Spatial Reference Memory is Associated with Modulation of Theta–Gamma Coupling in the Dentate Gyrus

Jean-Bastien Bott1,2,4, Marc-Antoine Muller1,2, Jesse Jackson3, Julien Aubert1,2, Jean-Christophe Cassel1,2, Chantal Mathis1,2 and Romain Goutagny1,2

1CNRS UMR 7364, Laboratoire de Neurosciences Cognitives et Adaptatives (LNCA), Strasbourg, France, 2Université de Strasbourg, UMR 7364, Strasbourg, France, 3Department of Biological Sciences, Columbia University, Columbia, NY 10027, USA and 4Present address: Douglas Mental Health University Institute, McGill University, Montréal, Quebec, Canada

Address correspondence to Romain Goutagny, CNRS UMR 7364, 12, rue Goethe, 67000 Strasbourg, France. Email: goutagny@unistra.fr

Abstract

Spatial reference memory in rodents represents a unique opportunity to study brain mechanisms responsible for encoding, storage and retrieval of a memory. Even though its reliance on hippocampal networks has long been established, the precise computations performed by different hippocampal subfields during spatial learning are still not clear. To study the evolution of electrophysiological activity in the CA1–dentate gyrus axis of the dorsal hippocampus over an iterative spatial learning paradigm, we recorded local field potentials in behaving mice using a newly designed appetitive version of the Barnes maze. We first showed that theta and gamma oscillations as well as theta–gamma coupling are differentially modulated in particular hippocampal subfields during the task. In addition, we show that dentate gyrus networks, but not CA1 networks, exhibit a transient learning-dependent increase in theta–gamma coupling specifically at the vicinity of the target area in the maze. In contrast to previous immediate early-gene studies, our results point to a long-lasting involvement of dentate networks in navigational memory in the Barnes maze. Based on these findings, we propose that theta–gamma coupling might represent a mechanism by which hippocampal areas compute relevant information.

Key words: hippocampus, gamma rhythm, mice, spatial memory, theta oscillations

Introduction

Spatial reference memory (a form of long-term memory representing the spatial, contextual, and factual aspects of a task that remains constant between trials) in rodents is widely studied as it provides insight into how the brain encodes, stores, and retrieves information. Hippocampal and parahippocampal cortices, widely accepted as the main brain areas sustaining this function, contain several cell types with distinct spatial firing patterns. More than 40 years ago, cells that code for the specific location of an animal in its environment were characterized in the hippocampus (the so-called place cells; O’Keefe and Dostrovsky 1971). Subsequently, place cells have been recorded in all the hippocampal subfields (for review, see Hartley et al. 2014). Extending the results obtained in the hippocampus, grid cells in the medial entorhinal cortex (MEC) encode the entire available environment of the animal (Hafting et al. 2005). When grid cells were discovered in the MEC, it was assumed that they were the primary spatial drive onto place cells through a feed-forward trisynaptic loop (entorhinal cortex → dentate gyrus (DG) → CA3 → CA1). Under this conceptual framework, the role of the DG was hypothesized to convert the grid cell code to a place code (Hafting et al. 2005; Leutgeb et al. 2007). However,
recent studies have surprisingly shown that grid cells of the MEC do not necessarily project to the DG and instead project mainly to CA1 (Ray et al. 2014; Tang et al. 2014). In addition, lesions of the MEC only partially disrupt hippocampal place cells (Hales et al. 2014), and a decrease in theta oscillations alters the periodic firing of grid cells but not of place cells (Koenig et al. 2011). Therefore, the precise computations performed by different hippocampal subfields in support of spatial learning remain unknown (but see Neunuebel and Knierim 2014). This paucity of data is surely related to the technical challenge underlying the study of neuronal activity across an iterative spatial learning paradigm (with few trials per day but across multiple days).

First, pyramidal cells of the cornu ammonis (CA) and granule cells of the DG are sparsely firing during exploratory behavior, drastically reducing the number of cells available for analysis. Second, it is challenging to record the same cell across multiple days of experiments.

