavioral features are visible including a marked slowing of running speed just after goal zone entry, quite similar speeds in the first and second halves of the goal period, and increased speed less than 1 s after the end of the goal period. Profiles are nearly identical for the two tasks.

2 s after the end of the goal period. The numbers of partial extinction trials used for computing these speed profiles were 298 and 250 trials in the place and cue tasks, respectively. An analysis of variance revealed no effect of task [$F(1, 7) = 0.0$, *n.s.*], a significant effect of time intervals [$F(1, 11) = 14.57$, $p < 0.00001$] and no significant interaction between the two factors [$F(1, 11) = 0.69$, *n.s.*], showing that the overall profiles were very similar in the two groups. Much as during rewarded trials (data not shown), both groups slowed down when reaching the goal zone and had low speeds during the goal period and, even more importantly, increased movement speed shortly after the end of the goal period, although the precise dynamics of such increases slightly differed. The similarity in speed profiles during partial extinction was confirmed by another analysis focusing on the two time intervals bracketing the end of the 2 s goal period, which yielded only a significant effect of time intervals [$F(1, 7) = 46.35$, $p < 0.002$] but no effect of task [$F(1, 7) = 1.09$, *n.s.*] and no interaction [$F(1, 7) = 2.45$, *n.s.*]. Since such increases occurred in the absence of any external signal, they likely reflect the rat's estimation that the usual 2 s time interval had elapsed. Finally, an analysis of speed profiles with higher time resolution revealed that the increase occurred in all rats between 300 ms and 400 ms after the end of the goal period (Fig. 4). Here again, the dynamics of speed changes differed with the place task rats displaying faster movements than the cue task rats, but there was no obvious link with the difference in firing patterns observed in the two tasks. Although it is possible that these dynamics reflect differences in the distributions of temporal estimation as measured by increases in motor activity, the number of trials is too small to build reliable distribution curves likely to support this hypothesis.
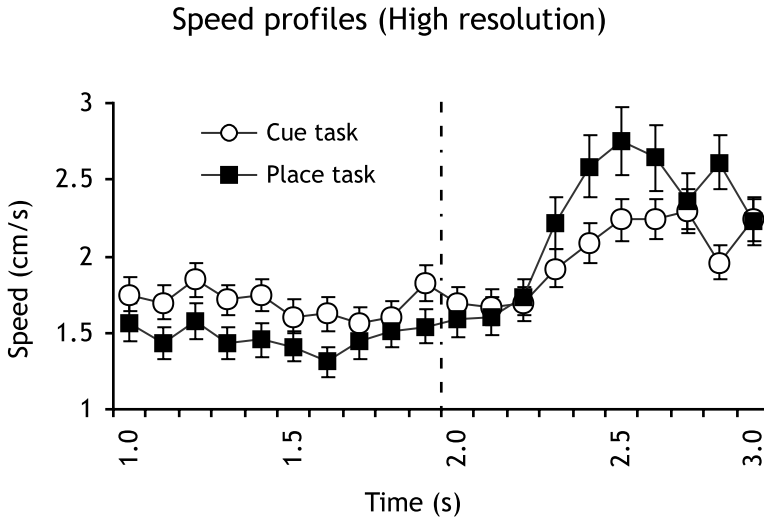
Speed profiles (High resolution)

Fig. 4.   Comparison of high time resolution (100 ms) speed profiles (mean and standard errors) during partial extinction periods. The end of the 2 s goal period (i.e., expected time for reward delivery) is at $t = 2$ s. In both place and cue tasks, a significant increase in activity occurred 300 ms–400 ms after the end of the goal period.

4. Discussion

In a recent report, we found that the timing of goal-related discharge by hippocampal place cells was offset when the rat had to solve a place navigation task compared to a cued navigation task [9]. In the present study, we examined the possibility that the differential timing of goal-related firing in the two tasks might be, at least partly, explained by a specific time estimation process arising in the place task. We reasoned that failure to obtain a reward after a fixed 2 s delay in the place task reliably reflected the rat's misplacement relative to the correct location, thus making time a valuable cue to help the rat perform the task. To test this hypothesis, well-trained rats were run on a partial extinction procedure in place and cue navigation tasks so that no feed-back signal was provided about their actual accuracy during extinction periods. The time estimation hypothesis predicts that only in the place task will the rat make correction movements at the end of goal periods during partial extinction. Contrary to this prediction, however, we found that rats did estimate time in both place and cue tasks. More specifically, in the absence of any sensory feedback related to feeder activation, rats in both groups displayed an increase in motor activity time-locked to the end of the 2 s dwell period in the goal zone. As a result, the specific temporal dynamics of goal-related discharge in the place task is not likely attributable to a different involvement of timing processes.

