Dynamic embedding of salience coding in hippocampal spatial maps

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21 **Summary**

22 Hippocampal CA1 neurons participate in dynamic ensemble codes for space and memory.
 23 Prominent features of the environment are represented by an increased density of place
 24 cells, but cellular principles governing the formation and plasticity of such disproportionate
 25 maps are unknown. We thus imaged experience-dependent long-term changes in spatial
 26 representations at the cellular level in the CA1 deep sublayer in mice learning to navigate
 27 in a virtual-reality environment. The maps were highly dynamic but gradually stabilized as
 28 over-representations for motivational (reward) and environmental (landmark) salience
 29 emerged in different time courses by selective consolidation of relevant spatial
 30 representations. Relocation of the reward extensively reorganized pre-formed maps by a
 31 mechanism involving rapid recruitment of cells from the previous location followed by their
 32 re-stabilization, indicating that a subset of neurons encode reward-related information. The
 33 distinct properties of these CA1 cells may provide a substrate by which salient experience
 34 forms lasting and adaptable memory traces.

35 **Introduction**

36 Navigation and spatial memory are essential elements of animal behavior that allow
 37 animals to forage, return home and avoid dangers. The hippocampus plays a crucial role
 38 in these cognitive processes, as hippocampal neurons fire when an animal is located in a
 39 particular part of an environment but not in others, providing an allocentric cognitive map
 40 of space (O'Keefe and Nadel, 1978). Although whether these "place cells" are indeed
 41 memory cells has been long debated, accumulating evidence supports this notion. One
 42 line of such evidence includes the knowledge that hippocampal place-specific firing
 43 exhibits dynamic changes according to context and experience on multiple time scales,
 44 ranging from a few minutes to days or weeks (Muller and Kubie, 1987; Bostock et al.,
 45 1991; Mehta et al., 1997; Lever et al., 2002; Leutgeb et al., 2005a). Moreover, studies
 46 have reported that a disproportionately large number of place cells are recorded at
 47 locations that are associated with reward, safety or walls or edges of the environment
 48 (O'Keefe and Conway, 1978; Wiener et al., 1989; Hetherington and Shapiro, 1997; Hollup
 49 et al., 2001; Dombeck et al., 2010; Dupret et al., 2010; Danielson et al., 2016), indicating
 50 that the environment surrounding an animal is not represented uniformly in the
 51 hippocampal map; their representations are strongly influenced by the motivational and
 52 environmental salience of the locations.

53 These findings imply that an increased number of place cells encode the presence
 54 of salience (i.e., something that draws attention) in the hippocampal map. This idea further
 55 proposes potential roles of such salience maps in not only spatial (Hollup et al., 2001;
 56 Dupret et al., 2010) or episodic-like memories (Komorowski et al., 2009; Eichenbaum and
 57 Cohen, 2014) but also goal-directed and landmark-based navigation (Gothard et al., 1996)
 58 because they can signal the subject's distance and direction relative to the represented
 59 salience by increases and decreases of population output activity to downstream neurons
 60 (Burgess and O'Keefe, 1996). Place cells are formed rapidly within minutes after initial

exposure to a new environment (Hill, 1978; Wilson and McNaughton, 1993; Frank et al., 2004). However, how the over-representation of salience is established and updated by experience has been poorly explored to date. Furthermore, mechanistic insights based on session-by-session comparisons of large-scale neuronal population data are also scarce. A few different but not mutually exclusive schemes are possible to answer this question. First, place cells are formed more preferentially for salient locations than non-salient locations from the beginning of place map formation (hereafter called the “direct formation” model) (Figure 1A). This model assumes that the probabilities of place cell formation are higher at salient locations. Second, place cells are initially formed uniformly for all locations, but cells that represent salient locations subsequently increase by recruiting the place cells that represent non-salient locations (“lateral recruitment” model) (Figure 1B). This model assumes that the probabilities of place cell formation are uniform across all locations and that the probabilities that place cells encoding non-salient locations turn into those encoding salient locations are higher than the probabilities that they continue to encode non-salient locations. Third, the place fields of all place cells potentially turn over dynamically, but spatial representations of salient locations are more stable than non-salient locations, leading to the persistence or accumulation of relevant spatial representations over time (“selective consolidation” model) (Figure 1C).