Neurons throughout the hippocampus are synchronized on a time scale set by local theta and gamma rhythms. Hippocampal theta oscillations (4–12 Hz) have long been linked to memory processes (Winson 1978; Buzsáki 2002; McNaughton et al. 2006). Beyond theta, the hippocampus also generates gamma (25–100 Hz) activity (Bragin et al. 1995; Colgin et al. 2009; Scheffer-Teixeira et al. 2011), which is thought to bind together groups of hippocampal pyramidal neurons to ensure coordinated firing during memory processes (i.e., cell assembles, Colgin and Moser 2010). Theta and gamma oscillations interact with each other: the phase of the ongoing theta oscillation modulates the amplitude of the gamma rhythm (Bragin et al. 1995). Recent studies have suggested that this phenomenon, known as cross-frequency coupling (Tort et al. 2010), plays a key role in learning and memory (Canolty and Knight 2010; Lisman and Jensen 2013). Interestingly, the theta–gamma coupling pattern depends on the task in which the animal is engaged (Tort et al. 2008; Tort et al. 2009) and the hippocampal subfield where recording is performed (Scheffer-Teixeira et al. 2011).

The goal of the current study was to characterize the dynamics of oscillatory activity in the CA1–DG axis of the dorsal hippocampus over an iterative spatial reference memory paradigm. The most commonly used behavioral test to assess spatial memory in rodents is the Morris water maze (Morris 1984). However, despite its popularity in cognitive neuroscience, it is challenging to couple this task with simultaneous electrophysiological recordings, as the animals have to swim to a hidden platform (but see Hollup et al. 2001). The cheeseboard-task has been used as a dry alternative (Dupret et al. 2010; Dupret et al. 2013), but it instead relies on spatial working memory, as animals learn new goal locations every day through numerous massed trials. We therefore developed an appetitive version of the Barnes maze (Barnes 1979). The Barnes maze is commonly used for assessing spatial reference memory and is more adapted to mice physiology and ethology than the Morris water maze (Whishaw and Tomie 1996). In the Barnes and Morris water mazes, animals have to learn a unique goal location over multiple days of training with few trials per day. Classically, learning is followed by a probe test 24 h after the last training day to assess long-term reference memory. In our version, the mouse is not required to enter a hole to reach a safe compartment that can hamper electrophysiological recordings. In our case, the classical holes are replaced by 12 identical cups and water-restricted animals have to find the only one filled with water (the target cup). Compared with standard measures of cerebral activity such as immediate early-gene studies, the outstanding time resolution offered by electrophysiological recordings enabled analyses of transient changes in network processing in relation to task-specific behaviors. Our results show that DG networks exhibit a transient learning-dependent increase in theta–gamma coupling specifically at the vicinity of the target area and thus point to a long-lasting involvement of DG networks in spatial reference navigational memory.

**Materials and Methods**

**Animals**

Male CD1 mice (n = 4) were used in accordance with the European Committee Directive (86/609/EEC), and the project was validated by the “Comité Régional d’Ethique en Matière d’Expérimentation Animale” from the University of Strasbourg (authorization: AL/58/65/02/13). Mice were housed in individual cages (42 × 25 × 15 cm), under a 12 h light/dark cycle (light on at 7:00 A.M.) with ad libitum access to food and water.

**Surgery**

Mice were anesthetized with sodium pentobarbital (70 mg/kg, i.p) and placed in a stereotaxic frame (Kopf, USA). A 16-channel linear probe (50 μm spacing between sites, Neuronexus) covered with DiI stain (Invitrogen Molecular probes, USA) for subsequent localization of the recording sites was lowered into the dorsal hippocampus (CA1–DG axis; location, −2 mm AP, +1.5 mm ML). The use of a linear probe allowed the simultaneous recording of 16 sites spanning 800 μm. Two screws were inserted in posterior and anterior portions of the skull to serve as ground and reference electrodes, respectively. The position of the electrode during the surgery was estimated with standard electrophysiological parameters, such as multunit activity in the CA1 “stratum pyramidale” and granular layer of the DG. The precise electrode location was assessed postmortem using the DiI staining (Fig. 1B).

