

Theta sequences are essential for internally generated hippocampal firing fields

Yingxue Wang¹, Sandro Romani^{1,2}, Brian Lustig^{1,3}, Anthony Leonardo¹ & Eva Pastalkova¹

Sensory cue inputs and memory-related internal brain activities govern the firing of hippocampal neurons, but which specific firing patterns are induced by either of the two processes remains unclear. We found that sensory cues guided the firing of neurons in rats on a timescale of seconds and supported the formation of spatial firing fields. Independently of the sensory inputs, the memory-related network activity coordinated the firing of neurons not only on a second-long timescale, but also on a millisecond-long timescale, and was dependent on medial septum inputs. We propose a network mechanism that might coordinate this internally generated firing. Overall, we suggest that two independent mechanisms support the formation of spatial firing fields in hippocampus, but only the internally organized system supports short-timescale sequential firing and episodic memory.

The hippocampus is necessary for both the storage and recall of life events^{1,2} (episodic memory). It is not fully understood how hippocampal network mechanisms support this function. Hippocampal firing is controlled not only by memory processing³, but also by sensory cues, and the respective contributions of these components to hippocampal activity have proven difficult to access^{4,5}. That makes it difficult to study the mechanisms used by the network for memory storage and recall. We aimed to identify the patterns of hippocampal firing (Fig. 1a) that are generated by the network (that is, internally) during memory processing by perturbing the internal clock of the hippocampal network while animals were exploring environments with varied demands on their memory and with varied availability of sensory cues.

First, we trained three rats to perform a two-arm, delayed alternation memory task^{6–9}, which was modified to include running on a wheel during the delay^{6–13} (Fig. 1b). The wheel was introduced to create an environment in which the animal was held in the same position while running, thereby minimizing the amount of changing sensory cues the animal was exposed to during the run. By contrast, the maze arms provided an environment in which the animals' movement through space induced changes in the sensory cues. Firing patterns of hippocampal neurons were very similar in the maze arms and on the wheel. In the maze arms, distinct neurons (place cells¹⁴) fired at particular locations. On the wheel, distinct neurons (episode cells^{10,15}, time cells^{11–14}) fired during particular periods of time since the start of the wheel run. Thus, in both environments, neurons were activated in a slow succession as the animal ran (Fig. 1a). Notably, on the wheel, firing fields were not formed unless the animal was engaged in an episodic memory task^{10,16,17}.

During running, the firing rate of neurons in the hippocampus rhythmically increased and decreased at ~8 Hz (theta rhythm)¹⁸. Theta rhythm is known to depend on inputs from the medial septum (MS)¹⁹. During individual theta cycles (~120 ms), neurons with overlapping

firing fields fired sequentially in the same order in which the animal traversed firing fields of the same neurons^{20–22} (Fig. 1a). Thus, hippocampal neurons had very similar firing patterns on the wheel and in the maze even though the prevalence of changing sensory cues was very different. This contradiction prompted us to ask whether firing patterns on the wheel and in the maze were internally generated as a result of the connectivity and interactions between neurons in the hippocampal network rather than as a result of sensory cues.

We perturbed the internally organized rhythmicity of the hippocampal network by inactivating MS and observed differential effects on the firing fields on the wheel, which were abolished, and on the firing fields in the maze, which remained mostly unchanged. In addition, theta sequences were eliminated both on the wheel and in the maze. These data suggest that firing fields on the wheel and theta sequences on both the wheel and the maze were all generated internally as a result of the hippocampal network connectivity and dynamics. We used a computational model to explore potential network mechanisms that could generate theta sequences and firing fields internally, that is, without any contribution from external sensory cues. We further experimentally explored the role these internal network mechanisms might have in the firing of neurons in the maze. We found that firing fields in a novel maze can be formed in the absence of the intrinsic mechanisms when distinct proximal sensory cues were available. However, no firing fields were formed in the absence of the intrinsic mechanisms when no distinct local cues were available. In summary, we suggest that the internally generated firing fields are built up from theta sequences as a result of the network's internal connectivity and interactions and support episodic memory of animals.

RESULTS

MS inactivation impairs memory and the wheel firing fields

To disrupt the internally coordinated activity of the hippocampal network, we perturbed the internal theta rhythmicity of the network by

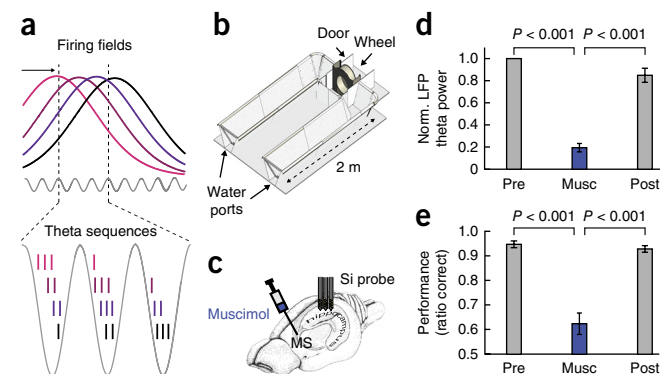
¹Janelia Research Campus, Ashburn, Virginia, USA. ²Center for Theoretical Neuroscience, Columbia University, New York, New York, USA. ³University of Chicago, Neuroscience Graduate Program, Chicago, Illinois, USA. Correspondence should be addressed to E.P. (pastak@janelia.hhmi.org).

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Figure 1 The effects of muscimol injection on theta rhythm and task performance. **(a)** Top, a cartoon of four firing fields (colored bell-like traces) and ongoing theta oscillation (gray trace). Arrow indicates the direction of the animal's run. Bottom, cartoon of theta sequences (colored tick marks) during three subsequent theta cycles (gray trace). **(b)** Two-arm alternation task maze with a wheel isolated from the maze arms by two doors. **(c)** Muscimol injection into MS and silicon probes in hippocampus. **(d,e)** Effect of muscimol injection on LFP theta power **(d)** and task performance **(e)**. 13 recordings from 3 animals. Error bars represent s.e.m.

injecting muscimol, a GABA receptor agonist, into the MS (3 rats, 15 muscimol recordings, 7 control recordings; **Fig. 1c**). After the injection, the amplitude of hippocampal theta rhythm was reduced by $81 \pm 14\%$ (Wilcoxon signed-rank test (WSR), $P < 0.001$; **Fig. 1d** and **Supplementary Fig. 1a,b**)^{23,24}. At the behavioral level, the animals' performance in the memory task was severely degraded^{24,25} (pre-muscimol: $94 \pm 5\%$ correct, muscimol: $61 \pm 15\%$ correct; WSR, $P < 0.001$; **Fig. 1e** and **Supplementary Fig. 1c**). The overall activity of the animals was not altered (**Supplementary Fig. 1d-h**), although running speeds were slightly reduced in both the maze and the wheel. The performance of the animals in the memory task was restored several hours after the injection²⁶.