That rats do estimate time in the cue task even though such estimation was not necessary for solving the task is intriguing but not totally unexpected since our recordings were conducted as rats had reached asymptotic levels of performance. This occurred after extensive training with all aspects of the task, which included

procedural, spatial and non-spatial components. Since a prolonged period of learning was required, it is very likely that identification of the regularities in problem space included the specific timing of the goal episodes. In other words, the rat came to expect the occurrence of some event at the end of a specific delay after entering the goal zone during correct trials. A remaining possibility, however, is that, even though the rat measured time in the two tasks (as reflected by its behavior), the hippocampus would only measure time in the place task. For example, early activation in the cue task could be induced by the sensory consequences (e.g., tactile, visual, olfactory) related to arrival to the cue disk whereas the late activation in the place task would be caused by timing. This is a very unlikely and nonparsimonious hypothesis, which cannot be discarded on the basis of the present data however.

Since a difference in time estimation processes is unlikely to be the basis for the difference in discharge timing in the place and cue tasks, one must find another explanation. Our previous work allowed us to discard differences in attentional processes or reward expectation, which both predict a continuous discharge increase during the goal period rather than the more phasic increases that were observed [9]. Similarly, we rejected the notion that neuronal responses during goal periods were caused by states of increased excitability since the hippocampal EEG did not reveal excess high-frequency events such as sharp waves and ripples, which are usually associated with large irregular activity and enhanced neural excitability [4]. Rather, the hippocampal EEG state during goal period was characteristically in theta mode [9]. Admittedly, other hippocampal EEG states could be linked to the rat's stops in the goal zone during task performance. For example, gamma rhythm (30–80 Hz) has previously been observed to increase when a rat would decelerate (W.E. Skaggs, personal communication). Interestingly, recent models have posited a role for oscillations in the theta and gamma frequency range in the readout from long-term memory of spatiotemporal sequences regarding upcoming places as cued by current position [14]. Nevertheless, the putative link between gamma oscillations and neuronal discharge would leave unsolved the issue of the differential discharge timing in the place and cue tasks, since locomotor activity looks similar in the two tasks.

This is why our previous interpretation of goal-related firing by hippocampal place cells emphasized the generation of a feedback signal about the rat's response. Briefly, we hypothesized that small amounts of excess goal activity occurring in many cells might provide a coherent output signal at system level, which could serve as a signature of the rat's awareness that it is at the correct location [9]. In this interpretation, the proposed signal appears earlier if the rat must locate a marked goal rather than calculate a goal location simply because it requires less processing time. In the rest of this discussion, we would like to provide another tentative explanation based on a consideration of hippocampal functional role.

Many current views of hippocampal function, largely based on behavioral and electrophysiological data, emphasize the idea that the hippocampus might basically serve to associate different events or types of events that may be offset in time. Thus, the hippocampus appears to be critical when the relevant stimuli do not

occur contiguously in time [2, 26]. Noticeably, recent accounts of place cell firing also stress their sensitivity to the sequence of spatiotemporal events (including places) experienced by the rat (e.g., [1, 3, 5, 7]). Extending this idea, Gaussier *et al.* [8] proposed that a major function of hippocampal pyramidal cells might be the coding of transitions between steady states. Such coding would allow for the prediction of the next state knowing the current state. In this view, place cells would not code just for the rat's current location, but also for the possible transitions available from this location, i.e., the locations that predictably can be reached from the current location. This transition model is not restricted to spatial events (i.e., places) however, but can be applied to any type of association. Thus, pyramidal cells would not be concerned with spatial coding *per se* as much as they would be with spatiotemporal coding. Turning to the present study, it becomes clear that the sequence of events before each correct response is slightly different in the place and cue tasks. Thus, each correct response in the place task reliably involved a transition between two states (stay at goal followed by reward delivery two seconds later), possibly leading to the buildup of a single association-prediction. In contrast, the cue task presumably involved one additional transition, namely that goal arrival was predicted by the visual cue disk on the apparatus floor. If we assume that each of these transitions comes to be coded by enhanced activity in the hippocampal network, then the following sequences, respectively obtained for the cue and place tasks, cue → goal → reward and goal → reward, would result in increased firing at the goal zone. This simple model explains why increased discharge would occur earlier in the cue task compared to the place task since the cue → goal transition precedes the goal → reward transition. Although the model predicts the occurrence of two activation peaks in the cue task corresponding to each transition, a single peak encompassing the whole goal period was observed instead [Fig. 2(b)]. Arguably, this difficulty can be explained if one supposes the existence of a ceiling effect in which the activation induced by the cue → goal transition obscured the activation induced by the goal → reward association.

In short, our analysis of goal-related firing in two closely related navigation tasks makes it clear that a range of processes is involved that could explain the overall patterns of neuronal discharge. More specifically, the results appear to be compatible with two very different models of hippocampal function. In the traditional spatial map model [19], hippocampal cells not only act as regular place cells but also provide a specific code for the goal location that may be used by the rat to perform the task. In the sequential learning model [8], hippocampal cells code for transitions between stimuli that may be used to predict future events and store spatiotemporal sequences. Although both models receive extensive empirical support, disentangling which most accurately reflects the actual computations performed by the hippocampal network remains a challenging task that has raised intense debate (e.g., [6, 17]). We contend that this objective will be reached only through a multidisciplinary approach combining behavioral and physiological methods with computational and modeling studies.

Acknowledgments

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