**Behavior**

Ten to 14 days after surgery, 4 mice were trained on the modified version of the Barnes maze (Fig. 1A). The apparatus consisted of a circular platform (1 m diameter) where 12 identical round cups (6.5 cm diameter, 3.5 cm height) were equally scattered at 1 cm from the edge of the platform. To ensure an adequate level of motivation, mice were water-restricted, a procedure more adapted to the physiology of mice than food restriction (Tucci et al. 2006). Restriction began 2 days before the start of the training and consisted of access to water 2 h per day only. Training lasted for 7 days, with 2 trials per day. After 30 s spent in a start box at the center of the maze, mice had 5 min to find the only target cup, filled with 0.05 mL of water. For each mouse, the target cup was at a fixed spatial location in the testing room, which provided various visual extra-maze cues. Between trials, the maze was wiped with a 70% alcohol solution and randomly rotated to disable direct odor-based strategy and favor spatial navigation based on allocentric cues. Each training day, and 2–3 h after the last trial completion, mice were given access to water in their home cage for 2 h, to avoid any hydric stress. Twenty-four hours after the last training session, reference memory was tested by a 120-s probe trial in which all cups were empty. The numbers of errors (visits to nontarget cups) as well as the latency to find the target cup were measured for all trials.

**Electrophysiology**

Electrophysiological recordings during the task were performed using a 16-channel Alpha-Omega SnR system (Alpha-omega)
synchronized with a video-tracking system (Noldus Information Technology). Local field potentials (LFPs) were amplified (200×), filtered (0.1–500 Hz), and digitized at 1395 Hz, and all analyses were conducted using custom-made scripts in Matlab (Matworks).

Before processing, the data were cleaned by removing artifacts (epochs of signal saturation) together with a 500-ms window around the selected artifacts. The theta epochs were detected as described previously (Klausberger et al. 2004). The theta (4–12 Hz) to delta (2–4 Hz) frequency power ratio was calculated in a 2-s window, and a ratio of >5 was considered as a theta period.

Power spectra were calculated during theta periods using the Chronux signal processing toolbox (Bokil et al. 2010) with a time–frequency product of 3 and 5 tapers. For time–frequency analysis, we used a 4-s window and moved across the data in 1-s increments. Spectral power was calculated as the integrated power throughout the data with 1-s increments. The relative MI was expressed as the MI between theta and FG per time bin divided by the grand mean of theta–FG MI for all theta periods across the entire trial.

Analyses across time were carried out with two-way ANOVA, and post hoc analyses used the Newman–Keuls or Bonferroni tests.