Next, we asked whether the injection, which perturbed the internal rhythmicity of the network and should therefore disrupt the internally generated firing patterns, would affect activity on the wheel and in the maze differently. Firing fields on the wheel (158 neurons with firing fields from 3 animals; **Supplementary Fig. 2a-d**) and their ordered



sequences were gone after the injection (**Fig. 2a,b** and **Supplementary Figs. 3a,b** and **4a,b**). Firing field information, a measure that quantifies the prominence of firing fields above baseline (Online Methods), decreased significantly after the injection (pre-muscimol: 1.7 ± 0.8 bits, post-muscimol: 0.6 ± 0.5 bits, Kolmogorov-Smirnov test (K-S), $P < 0.001$; **Fig. 2c** and **Supplementary Fig. 4c**). We observed only a few cells that maintained or formed firing fields after the injection (**Supplementary Fig. 4a**). Notably, all of these fields were located in the first second of wheel running. The average firing rate of cells on the wheel decreased from 1.2 ± 0.1 Hz to 0.9 ± 0.1 Hz (WSR, $P < 0.001$; **Fig. 2d** and **Supplementary Fig. 4c**). In addition to firing fields, theta sequences on the wheel were also lost after the injection (see Online Methods; pre-muscimol: $7.8 \pm 0.4\%$, muscimol: $5.5 \pm 0.5\%$;

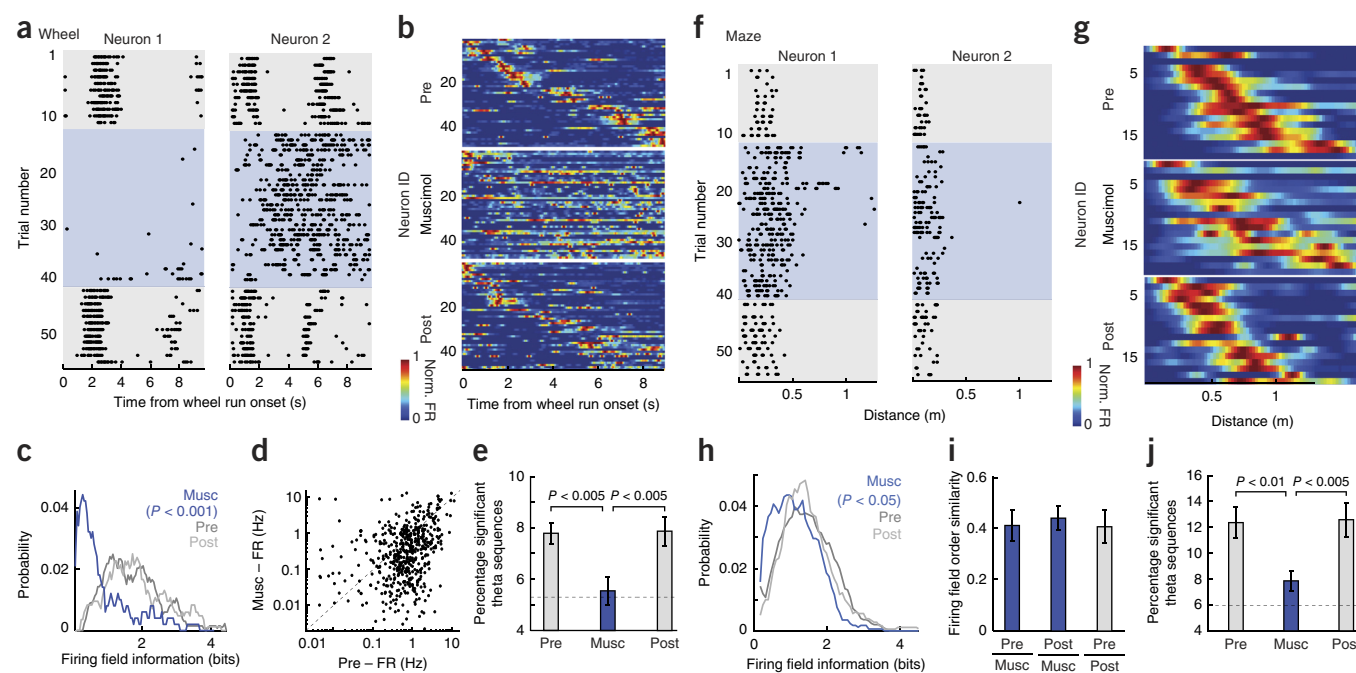
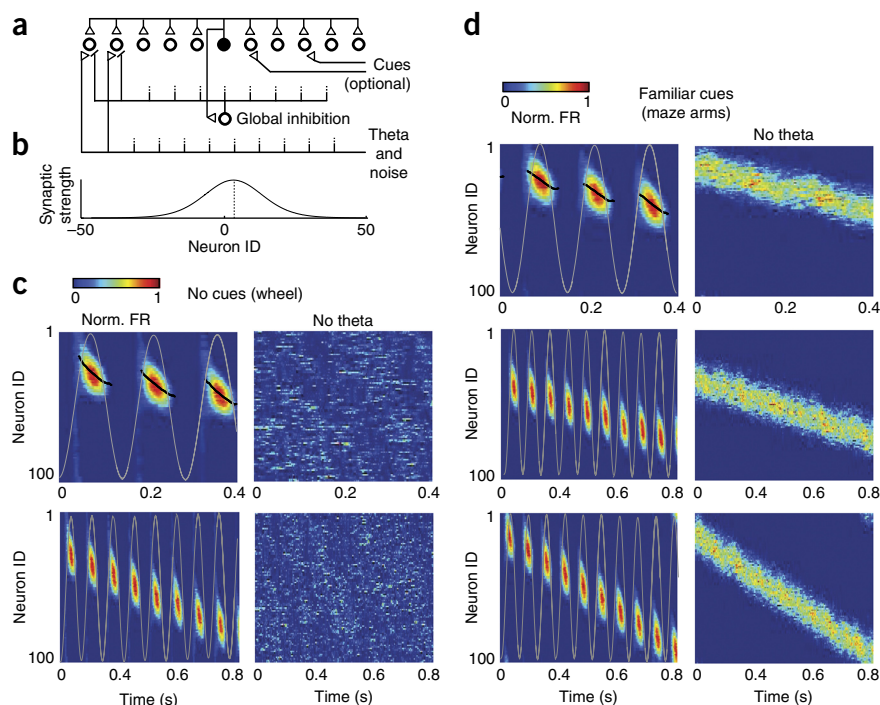


Figure 2 The effects of muscimol injection on the cell firing on the wheel (top) and in the maze (bottom). **(a)** Activity of two cells on the wheel before (trials 1–11), during (trials 12–41) and after (trials 42–51) the effect of muscimol injection. **(b)** Example sequences of firing fields on the wheel before (top), during (middle) and after (bottom) the effect of muscimol injection. **(c)** Firing field information on the wheel before (dark gray), during (blue) and after (light gray) the effect of muscimol injection. 158 neurons from 3 animals. **(d)** Firing rate of cells on the wheel before and during the effect of muscimol injection. 158 neurons from 3 animals. **(e)** Percentage of time windows with theta sequences during wheel running. 13 recordings from 3 animals. **(f)** Activity of two cells in the maze before (trials 1–11), during (trials 12–41) and after (trials 42–51) the effect of muscimol injection. **(g)** Example sequences of firing fields in the maze before (top), during (middle) and after (bottom) the effect of muscimol injection. **(h)** Cell firing field information in the maze before (dark gray), during (blue) and after (light gray) the effect of muscimol injection. 160 neurons from 3 animals. **(i)** Firing field sequence similarity between conditions. 160 neurons from 3 animals. **(j)** Percentage of time windows with theta sequences during maze running. 13 recordings from 3 animals. Error bars represent s.e.m.