To elucidate the single or multiple forms of cellular dynamics that govern the formation and plasticity of the salience representation in the hippocampus, we longitudinally imaged place maps of the CA1 deep sublayer in a neuron-specific G-CaMP7 transgenic mouse line during training on a virtual linear track, in which two distinct locations were associated with either reward or a visual landmark as motivational or environmental salience, respectively. The experiments allowed us to track the neuronal activities and anatomical positions of a large population of pyramidal cells within a particular area of the hippocampus over days and thus to examine the dynamic cellular

changes predicted differently by each of the above hypotheses. We found that initial establishment of over-represented salience maps is dominated by selective consolidation of the cells that encode each salient location but not by direct formation from non-place cells or lateral recruitment of the cells that encode non-salient locations. We also found that over-representations of motivational and environmental salience emerge with experience in different rapid versus delayed time courses, respectively. By contrast, robust reorganization of pre-established maps by rearrangement of salient features occurred via a coordinated interplay of these three processes. These findings reveal the distinct engagement of multiple forms of cellular dynamics in the establishment and reorganization of hippocampal salience maps and provide a mechanism by which experience of salient environmental features form lasting and adaptable traces in these maps.

Results

Mice and behavioral task

To reliably perform longitudinal imaging of large-scale hippocampal functional cellular maps, we generated a new transgenic mouse line that coexpresses the fluorescence calcium indicator protein G-CaMP7 and the calcium-insensitive red fluorescence marker protein DsRed2 via 2A peptide-mediated bicistronic expression under the neuron-specific Thy1 promoter. G-CaMP7 is an improved, highly sensitive G-CaMP variant that exhibits large fluorescence changes and rapid kinetics in response to a wide range of intracellular calcium concentrations (Ohkura et al., 2012; Poder et al., 2015). We selected one mouse line, here termed Thy1-G-CaMP7, that expresses G-CaMP7 and DsRed2 in widespread brain areas at a high level in the adult brain (Figure 1D). In the dorsal CA1 hippocampus, the population of calbindin-negative pyramidal cells in the deep pyramidal cell sublayer was preferentially labeled with G-CaMP7 (Mizuseki et al., 2011; Kohara et al., 2014; Lee et al., 2014; Valero et al., 2015; Danielson et al., 2016) (Figure

1E). Immunofluorescence labeling against glutamic acid decarboxylase 65/67, parvalbumin or somatostatin revealed that interneurons positive for these markers were devoid of G-CaMP7 expression (Figure 1E and S1A). In addition to strong hippocampal expression (Figure S1B), G-CaMP7 expression was found in diverse brain areas, including the cerebral cortex, olfactory bulb, brainstem and cerebellum (Figure S1C-N), making Thy1-G-CaMP7 mice an attractive alternative to our previously reported TRE-G-CaMP7 mice in various imaging studies (Sato et al., 2015).

To allow for imaging of hippocampal maps during repeated training of spatial behavior in a controlled environment, we used a head-fixed virtual reality (VR) system that consisted of an air-supported Styrofoam treadmill and a wide LCD monitor placed under a two-photon microscope (Sato et al., 2017) (Figure 1F). Thy1-G-CaMP7 mice were trained to run along a virtual linear track for water reinforcement (Figure 1G). In this task, a head-fixed mouse in a virtual environment ran unidirectionally through an open-ended linear track segment whose walls were textured with different patterns. The mouse started running from the origin of the segment, passed under a green gate as a visual landmark, received water at a reward point and returned to the origin after reaching the other end (for details, see Methods). The visual landmark and reward delivery were associated with two distinct locations in the track to examine the effects of two different kinds of salience separately. Because the transition was instantaneous and the patterns of the walls and floor appeared seamless, the mice kept running forward as if they ran along an infinitely long repetition of a corridor, similar to a real-world circular track and a head-fixed treadmill belt used elsewhere (Hollup et al., 2001; Danielson et al., 2016). In training, behavioral performance measured by distance traveled and time spent running during 10-min sessions markedly increased as training proceeded (Figure 1H-I; Distance, $p < 0.0001$, $F_{(14,154)} = 7.30$; Run period, $p < 0.0001$, $F_{(14,154)} = 5.26$; $n = 12$ mice from 3 groups, one-way ANOVA). In addition, running speed as a behavioral measure of familiarity (Frank et

al., 2004) also significantly increased during training ($p < 0.0001$, $F_{(14,154)} = 11.6$). This simple task thus allowed us to investigate experience-dependent changes in hippocampal representations of salient locations by cellular resolution functional imaging.