**Results**

**Hippocampal Theta and Gamma Oscillations during Learning**

When the start box opened, mice had 5 min to explore the maze and find the only rewarded target location. To assess learning, we calculated the number of spatial errors (visit to a nontarget cup) across training (Fig. 2). As expected, the number of errors decreased during learning (F_{6,18} = 7.267, P = 0.0002), but this effect relied mainly on differences between the first and the other days, as confirmed by post hoc analyses (Neumans–Keuls, P < 0.05 at least). In hippocampal networks, it is well established that robust theta is only observed when the animal is actively engaged in the task, whereas large-amplitude irregular activity is observed during resting and consummatory behavior (Buzsaki et al. 1983). The duration of time spent in the theta state was not homogeneous in all hippocampal subfields. Indeed, whereas it did not change across training in the CA1 “stratum radiatum” (CA1rad), we found a significant decrease in time spent in the theta state in the molecular layer of the dentate gyrus (MoDG; interaction day × recording location: F_{6,18} = 5.723, P = 0.0018, Supplementary Fig. 1). To ensure adequate comparison, we therefore computed spectral power only during the theta state in CA1rad and MoDG areas. In CA1rad, theta and gamma power were constant across the 7 training days (F_{6,18} = 1.903, P = 0.1354 for theta, F_{6,18} = 0.6644, P = 0.6792 for slow gamma (SG: 25–45 Hz) and F_{6,18} = 0.7694, P = 0.6037 for fast gamma (FG: 60–100 Hz; Fig. 3A). In contrast, in the MoDG, theta and FG power decreased over days (F_{6,18} = 6.910, P = 0.006 for theta and F_{6,18} = 7.605, P = 0.0004 for FG; Fig. 3B) with Day 1 being significantly higher than all other training days (P < 0.01 at least, Newman–Keuls post hoc analysis). However, this was not the case for SG power (F_{6,18} = 1.838, P = 0.148; Fig. 3B). Since variations in theta amplitude have been linked to...
locomotion speed (Wyble et al. 2004), we also determined whether changes in velocity across trials could explain the decrease in theta power. As expected with increased familiarity of the testing apparatus, mice spent a growing amount of time immobile (linear regression between day of training and percent time spent immobile: $R^2 = 0.7493$, slope different than 0: $F_{1,26} = 77.7$, $P < 0.0001$, Fig. 4A). However, average speed computed only during motion did not change ($F_{6,18} = 2.298$, $P = 0.0801$, Fig. 4B). To look at the relation between speed and MoDG theta power, we first divided speed (assessed in 1-s bins) in 4 quartiles during each trial and for each mouse. We then computed the mean MoDG theta power corresponding to these speed quartiles. We showed that MoDG theta power was not linearly related to speed ($R^2 = 0.6095$, slope different from 0: $P = 0.2193$, Fig. 4C). This was also the case on second by second correlations for each mouse during all trials (see examples in Supplementary Fig. 2). Therefore, and as previously shown, theta power in the MoDG is not strongly modulated by running speed (Montgomery et al. 2009).

Altogether, these data indicate that theta and gamma oscillations do not homogeneously change over the course of learning in the CA1–DG axis of the hippocampus (Montgomery and Buzsáki 2007) and tend to show a progressive disengagement of the MoDG across learning.

**Hippocampal Theta–Gamma Coupling during Learning**

In addition to the oscillatory activity per se, recent studies have suggested that cross-frequency coupling (Tort et al. 2010) between theta phase and gamma amplitude plays a key role in learning and memory (Canolty and Knight 2010; Lisman and Jensen 2013). We therefore examined whether theta-gamma coupling over the whole trial was modulated during learning. To ensure that the recorded traces were local (especially given
the proximity between the DG and CA3 region, the latter being known to generate strong gamma activity (Bragin et al. 1995), rather than volume propagated, we took advantage of the use of the 16-channel linear probe and derived the gamma trace from CSD rather than from local field potential, as already described (Laszotoczi and Klausberger 2014).

As shown in Figure 5A for CA1rad, only the 60–100 Hz gamma-band amplitude was modulated by theta phase. The modulation index (MI) was therefore calculated as the coupling strength between theta and FG. Theta–FG coupling was not modulated by learning in the CA1rad (F_{6,18} = 0.479, P = 0.8181, Fig. 5B). In contrast, we found a significant decrease of theta–FG coupling in the MoDG with learning (F_{6,18} = 16.98, P < 0.0001, Fig. 5C) with Day 1 being significantly higher than all the other days of training (P < 0.0001, Newman–Keuls post hoc
analysis). We next examined whether the decrease in theta power could account for the decrease in theta–gamma coupling, as it was previously shown that the theta–gamma coupling magnitude depends on theta power (Tort et al. 2009; Scheffer-Teixeira et al. 2011). To this end, we added both theta and FG power as covariates in the analysis. Using this approach, we still found a significant decrease of theta–FG coupling in the MoDG with learning (ANCOVA for theta–FG MI with theta and FG power as covariates: \( F_{6,19} = 10.643, P = 0.0003 \)). Therefore, and even if theta power does influence theta–FG coupling (Supplementary Fig. 3), the decrease in theta and gamma power across training days is not sufficient to explain the overall decrease in theta–FG coupling.