Figure 3 Structure and firing patterns of the network model. **(a)** Array of neurons (circles), synaptic connections of one neuron (filled circle) and global feedback inhibition. External inputs: theta, noise and optional cues. **(b)** Synaptic strength as a function of the distance between pairs of neurons. **(c)** Network firing rate (color coded) at the high (top) and low time resolution (bottom) in the presence (left) and absence (right) of theta input. Gray trace: ongoing theta input. **(d)** Data are presented as in **c**, but in the presence of slow (middle) and fast (bottom) changing sensory cues. Gray trace: ongoing theta input.



chance level = 5.3%, WSR, $P < 0.005$; **Fig. 2e** and **Supplementary Fig. 5a–h**). These data indicate that the memory-dependent firing fields and theta sequences were mostly lost during wheel runs after the injection.

Unlike firing fields on the wheel, firing fields in the maze arms (150 cm long, 25 cm wide) were maintained^{23,24,27} (160 neurons with place fields from 3 animals; **Fig. 2f** and **Supplementary Fig. 6a,b**). There was a small, but significant, decrease in firing field information (pre-muscimol: 1.4 ± 0.67 bits, muscimol: 1.2 ± 0.8 bits; K-S, $P < 0.05$; Online Methods, **Fig. 2g** and **Supplementary Fig. 7**), but the order of firing fields along the maze arms was preserved ($r = 0.4 \pm 0.06$, Kruskal-Wallis test (K-W), $df = 2$, $P > 0.05$; **Fig. 2h,i**). In contrast with the firing fields, theta sequences were severely affected by the injection (see Online Methods; pre-muscimol: $12.3 \pm 1.2\%$, muscimol: $7.9 \pm 0.7\%$, chance level: 6%; WSR, $P < 0.001$; **Fig. 2j** and **Supplementary Fig. 8a–e**) despite the presence of abundant familiar sensory cues in the maze. Taken together, these data indicate that, although firing fields formed in the presence of sensory cues were preserved^{23,24,27}, firing fields on the wheel were mostly disrupted. Theta sequences were disrupted independently of the presence or absence of cues, suggesting that firing fields on the wheel and theta sequences in both parts of the maze depend on internally organized processing.

Network model reproduced the effect of MS inactivation

It was interesting to us that, unlike firing fields in the maze, firing fields on the wheel disappeared along with theta sequences following the injection. Given that there is no model available in the literature that would generate both theta sequences and firing fields in the absence of sensory inputs, we explored plausible mechanisms using a recurrent network model. We used an array of asymmetrically connected neurons^{28,29} (**Fig. 3a**). The strength of the synaptic connections was dependent on the distance between neurons (**Fig. 3b**). We modulated the excitability of the network with an oscillatory input (theta rhythm). All excitatory synapses were endowed with short-term synaptic depression and facilitation^{30–32} (**Supplementary Fig. 9a,b** and **Supplementary Modeling**). At the beginning of every theta cycle, as the excitability in the network increases, a few of the most excitable neurons in the network become active, initiating a ‘bump of activity’ built on the strong local connections. In the course of one theta cycle, this bump travels along the network as a result of the fact that the previously active synapses ‘behind’ the bump become depressed and the synapses in front of the bump are stronger because of the connection asymmetry. Thus, the traveling bump forms a theta sequence in the course of each theta cycle (**Fig. 3c**). Simultaneously, as the bump travels,

the excitability of currently active neurons increases as a function of the firing rate as a result of the synaptic facilitation so that neurons that were most active during the last few theta cycles tend to fire first at the beginning of the next cycle (**Fig. 3c** and **Supplementary Fig. 9c,d**). As a result, individual neurons fire during several subsequent theta cycles, forming theta modulated firing fields (**Fig. 3c** and **Supplementary Fig. 9e–g**), despite complete absence of sensory cues. Thus, in contrast with other models of theta sequences^{28,29,33}, all of which require neuron-specific (sensory) external inputs, this model provides a plausible mechanism for the construction of firing fields without any sensory cues.

We further verified that the same class of models (that is, short-term plasticity based attractor networks) was general enough to be used by animals navigating in a two-dimensional environment to generate firing fields and theta sequences in the presence of sensory cues (**Supplementary Fig. 10a**).

To test the validity of our model, we simulated the effect of MS injection by silencing rhythmic theta input into the network. This manipulation resulted in the disruption of firing fields and theta sequences in the model (**Fig. 3c**), an effect that mimicked our experimental data (**Fig. 2a–e**). This result suggests that the synchronized increase of excitability at the beginning of every theta cycle is important for the generation of theta sequences; namely, it sets up fair competition between all neurons so that the most excitable neurons were activated first, and therefore allowed to initiate the formation of the bump of activity. Without the synchronized change in the network excitability, neurons in the network were activated randomly, exerting uncoordinated excitatory and inhibitory drive on all of their neighbors. We tested whether an increase of the nonspecific input to the network would be able to support firing fields in the absence of theta, but the model generated fast sequential activity (**Supplementary Fig. 10b,c**) instead of firing fields. Thus, the firing field formation is dependent on the presence the theta input.

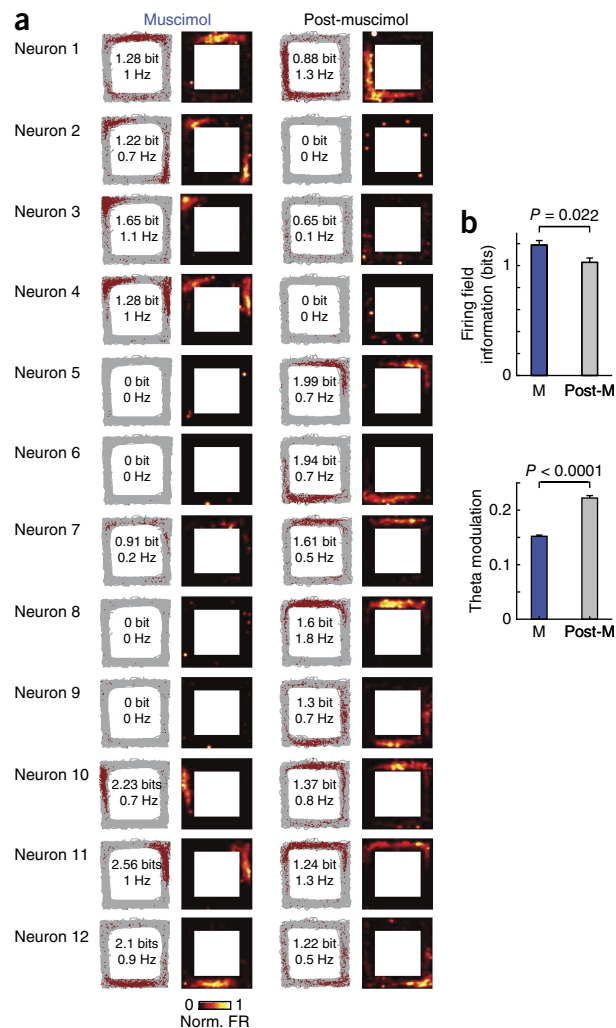
One possible cause for the differential effect of MS inactivation on the integrity of the cell firing on the wheel and in the maze is the different amount of changing familiar sensory cues available in these