Initial establishment of hippocampal salience maps

We next sought to visualize how hippocampal CA1 place maps emerge from a naive state during training on the virtual endless linear track task, with a particular interest in whether and when the representations of the two salient locations become prominent in the map. Two-photon imaging of the CA1 pyramidal cell layer through an optical window in Thy1-G-CaMP7 mice provided an image of a large number of G-CaMP7-labeled pyramidal cells across the entire field of view (Figure 2A). Because the neuronal density of the dorsal CA1 pyramidal cell layer is very high, we computationally extracted the morphology and activity of individual neurons from time-lapse movies using a modified non-negative matrix factorization algorithm to ensure objective and reproducible image analysis (Vogelstein et al., 2010; Pnevmatikakis et al., 2016; Takekawa et al., 2017) (Figure S2). This algorithm assumes that time-varying fluorescence signals of each cell can be decomposed into the product of a spatial filter and a time variation of fluorescence intensity, which are estimated by two alternating iterative steps (for details, see Methods). The spatial filter represents the position and shape of the cell (Figure S2A-B). The timing of spiking activity is inferred using the assumption that each spike evokes transient elevations of fluorescence intensity with a double-exponential shape (Figure S2C).

Using these activity time traces, we determined the virtual location-specific activity by statistical testing of the mutual information content calculated between each cell's neuronal activity and the animal's virtual locations (Figure S2D-E). Accumulating evidence has demonstrated that “place cells” in the VR recapitulate many, though not all, place cell properties observed in real environments (Chen et al., 2013; Ravassard et al., 2013). We

thus called the cells that exhibited place cell-like virtual location-specific activity “virtual place cells (vPCs)” in this study and used them as a means to understand how information regarding the spatial aspect of the external world is represented in the hippocampal map.

Consistent with previous studies in real and virtual environments, vPCs were formed rapidly within the first session on the virtual linear track (Hill, 1978; Wilson and McNaughton, 1993; Frank et al., 2004; Chen et al., 2013) (Figure 2B-C). The initial fractions of vPCs were low but then increased as the training proceeded. The fraction of vPCs and that of time spent running showed a good overall correlation (Figure S3A). The parallel increase in these two factors during training thus suggested that the observed increase in vPCs might be simply due to more sensitive detection of vPCs, which was enabled by an increase in the length of data for virtual place field calculations. Thus, we created scatter plots of the fraction of vPCs against that of the time spent running for all seven animals for each of the 15 sessions and determined the slopes of the regression lines to obtain indices (termed the vPC formation factor) representing the amount of vPCs formed by a unit length of time spent running (Figure 2D-F). The vPC formation factor significantly increased in the late phase of training compared with the early phase (Figure 2D-F). These results indicate that training indeed facilitated more effective formation of vPCs for a given amount of spatial experience. A separate analysis demonstrating that the sessions in the late phase contained larger fractions of vPCs than the sessions in the early phase with a comparable extent of running time also supports this idea (Figure S3B-C).

We then examined whether the virtual locations associated with salience were disproportionately over-represented in the hippocampal spatial map. The histograms of vPCs against the track position typically exhibited two large peaks, which were clearer in the late training phase; one peak corresponded to the location of the landmark (i.e., the green gate), and the other corresponded to that of the reward (Figure 2G). While the first peak closely matched the landmark location, the second peak was slightly shifted to the

direction of the mouse's running, which likely reflects that the animals recognized or anticipated the rewards at places that were slightly past the delivery point, as suggested by slowing down of running speed around this zone (data not shown). Importantly, the over-representation of the location for the reward was discernible even in the first session of training, whereas that of the location for the landmark gradually developed as the training proceeded (Figure 2G-I). The fraction of vPCs that encoded the location of the reward (here termed "reward cells" (RW) for convenience) was not significantly different between the early and late phases of training, whereas that of vPCs that encoded the location of the landmark (similarly termed "gate cells" (GT)) increased significantly with a complementary decrease in the fraction of vPCs that encoded other locations (termed "non-reward, non-gate vPCs" (NRNG)) (Figure 2H-I). The slower increase in vPCs that encode locations associated with salient visual cues is further supported by a more delayed and smaller increase in vPCs that encode a location with less visual salience, such as a boundary of different wall patterns (termed "wall cells" (WL), Figure S4). Collectively, these results demonstrate that the over-representation of salient locations is formed and maintained at a population level, even though the maps develop dynamically throughout the training period. The establishment and refinement of representations of salience depend on its nature; the representation of motivational salience is established rapidly, whereas that of environmental salience develops over the course of training.