Hippocampal Theta–Gamma Coupling at the Vicinity of the Target

To take advantage of the high temporal resolution allowed by electrophysiological recordings, we then performed the theta–FG MI analysis on a finer temporal scale between LFP theta phase and CSD gamma amplitude. A detailed evaluation of the theta–FG coupling in the CA1rad and the MoDG across time revealed the presence of transient periods of high coupling even during the last day of training, specifically in the MoDG (Supplementary Fig. 4). Analyses of video recordings by experimenters blind to electrophysiological results revealed that these transient high coupling epochs were mostly related to target visits. We therefore analyzed dynamics of MoDG theta–FG coupling when the animals reached the target for the first time during the first and last day of learning (from \(-10\) s to \(+5\) s relative to target visit). We found that the relative theta–FG coupling at the vicinity of the target increased in the last day compared with the first one (interaction, time relative to target × day of training: \( F_{15,45} = 8.116, P < 0.0001, \text{Fig. 6A,B} \)). Interestingly, this increase in relative MoDG theta–FG coupling appeared \(3–6\) s before the animal reached the target (Bonferroni’s post hoc analysis, \( P < 0.0056 \) at least). We therefore analyzed dynamics of MoDG theta–FG coupling increased with training when the animal approached the target whereas the strength of relative theta–FG coupling decreased across training days both before and after mice visited the target. On the other hand, FG power did not change with training. (F) During the probe test, there was a sharp increase in relative MI when the mice reached the now-empty target cup (mean ± SEM; **\( P < 0.01 \), Day 7 target vs. probe). Dashed lines represent individual mice.
coupling after reaching the target did not change (interaction, time relative to target × day of training: $F_{6,18} = 3.321$, $P = 0.0222$, Fig. 6A,B). To address whether this dentate theta–gamma coupling dynamically “coded” for the target location and not only for a cup location, we computed theta–gamma coupling around the first visit to a nonrewarded cup (i.e., spatial errors) during the last day of training. We found that the transient increase in theta–FG coupling was absent for visits to nonrewarded cups (interaction, time relative to target × cup: $F_{1,45} = 3.616$, $P = 0.004$, Fig. 6C, Supplementary Fig. 5). These dynamics of theta–FG coupling cannot be accounted for by changes in speed (day effect for speed: $F_{1,3} = 1.529$, $P = 0.3042$; Fig. 6D; Supplementary Fig. 5). In addition, theta power decreased with learning (day of training effect: $F_{6,18} = 2.827$, $P = 0.0405$; Fig. 6E); being significantly higher on Day 1 than all other days before and after the target visits (except for Day 5 before the target, $P < 0.0001$, Newman–Keuls post hoc) and therefore could not account for the increase in theta–FG coupling. Finally, there was no modulation of FG power at the vicinity of the target with learning (day of training effect: $F_{6,18} = 1.777$, $P = 0.1622$; Fig. 6F), indicating that FG power could not account for the increase in theta–FG coupling. To confirm that this dentate theta–gamma coupling dynamic was related to the target location, we computed theta–gamma coupling during the probe test, where the target cup was empty. During the 10 s, immediately preceding the target visit, the theta–FG coupling dynamics was similar to that of training days but increased from 1 to 2 s after reaching the empty target (interaction, time relative to target × day: $F_{1,45} = 2.823$, $P = 0.0036$; Fig. 6F). Together, these data indicate that the transient increase in theta–FG coupling in the MoDG is related to the location of the target in our modified version of the Barnes maze. In addition, our results suggest that dentate networks might also have triggered a re-encoding of spatial information when the recalled memory trace led to an “error.”