Figure 4 The effects of muscimol injection on the spatial firing pattern of neurons on a novel linear track. (a) Spike maps (first and third columns) and firing rate maps (second and fourth columns) of 12 neurons simultaneously recorded on a novel linear track during muscimol (left two columns) and post-muscimol recordings (right two columns). Insets, firing field information and mean firing rate. (b) Firing field information (top) and theta modulation (bottom) during muscimol (M) and post-muscimol (Post-M) recordings. 290 neurons from 2 animals. Error bars represent s.e.m.

two environments. We tested this possibility by including familiar sensory cue input in the model: an excitatory drive that affects only a few neurons at the time and slowly moves along the network. This cue input was able to control the speed of the model firing field sequence, thereby effectively prevailing over the effect of the short-term facilitation, which supported the firing of subsets of neurons at the beginning of individual theta cycles before the introduction of the cues (Fig. 3d). Once we removed the theta input, the theta sequences were disrupted despite the presence of sensory cues, similar to the effect observed in the absence of the cues. However, firing fields were maintained with a sufficiently strong cue input (Fig. 3d), just as in our experimental data (Fig. 2f–j). Thus, these results (Fig. 3c,d) are consistent with the hypothesis that theta sequences depend on hippocampal network dynamics paced by MS inputs regardless of whether the animal is on the wheel (with no changing sensory cues) or in the maze arms (with changing familiar sensory cues). These results (Fig. 3d) also suggest that familiar sensory cues in the maze arms were responsible for the maintenance of firing fields in the maze after the injection. In summary, the short-term plasticity model provides a plausible mechanism for the generation of firing fields and theta sequences in the absence of sensory cues.

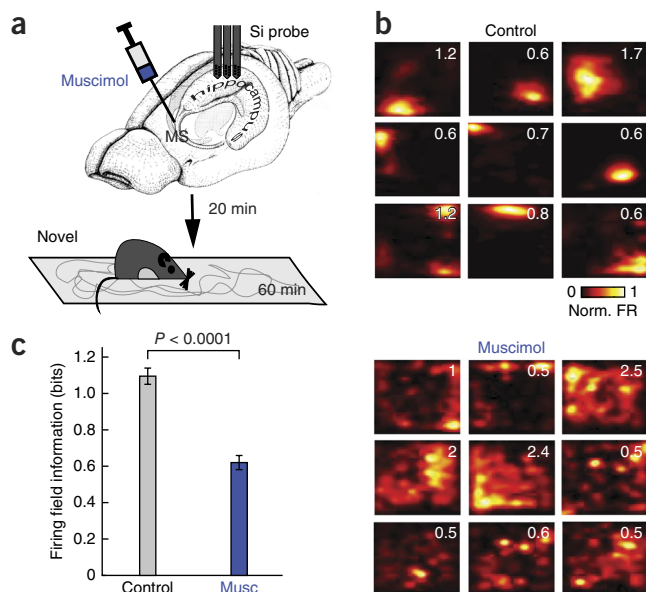
Place fields on a novel linear track after MS inactivation

Given these modeling results, which suggest that firing fields in the maze are maintained by sensory cues after MS inactivation, we asked whether firing fields can also be newly established in the absence of the internally organized activity when an animal enters a novel environment. To answer this question, we recorded the activity of dorsal CA1 neurons in animals exploring a novel linear track (1.5 × 1.5 m, 25–30 cm wide; muscimol: 290 neurons from 2 animals; control: 136 neurons



from 2 animals) after MS inactivation (muscimol recording) and then several hours later, once animals recovered from the effect of the injection (post-muscimol recording). We observed many firing fields with high firing field information on the novel linear track during both muscimol and post-muscimol recordings (firing field information, muscimol: 0.95 ± 0.04 bits, post-muscimol: 0.81 ± 0.03 bits; WSR, $P = 0.23$; Fig. 4). To ensure that the injection was effective, we compared theta modulation of the cell firing between the conditions and found a significant reduction of theta modulation during the muscimol recordings (theta modulation, muscimol: 0.15 ± 0.002 , post-muscimol: 0.22 ± 0.004 ; WSR, $P = 1.2 \times 10^{-25}$). Notably, despite the fact that the sensory cues were the same during the muscimol and post-muscimol recordings, firing fields between the muscimol and post-muscimol recordings completely remapped (Fig. 4), suggesting that firing fields formed in the absence of MS inputs are less stable than firing fields formed under controlled conditions (Supplementary Fig. 11a,b).

Figure 5 The effects of muscimol injection on the spatial firing patterns of neurons on a large novel platform. (a) An experimental design. (b) Firing rate maps of nine representative neurons obtained on a large novel platform after saline (control) and muscimol injection (muscimol). Gray numbers: mean firing rate (Hz). (c) Firing field information (control: 372 neurons from 7 recordings in 4 animals; muscimol: 223 neurons from 5 recordings in 5 animals). Error bars represent s.e.m.



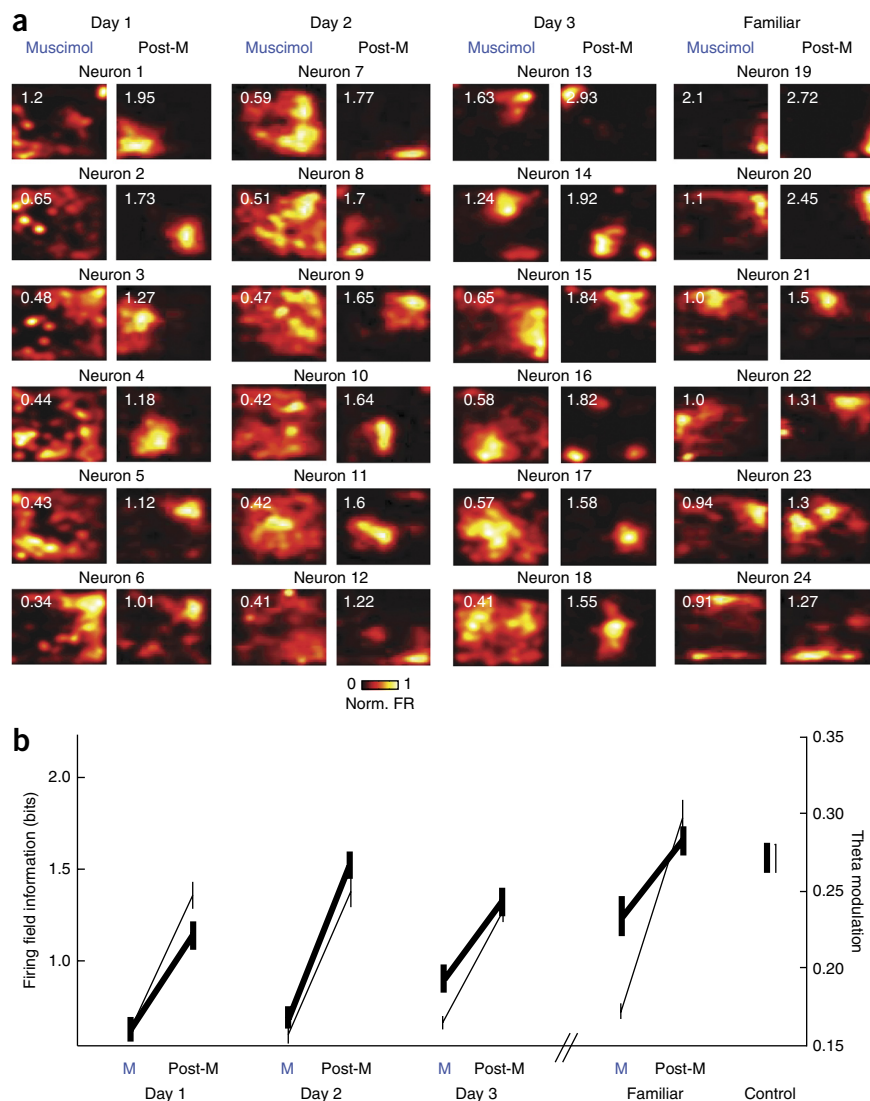


Figure 6 The effects of muscimol injection on the spatial firing pattern of neurons during repeated visits of a large novel platform. **(a)** Example firing rate maps from repeated visits of the same, originally novel environment. **(b)** Firing field information (left axis and thick lines) and theta modulation (right axis and thin lines) during repeated visits of an environment. M, muscimol; post-M, post-muscimol; Hash marks indicate 3-d familiarization procedure. Range of 39–75 neurons from 3 animals. Error bars represent s.e.m.

findings on the wheel and on the linear track (**Supplementary Figs. 4a** and **13**). After the injection, the firing field information was significantly lower (0.62 ± 0.04 bits) than in the control group (1.1 ± 0.04 bits; K-W, $H = 81.35$, $P = 1.6 \times 10^{-17}$; **Fig. 5c** and **Supplementary Figs. 12a–g** and **13**). Given that our recording sessions were relatively long (60 min), it is possible that firing fields formed, but were unstable in time or space. We therefore compared activity of neurons in 15-min blocks. However, we did not observe any stable firing fields (K-W, firing field information between four 15-min segments, $df = 3$, $\chi^2 = 6.22$, $P = 0.1$; **Supplementary Fig. 14**). We observed several changes in the behavior of animals after the injection, such as higher running speed (**Supplementary Fig. 15a–c**) and stereotyped patterns of exploration, but none of these changes could explain these results because they were not specific to the novel environment.

To confirm the idea that the size of the platform is one of the key parameters in these experiments and to reproduce previously published data³⁵ obtained on a small platform (circular: 90-cm diameter, rectangular: 90×90 cm), we repeated the key experiments on a platform that was about three-fourths the size of our original platform (223 neurons from 2 animals; **Supplementary Fig. 16a,b**). We reproduced data from the published study³⁶ in that one animal formed and one animal did not form place fields on a small novel platform. We suggest that the group statistics differences between our data and the previous study's data³⁶ might diminish once the same criteria for the cell selection are used, that is, when firing information in all well-isolated neurons is taken into account.

Multiple visits are required to form compound place fields

We found that no firing fields were formed when an animal entered a large novel environment after MS inactivation. However, muscimol injection did not prevent reactivation of previously established firing fields²⁷ (**Fig. 2**). How much experience with the environment did animals have to have before firing fields become independent of MS inputs? To answer this question, we exposed three animals to the same environment repeatedly. During recording days 1, 2 and 3, we injected MS with muscimol and let animals explore a large novel platform (range of 39–75 neurons from 3 animals). Firing field information remained low during all three muscimol recordings (day 1: 0.63 ± 0.07 bits, day 2: 0.69 ± 0.06 bits, day 3: 0.9 ± 0.08 bits; **Fig. 6**). However,

No place fields on a novel platform after MS inactivation

We found that MS inactivation prevented the generation of firing fields during wheel running. Based on our model, fields on the wheel were formed as a result of the network's ability to transiently store information about its previous activity in the strength of the synapses and to recall this information at the beginning of every theta cycle. Thus, theta sequences in the model were dependent on the network's ability to integrate and store information about its recent activity. In contrast, we found that MS inactivation did not affect firing fields on a narrow linear track with an abundance of sensory cues, where integration of the internal state of the network might not be necessary. Thus, we asked whether the MS-paced network activity is required for the generation of firing fields under the conditions in which animals had to rely to a greater extent on their memory and to a lesser extent on sensory cues³⁴.

To test this, we injected muscimol into the MS and let animals explore a large novel platform (1.5×1.5 m; **Fig. 5a**). We found that hippocampal neurons were active almost independently of animals' position (muscimol: 145 neurons from 5 animals, control: 247 neurons from 4 animals; **Fig. 5b** and **Supplementary Figs. 12a–g** and **13**). In some recordings, a few neurons formed firing fields in close proximity of walls and corners of the platform, corresponding well with our

when animals returned onto the same platform at the end of each day, once they recovered from the injection, we observed normal firing fields with high firing field information (day 1: 1.1 ± 0.08 bits, day 2: 1.5 ± 0.07 bits, day 3: 1.3 ± 0.08 bits; **Fig. 6**). Given that the firing fields were sensitive to MS inactivation even after 3 d of exploration, we familiarized animals with the platform. Only after a 3-d familiarization without any injection did firing fields become impervious to the effect of the inactivation (1.24 ± 0.1 bits; **Fig. 6**).

DISCUSSION

In summary, we found that hippocampal firing fields can be formed by two distinct mechanisms. First, firing fields can be formed internally as a result of network interactions during wheel running (**Fig. 2**). Generation of the internally generated fields is closely linked with episodic memory, as these fields were formed only when animals were engaged in an episodic memory task^{10–13,15–17}, their initial development was accompanied by an improvement of animals' performance in the task^{13,15} and they were eliminated by the same manipulation that impaired animals' ability to solve the memory task (**Figs. 1 and 2**). Notably, even well-established internally generated firing fields were abolished by MS inactivation (**Fig. 2**). We propose a network mechanism that might be responsible for the formation of these fields (**Fig. 3**). Specifically, using a network model, we found that firing fields can be built up from theta sequences, in other words that the combined effect of short-term plasticity, recurrent connectivity and theta input can create 'meta-field' structure in the firing of neurons. Thus, we suggest that theta sequences are the hallmark of the internally generated firing fields and that theta sequences, along with the internally generated fields, are the physiological substrate of episodic memory³⁷.

Second, firing fields can be supported by sensory cues (**Fig. 4**). Sensory cue-supported firing fields appeared to be located very close to dominant cues such as walls and corners of an environment³⁶ (**Fig. 4**). Sensory cue-driven firing fields were stable over at least 1-h-long recordings. These fields were formed while MS was inactivated and the power of theta oscillations was very low (**Fig. 1**), that is, under the conditions when no theta sequences were likely formed (hollow firing fields; **Fig. 2**) and when episodic memory of animals was likely impaired (**Fig. 1**). Notably, however, these sensory cue-based fields completely remapped once an animal entered the same environment with MS recovered (**Fig. 4**), that is, once the mechanisms generating theta sequences and internally organized firing fields were likely restored. It is possible that firing fields observed in bats³⁸, which also seem to be formed in the absence of theta oscillation, are driven by sensory cues.

We suggest that, under normal conditions, as on a familiar platform, firing fields are supported by both an internally organized network mechanism and sensory cues (compound firing fields), given that, on the one hand, they did not form in the novel environment in the absence of MS inputs (**Fig. 5**) and, on the other hand, once they were well-established they were maintained in the absence of septal inputs²⁷ (**Figs. 2 and 6**). Notably, in contrast with sensory-based firing fields, compound fields did not remap after MS inactivation^{27,39} (**Fig. 2**), suggesting that firing fields formed in the presence of MS inputs are more robust.