Is this type of coding, using theta–FG coupling, specific to the MoDG or is it a more common feature of hippocampal networks? When we analyzed the dynamics of CA1rad theta–FG coupling at the vicinity of the target, we found an effect of time (time relative to target effect: $F_{15,45} = 2.180$, $P = 0.0224$, Fig. 7A) but no effect of training (training day effect: $F_{1,3} = 4.634$, $P = 0.1279$, Fig. 7A) nor an interaction between the 2 factors (interaction, time relative to target × day of training: $F_{15,45} = 1.687$, $P = 0.0886$; Fig. 7A). Since there is a “time relative to target” effect, we computed the relative theta–FG coupling for each learning day and each mouse in 2 time windows: before (from −6 to −1 s) and after the target (from 1 to 5 s), we found that the relative theta–FG coupling tends to increase with training when the animal approached the target whereas the strength of relative theta–FG coupling during target exploration did not change (significantly different from Day 7 for pretarget: “$P < 0.05$ and “$P < 0.01$). (C) This increase in relative theta–FG MI was not specific to the target as approaching other cups in the last day of training did not induce significantly different coupling dynamics. Dashed lines represent individual mice.

Discussion

We demonstrate here that the molecular layer of the DG exhibits a learning-dependent increase in theta–gamma coupling specifically at the vicinity of the target area in an appetitive version of the Barnes maze. In contrast to previous immediate early-gene studies, our results point to a long-lasting involvement of DG networks in spatial reference memory.

It is well known that the CA1 and CA3 areas of the hippocampus, through the presence of place cells (O’Keefe and Nadel 1978), play a key role in spatial reference memory. However, the DG seems to also be implicated in spatial learning. DG neurons exhibit spatially selective firing fields (Jung and McNaughton 1993; Neunuebel and Knierim 2012), and altering the activity of the DG disrupts spatial learning (Hunsaker and Kesner 2008; Hunsaker et al. 2008). Recent immediate early-gene studies have shown a progressive disengagement of dentate network with increased mastery of a spatial memory task (Poizier et al. 2008), indicating that they might rather be involved in the initial stages of learning. Our results confirm these findings by showing that MoDG oscillatory power decreases with learning when the whole trial is taken into account. Indeed, we found that MoDG theta and FG power as well as theta–FG coupling decrease with learning, over these extended periods of time. These results might seem to contradict those of Tort and colleagues who
showed a continuous increase in CA3 theta–gamma coupling during learning of a context–item association task (Tort et al. 2009). Our study (CA1 vs. DG coupling) and others (Tort et al. 2008; Scheffér-Teixeira et al. 2011) nevertheless suggest that different patterns and temporal modulation of theta–gamma coupling might co-exist within and between different brain areas.

Despite its global disengagement, a more detailed analysis of MoDG activity revealed that with training, theta–gamma coupling increased specifically just before the animal reached the target cup. We interpreted this increased cross-frequency coupling as a “memory code” dependent on task mastery that discriminates the target position as it was 1) specific to the target and 2) not directly sensory-driven by nonspatial cues since it was still present during the probe trial, when the target was empty. Moreover, since such changes can neither be attributed to changes in running speed, theta nor FG power, these results strongly support a long-lasting functional role for theta–gamma coupling in dentate networks during spatial navigation. These findings are consistent with previous studies in rodents, primates, and humans showing a role for theta–gamma coupling in memory processes (for a review, see Canolty and Knight (2010)). However, to the best of our knowledge, this is the first evidence that transient theta–gamma coupling is reported in the DG in line with a putative behavioral correlate. Together, these data indicate that theta–gamma coupling might represent a common mechanism by which hippocampal, but also cortical (Sauseng et al. 2009; Scheffér-Teixeira et al. 2011) and subcortical areas (Tort et al. 2008), compute behaviorally relevant information.

It was recently shown that action potentials in granule cells are phase locked to nested theta–gamma LFP oscillations, suggesting that specific populations of granule cells are recruited to implement task-dependent memory function (Pernia-Anrada and Jonas 2014). In addition, recent models of dentate networks suggest that rhythmic oscillations, particularly gamma oscillations could contribute to the selection of cells that receive the highest excitation level by a “winner takes all” mechanism: granule cells that received the maximum excitation being the only ones that fire (de Almeida et al. 2009). Therefore, it might be the transient increase in MoDG theta–gamma coupling with learning at the vicinity of the target area that selects which specific subset of cells fires and participates in the formation of cell assemblies. Indeed, the increased theta–gamma modulation that immediately precedes visits to the target differentiates the goal location from other highly similar cups (ambiguous clues), positioned at nonrewarded locations in the maze. This phenomenon can be conceptualized as a form of “pattern separation,” a mechanism by which the hippocampus is thought to discriminate highly similar representations and process them in a distinct, nonoverlapping fashion (Yassa and Stark 2011). The DG has been proposed to be especially involved in this process in various experimental conditions (Leutgeb et al. 2007; Treves et al. 2008; Neunuebel and Kneierim 2014). If “pattern separation” has mostly been connected to the encoding of new information, our results suggest a long-lasting involvement of the DG in spatial reference memory, through “on-line” spatial pattern separation. Indeed, the design of our version of the Barnes maze induces a persistent ambiguity through the presence of 12 identical prominent cups whereby only one contains the reinforcement. This is in line with results showing that the DG is particularly necessary for place learning in high-ambiguity conditions (Morris et al. 2012). Moreover, Lee and collaborators (Lee et al. 2009) have suggested that the DG may be necessary in aligning the internal spatial map to distal visual landmarks. Another possible role for the increase in MoDG theta–gamma coupling with learning at the vicinity of the target area is that this coupling dynamic directly codes for the goal location. Indeed, it is known that place cells of the CA1 area of the hippocampus exhibit secondary firing fields that code for the goal location (Hok et al. 2007). We may therefore postulate that the increase in theta–FG coupling before the animal reaches the target may provide information about the success of navigation toward the goal. However, as theta–FG coupling strength immediately decreased when the mice reached the target position, dentate theta–FG coupling likely reflects an active spatial computation relative to the goal position rather than a “passive” coding of its absolute position. Indeed, during the probe trial, the strength of theta–FG coupling in the MoDG immediately increased again when mice no longer found the water reward at the predicted location. Thus, the target-specific modulation of theta–FG coupling in the MoDG appears to reflect the recruitment of dentate networks in a goal-directed way during the specific approach of its position. Beyond its implication in the early phases of encoding, the DG is implicated in navigation by enabling the retrieval of discrete spatial representations of relevant points in the environment, such as goal locations, perhaps by distinguishing them from other less significant yet ambiguous positions through pattern separation.

Another striking result of our study is the dynamics of theta–FG coupling in the CA1rad region. We found that theta–gamma coupling increased with learning at the vicinity of the target cup. However, this increase in theta–FG MI was comparable between visits to the target and nonrewarded cups and therefore did not appear to code specifically for the target location. Rather, theta–gamma coupling in the CA1rad is probably more related to a global coding of the environment, as suggested by the presence of place cells that together code for all possible locations in the environment. Altogether, and as previously shown in a delayed spatial alternation task (Montgomery and Buzsáki 2007), our results point to differential modulation of gamma as well as theta–gamma coupling in different hippocampal networks during behavior. In addition, this differential modulation depends on the task in which the animal is engaged (Montgomery and Buzsáki 2007).

Therefore, our results expand the role of the DG by indicating that theta–gamma coupling in dentate networks might be implicated in spatial reference memory, enabling the retrieval of discrete spatial representations of relevant points in the environment such as goal locations.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

Funding
The authors wish to thank Dr. Adriano Tort for his excellent routine used to calculate the theta–gamma coupling, as well as C. Strittmatter, O. Bildstein, G. Edlmanny, and O. Egesi for animal care. This work was supported by a Career Integration Grant from the Marie Curie program of the European Research Council (grant # PCIG10-GA-2011-303573 to RG), the CNRS, the French Ministry of Research and Education (JBB), and the Université de Strasbourg.

Notes
Conflict of Interest: None declared.
References