We did not address the question of whether self-generated cues⁴⁰ are required for the formation of either type of firing fields. It is likely that some kind of coordination between hippocampal activity and body movement is required when the sensory and compound firing fields are generated so that the hippocampal activity can account for changes in the running speed, head direction, etc. However, we do not know how such coordination might be implemented. For example,

it is unknown whether firing of hippocampal neurons coordinates and guides body movements^{38–41} or whether body movements guide hippocampal cell firing. In addition, we do not know how to differentiate between impaired ability to integrate self-generated cues⁴² and impaired ability to remember own past actions (**Fig. 1**). Thus, the potential role of self-generated sensory cues will have to be investigated in future experiments. It is interesting to note, however, that grid cells, which have been suggested to serve as a substrate of path integration⁴³, are perturbed by MS inactivation^{27,39}.

We used a short-term plasticity based attractor network model to propose plausible mechanism that can generate meta-firing fields and theta sequences in absence of sensory inputs (**Fig. 3**). The same class of models can account for several experimental results on hippocampal dynamics as two-dimensional firing fields⁴⁴, reactivation of short-timescale (ripple) sequences⁴⁴ and phase precession^{28,29,33,45}. However, our model is unique because it can generate theta oscillation-dependent and sensory cue-independent firing fields (**Fig. 3**).

Based on our model we proposed that the role of the rhythmic inputs from MS is to reset the network activity and thus to enable generation of theta sequences. Our model is in stark contrast to previously proposed mechanisms^{28,29,33} in that it predicts that the order of neurons in the theta sequences dictates the order of internally generated firing fields, not the other way around. Our model also suggests that this order is determined by the recurrent connectivity within the CA3 network⁴⁶ and then inherited by CA1 neurons⁴⁷. This prediction is in line with two findings. First, that short-time scale sequences observed during a novel environment exploration are 'pre-played' during the CA3 triggered sharp-wave ripple events that precede exploration of a novel environment⁴⁸ and second, that the sensory cue based firing fields remapped once theta sequences were activated (**Fig. 4**).

How might the theta sequence-based, internally generated firing fields be associated with sensory cues during the formation of the compound firing fields? We propose that the CA3-based internally organized activity of specific cells is associated with sensory cues perceived at specific locations through experience. Given that MS inactivation can eliminate a large portion of established CA3 firing fields²⁴, it is possible that the association between the internally generated fields and sensory cues is established on CA1 rather than on CA3 neurons. Specifically, it is possible that information about the sensory cues is brought onto the distal dendritic tufts of CA1 hippocampal neurons through cortical inputs⁴⁹. We suggest that CA1 neurons associate the internally generated, spatially tuned CA3 inputs with the entorhinal cortex sensory inputs as the animal explores the large novel environment. Once this association is established through the adjustment of synaptic plasticity⁵⁰, the cortical inputs might become sufficiently powerful to support firing fields in a maze, even when the CA3 network dynamics are compromised.

In the absence of the spatially tuned CA3 inputs, strong sensory cue-driven inputs alone might be able to support firing field formation, as on a novel linear track after MS injection. However, once the CA3 inputs become available, they have a strong influence over CA1 cell firing as a result of the proximity to the cell bodies. Thus, the internally organized firing patterns might over-write the original, sensory cue-based firing fields.

In summary, we propose that firing fields in hippocampus can be supported by proximal sensory cues in the absence of MS inputs, theta and theta sequences. However, these fields likely do not support episodic memory of animals and are eliminated once the internally organized mechanisms are activated. In contrast, compound firing

fields support episodic memory as a result of their internally organized component, the signature of which is theta sequence.

METHODS

Methods and any associated references are available in the [online version of the paper](#).

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

Y.W. and E.P. design the study. Y.W., E.P., A.L. and B.L. analyzed the data. S.R. built the model. All of the authors discussed the data analysis and the model and commented on the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

All procedures were approved by the Janelia Research Campus Institutional Animal Care and Use Committee.

Alternation task training. Three male Long-Evans rats (~300 g) were housed in cages (Tecniplast) with custom-made running wheels. Animals were water-restricted following standard procedures. During training, animals were placed into an in-house built maze (Fig. 1b) controlled by an Arduino-based board (Arduino mega 2560). The maze contained a running wheel (10-cm depth, 29.5-cm diameter, Lafayette Instrument, 8086W) located in the delay area of the maze was equipped with an optical motion detector (HB6M-500-500-I-S-D, US Digital). The delay area was isolated from the maze arms by two automated doors. The wall of each maze arm housed beam breaks (Omron E3F2-R4C4-P1) to detect the presence of the animal (Coulbourn H20-94). The end of each arm was equipped with water ports with lick-detectors for water reward delivery. Behavioral events, including instantaneous wheel running speed, door closing and opening, beam breaking, water delivery, and licking were recorded during every training session.

Before the training started, animals were allowed to explore the maze and discover the locations of water ports while both doors were kept open. Once the animals were familiar with the environment, they were placed into the delay area with the doors closed. To open the doors, animals were required to run in a wheel for a predefined period of time. This time period was initially set to 2 s and was increased at the beginning of each training session until 9 (rat 1) or 10 (rats 2 and 3) s. If the running speed dropped below 18.5 cm s^{-1} at any point during a wheel run, the time counter reset to zero, and a new wheel run needed to be initiated. At the end of running, both doors opened and animals were free to choose the left or right arm. Animals were rewarded with a drop of water (about 0.15 ml) for every correct (left-right alternating) choice. Animals typically reach 85–95% correct performance in about 10 d.

Surgery. Ten animals were implanted with a guide cannula (26G, Plastic One) aimed 1 mm above MS (AP: +0.6, ML: –1, V: 4.7, angle 10 degree toward mid-line) and two 64-channel silicon probes targeting the CA1 region of hippocampus in both hemispheres (AP: –4, ML: ±3) (Neuronexus or Janelia RC). Details of the surgical procedure were described previously⁵¹. Recording sites of the in-house made probes were coated with poly(3,4-ethylenedioxythiophene) film to lower impedance. We did not observe any noticeable differences between signals recorded with different probes. The silicon probes were slowly lowered into hippocampal CA1 pyramidal layer 1–2 weeks after the surgery.

Opened field and linear track recordings. After animals fully recovered from the surgery (approximately 2 weeks after surgery), we restricted their access to water. Animals were brought to a familiar recording room and plugged into the recording system. Then they were placed onto either novel or familiar linear track (150 cm × 150 cm, 25–30 cm wide), small platform (circular: 90 cm diameter, rectangular: 90 × 90 cm), or large rectangular platform (150 cm × 150 cm) with walls. To motivate exploration, water was sprinkled onto the surface by an experimenter approximately every 2–4 min.

Recordings. During physiological recordings, data from all channels were filtered (0.3 Hz to 10 kHz), amplified (gain = 400) and continuously sampled at 20 kHz using the Ampliplex system⁵² (16-bit resolution). One small light-emitting diode was fixed on the top of the implant to track the animal's position with an overhead camera (30 Hz, Ampliplex⁵²). Time stamps of behavioral events from the maze, electrophysiological recordings, and tracking data were synchronized, recorded and stored on a computer. A typical alternation task recording with muscimol injection was composed of three sessions. Pre session: 25–30 trials. Muscimol session: ~20 min after injection, over 40 trials. Post session: ~4 h after injection, 25–30 trials. A typical open field recording was composed of two sessions. Muscimol session: ~20 min after injection, recorded for ~1 h. Post session: ~6 h after injection, recorded for ~1 h.

Intracranial injections. Muscimol injections were carried out as follows. First, an injection cannula (33 Ga, Plastic One) was connected to a 10- μl Hamilton syringe through Tygon tubing (Tygon 720993) and filled with muscimol (0.125 mg ml^{–1}) or saline (0.9%). Then the syringe was mounted into a microinjection pump

(UMP3 with SYS-Micro4 controller). At the beginning of the injection procedure, the dummy cannula was removed from the guide cannula and replaced by the injection cannula which extended 1 mm deeper into the brain than the guide and dummy cannulae. Then 500 nl muscimol or saline were slowly injected (80 nl min^{-1}) into MS and the injection cannula was left in place for another 2 min after the injection was complete. Next, the dummy cannula after cleaned with alcohol and dipped in sterile mineral oil was inserted back into the guide cannula. Animals explored freely and were fed with treats during the injection. Animals did not show any sign of stress or discomfort during the procedure. ~20 min after the injection, animals were placed into the delay area of the maze or onto the opened field and recording started.

Spike sorting. To identify spikes from the same neurons across pre, muscimol and post sessions, we first merged data from all three sessions. We performed off-line spike sorting on the merged files following published methods using Klustakwik⁵³.

Data analysis. We analyzed data during wheel and maze running period separately. Each wheel run was aligned to the beginning of the 9 or 10 s of running before the doors opened. A maze run was defined as the period between when the animal entered the straight part of a maze arm and arrived at the water port. We separately analyzed out-bound (toward the water port) and in-bound (toward the wheel) maze runs because different sets of neurons can be involved.

Statistics. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications^{11,23,24,29,42}. The data collection was not randomized since each animal was tested during both, control and testing conditions. Muscimol and saline injection experiments were interleaved. Data collection and analysis were not performed blind to the conditions of the experiment, however all data collection and data analysis was performed by two experimentalists to ensure reproducibility of the results. The same experiment was repeated with each animal at least three times to ensure reproducibility of the results. We did not see much difference in s.e.m. among pre, muscimol and post sessions. We used non-parametric statistical test to estimate significant differences between groups: WSR test, Spearman correlation and K-S test. All of our tests were one sided.

LFP power. Local field potential power spectra were estimated using Thomson multi-taper methods (NW = 4, 30). The peak power in the theta frequency range (6–10 Hz) was detected, normalized by the value recorded during the pre-session and averaged across trials (Fig. 1d).

Episode cell firing fields. Time stamps of all spikes (down-sampled to 1,250 Hz) generated during the 9–10-s wheel running were isolated. In each trial, spike times were referenced to the beginning of the wheel run. The spike train of each neuron was first smoothed with a Gaussian function (s.d. = 75 ms) and then averaged across trials. This smoothed firing rate was used to identify firing fields (episode fields).

To identify episode fields, we used the following set of criteria (Supplementary Fig. 17a): for each neuron we calculated the mean firing rate (FRm) and ratio of the peak to mean firing rate (FRp2m). We selected neurons with FRm ≥ 0.1 Hz, and with a FRp2m ≤ Thp2m (with Thp2m = 3, see below). Then, for each neuron, we identified segments in the smoothed firing rate profile where the firing rate was larger than or equal to a threshold determined by the FRm * Thp2m. To make sure the firing rate fell back down outside the fields, we used two thresholds Thl and Thh, with Thl = 0.3*FRm, and Thh = 0.9*FRm [1]. On each side of a high firing rate segment, we detected the time points at which the firing rate fell below Thh and Thl for the first time. If the firing rate dropped below Thl and the time spent between Thh crossing and Thl crossing (field margin) was small, then a potential field existed between the two Thl crossing time points. The potential field was considered a real field if it also fulfilled the following criteria: there were enough spikes within each field (at least two spikes per trial on average), the size of the field was not larger than 4 s, and in at least 40% of the trials there were spikes within the field.

We separately analyzed wheel runs before the left and right arm maze runs, as some neurons have shown different activation patterns before the left and right maze trajectories¹¹. On average, we isolated the following number of episode cells per recording: rat 1, 42.8; rat 2, 15; rat 3, 16.38.

Default parameter values are given. The field detection algorithm was run based on these default values and the results were visually inspected. Some parameters were adjusted if there were obvious false positive or true negative errors in the field detection.

Firing field sequence of episode cells. We took all the excitatory neurons with at least one episode field in either pre- or post-session, and ordered them according to the latency to the center of their episode field(s) (T_c ; Fig. 2b). If a neuron had multiple episode fields in pre-session, T_c would be measured based on the field with the largest peak firing rate. If no episode field was identified in pre-session, T_c would be calculated based on post session.

Firing fields of place cells in the alternation task. Firing fields of place cells were detected using the same method as described above using firing rate profile in time. We also constructed the spatial firing rate map for each neuron by identifying the animal's location in the maze at the time of each spike, smoothing spikes with a Gaussian function (s.d. = 17.5 pixels), and normalizing by time spent within each location. The difference between the firing profile in time and distance is small due to the short length of the maze arms. On average, we isolated the following number of place cells per recording: rat 1, 19.6; rat 2, 11; rat 3, 10.33.

Firing field sequence of place cells. See firing field sequence of episode cells above.

Firing field sequence similarity. For each session (pre, muscimol, post), we first generated a vector, each element of which is the latency to peak firing rate (or the spatial location of peak firing rate) of each neuron in the episode (or place) field sequence. The field sequence similarity is defined as the Spearman correlation between each pair of vectors from different (pre, muscimol, post) sessions.

Firing field of place cells in the opened field. The position of an animal in the open field was detected at the time of each spike. A spike map and a firing rate map were constructed for each cluster. To smooth the firing rate map, we used the adaptive binning technique⁵⁴.

All clusters that satisfied the following criteria were used for the analysis: mean firing rate > 0.4 Hz and < 5 Hz; refractory period violation < 0.3; Mahalanobis distance from noise cluster > 10 and minimal spike amplitude 200 μ V. Note that even cells with no clear place field were included in the analysis.

Firing field information. $I = \sum \lambda(x)/\lambda * \log_2(\lambda(x)/\lambda) * p(x)$ (ref. 55), I : information (bit), $\lambda(x)$: firing rate in a time bin (or spatial pixel x), λ : mean firing rate across firing rate profile, $p(x)$: probability of an animal being in time bin (or spatial pixel x).

Firing field information (Figs. 2c,h, 4b, 5c and 6b, and Supplementary Figs. 4c, 7, 11b, 12b and 16b) was calculated either on the episode cell firing field profile over time as defined above, or on the place cell firing field map over distance as defined above.

Sparsity. $S = \sum (\sum p(x) * \lambda(x))^2 / \sum p(x) * \lambda(x)^2$ (ref. 55), S : sparsity (ratio), $\lambda(x)$: firing rate in pixel x , $p(x)$: probability of an animal being in pixel x (Supplementary Fig. 12c).

Frequency of single unit oscillation and theta modulation of spike trains. Each spike was convolved with a narrow Gaussian function (s.d. = 1 ms) and an auto-correlogram of spikes was calculated for each neuron. An auto-correlogram of a theta modulated neuron showed firing rate peaks symmetrically distributed around zero at multiples of ~120 ms. Power spectra density of each auto-correlogram was estimated using the Thomson multi-taper method (time-bandwidth product $NW = 4$)^{56,57}. Theta modulation was calculated as a ratio between the sum of the power in the theta frequency range (6–9 Hz) and in a broader frequency range (5–40 Hz) (Figs. 4b and 6b, and Supplementary Figs. 3b, 4c, 7, 11b, 12d and 16b).

Theta phase estimation. One LFP trace recorded from the middle of the CA1 pyramidal layer was filtered with a second order Chebyshev Type II filter (4–25 Hz). The peaks, valleys and zero crossing points were detected and assigned as

phase 0, 180 and 90 or 270, respectively. Phases between these points were linearly interpolated⁵⁸. Peaks in the LFP corresponded to the minimum firing of CA1 pyramidal cells. This method has been shown to give a more sensitive estimate of theta phase than instantaneous phase estimated based on the Hilbert transform, because the shape of theta cycles is frequently asymmetric⁴⁴.

Theta sequence detection using a sliding window (Fig. 2e,j and Supplementary Figs. 5a,b and 8a,b). To make sure we have enough neurons covering the entire wheel and maze running period and sufficient overlap between firing fields of different neurons, we only selected recordings with more than eight episode or place cells.

After reduction of theta oscillations under muscimol condition, identification of theta cycles becomes unreliable. As a consequence, it is problematic to use the identified theta cycles as individual units to detect theta sequences^{59,60}. Therefore, we chose to use a sliding window with a constant length. This window length was determined by the averaged theta cycle length in each individual session (pre, muscimol or post). Although detecting individual cycles precisely is hard, the averaged theta cycle length should reflect the actual cycle length more accurately. The time window slid with a step size of 25 ms.

We only selected those time windows in which the averaged running speed was above threshold (wheel > 18.5 cm s^{-1} , maze > 30 cm s^{-1}), and which had at least two active neurons ($N \geq 2$). Controlling the number of active cells helped (at least partially) to compensate for the firing rate change and the change in number of active cells per window between pre/post and muscimol conditions.

Our sequence detection method was similar to the algorithm described previously^{45,46}. The detection was separated into three steps. In step 1, we derived a sequence score for each time window. The sequence score described how similar the order of spikes within that time window was to the order of firing fields of the same neurons. Specifically, for each pair of spikes we added +1 to the sequence score, if the order of spikes was the same as the order of the episode/place cells in the field sequence. Otherwise, we added -1. The sum of scores across all pairs of spikes within the time window determined the sequence score.

In step 2, we determined whether the sequence was a good sequence by comparing the calculated sequence score to the scores from the randomly shuffled data (Supplementary Fig. 17b). We shuffled spikes in the time window, and re-calculated the sequence score as described in step 1. Shuffling was carried out by randomly reassigning the identity of each spike to a neuron which was active in the current time window, while preserving individual spike timing. The shuffled sequence was then checked to make sure it had the same number of active neurons as the un-shuffled sequence. This method shuffles both the identity of the cells and the order of spikes. It allows sequences with as few as two cells to be identified, providing that there are enough spikes in the sequence. The shuffling was repeated 10,000 times. If the un-shuffled sequence score was > 95% of the shuffled sequence scores, then the sequence was considered as a significant sequence. This means only significant sequences with $P < 0.05$ were considered in the analysis (Fig. 2e,j).

In step 3, we calculated the percentage of significant sequences, and compared the value with that detected from the randomly shuffled data (Supplementary Fig. 17b). The percentage of significant sequences is defined as the number of detected significant sequences divided by all qualified sequences. A sequence is 'qualified' if, after properly ordering all the spikes in the sequence, the sequence score can reach the significant level. We then took the shuffled data in step 2 and calculated the percentage of significant sequences using each shuffle across all the time windows. Based on the 10,000 shuffles, we estimated the distribution of percentage of significant sequences detected by chance. If the percentage calculated using true data was located above 95% of this distribution, then the recording session is considered to have significantly more theta sequences than chance. The chance level in Figure 2e,j showed the 95% level estimated with all the shuffled data across all recordings.

In our analysis (Fig. 2e,j), a recording was selected only if it satisfied the following criteria. First, the recording should have significantly more theta sequences than chance level in the control sessions (pre or post session). Second, the number of qualified sequences in each session is >100 to assure that the percentage of significant sequences is not biased by small number of samples. Third, the percentage of qualified sequences (number of qualified sequences/total number of time windows) is not substantially different (ratio ≤ 7) between sessions in the same day. As a result, there were nine recordings from three different animals

satisfied all these criteria in the wheel. In the maze, five recordings from two different animals satisfied all the criteria.

Compression index (Supplementary Figs. 5c–e and 8c). A theta sequence can also be described by compression index²³, which is the correlation of the distances between pairs of firing fields and the mean temporal offsets of the action potentials of the same pairs in a theta cycle.

Only pyramidal neurons with identified firing fields and pairs of pyramidal neurons with sufficiently overlapping firing fields (more than 50 counts in a ± 0.7 -s-long cross-correlogram, CCG) were used for this analysis. We used 3-ms time bins to calculate CCGs. To calculate the theta time scale temporal offsets between pairs of neurons, each CCG was smoothed with a narrow Gaussian function (SD-narrow = 12 ms) and the peak of the smoothed CCG was detected within ± 210 ms. To derive the distances between pairs of firing fields, the same CCG was also smoothed with a wide Gaussian function (SD-narrow = 150 ms) and the peak of this broadly smoothed CCG was detected within $\pm 1,650$ ms. Theta-time scale peaks larger than 195 ms were discarded. Firing field peaks larger than 1,500 ms were discarded. The theta timescale and firing field time scale peak values were plotted against each other for pre, muscimol and post condition. Spearman correlation between theta timescale peaks and firing field peaks was calculated for each recording.

Multiple-cell CCG derived sequence (Supplementary Figs. 5f–h and 8d,e). The idea of this analysis is to find subsets of episode/place cells (at least three cells) using CCGs, in which the offset of CCG peak between each pair of cells on the theta cycle time scale always followed the same order as the order of episode/place cells in the slow sequence.

First, CCGs between each pair of place/episode cells were calculated using 10-ms time bins, and normalized by the number of trials in the session. CCGs were ordered according to the place/episode sequence, and formed an $N \times N$ CCG matrix (N is the number of place/episode cells in one recording). Each of

the CCGs was smoothed within ± 80 -ms window and the offset of the peak was detected, if the offset of the CCG peak was further from zero ($+1$), the offset of the CCG peak was the same as the last neuron in the sequence ($+0.5$), the CCG did not have enough of an overlap or the peak to mean ratio was < 2.5 ($+0.1$).

The resulting candidate sequence was considered a real sequence when it satisfied the following criteria: length of the sequence was ≥ 3 cells, the sequence was not a sub-sequence of previously detected sequences, the score was ≥ 2 for 3-cell sequences or $\geq (\text{length}(\text{sequence}) * \text{length}(\text{sequence}) - 1)/4$ for sequences longer than 3 cells.

A **Supplementary Methods Checklist** is available.

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