What is the benefit of this salience representation in encoding and storage of information about the animal's environment? We trained a Bayesian decoder from the first 150 s of the running period and reconstructed the animal's trajectory in the following 90 s using the activities of vPCs in the same sessions (Zhang et al., 1998; Ziv et al., 2013) (Figure 3A). The average median errors across all track positions were initially large in the early phase of training and decreased steadily as the training proceeded (Figure 3B), likely due to the training-induced increase in vPC number (Figure 3C). A more detailed analysis

of well-decoded sessions (average median error across all positions < 10 cm) revealed that the errors for the locations associated with the landmark and reward were significantly smaller than those for other locations (Figure 3D). These results demonstrate that the established salience maps enable more accurate population coding for these locations.

Experience-dependent map consolidation

The vPC maps imaged at each session appeared rather different from each other, even within the same animals, implying that hippocampal spatial representations are highly dynamic while being established (Figure 2B). This result further suggests that while new representations were created by each experience, at least in parts of the maps, pre-existing representations are either eliminated or stabilized. To gain insight into whether repeated training stabilizes the maps, we next investigated training-induced changes in the maps at an individual cell level by comparing the virtual place fields of the same cells across different sessions (Figure S5). In this analysis, we conservatively focused on comparisons between two consecutive sessions (i.e., sessions 1 and 2, 2 and 3, etc.) because the quality of the image alignment was gradually reduced as the number of sessions that separated the two images increased ($P < 0.0001$, $F_{(13, 721)} = 10.94$, one-way ANOVA, Figure S5F). In the early maps, which contained a relatively small fraction of vPCs, only a small number of vPCs were identified as common in both sessions (hereafter called “common vPCs”), whereas the fraction of common vPCs increased significantly as more vPCs were imaged in the late phase of the training (Figure 4A-C). Moreover, the fraction of vPCs that had stable virtual place fields (< 10 cm difference) in both sessions (“stable vPCs”) also increased markedly as the training proceeded (Figure 4A-B, D-E), indicative of experience-dependent map consolidation. Image comparisons between adjacent sessions showed that fractions of common cells (i.e., cells that were identified in common in the two sessions) were constant over time ($P = 0.57$, repeated measures one-

way ANOVA) (Figure S5E), verifying that the increased stability of vPCs was not caused by differences in image alignment. Furthermore, the fractions of common and stable vPCs normalized by the number of vPCs also increased significantly as the training proceeded, revealing that the training-induced increase of vPC stability was not simply due to the increase of the number of vPCs (Figure S6). We then asked whether representations of salient locations are more stable than those of non-salient locations. We calculated the fractions of stable vPCs with respect to the number of common vPCs as an index for stable representations at each location and found that this index was significantly higher for locations associated with the landmark or reward than for other locations (Figure 4F-G). Finally, we tested the hypothesis that the stability of the maps predicts the performance of animals in the behavioral task. We found that the gain of vPC stability between the early and late phases of the training in individual animals exhibited a good linear correlation with their differences in time spent running between the two phases of the training ($r = 0.84$, Figure 4H). The results suggest that hippocampal place maps are more strongly stabilized if the animals more effectively learn to run the virtual linear track task.

Cellular dynamics for map establishment

To elucidate the cellular mechanism for the map establishment, we conducted two analyses of the functional transitions of cells belonging to different virtual location-related cell categories between sessions (Figures 5 and S7). In the first analysis, common cells in a reference session N were classified into vPCs or non-vPCs (NvPC) according to their virtual location-related activities, and the probabilities of transitioning to the same or the other categories in the subsequent session N+1 were calculated (for details, see Methods) (Figure S7A). The probability of vPC to remain as a vPC, $P_{\text{vPC-vPC}}$, and that of “*de novo*” vPC formation from NvPC, $P_{\text{NvPC-vPC}}$, increased significantly between the early and late phases of training (Figure S7B-C), with complementary decreases in the probabilities

269 of vPC disappearance $P_{\text{vPC-NvPC}}$ and NvPC stabilization $P_{\text{NvPC-NvPC}}$ (Figure S7B). In
 270 addition, $P_{\text{vPC-vPC}}$ was significantly greater than $P_{\text{NvPC-vPC}}$ in both training phases (Figure
 271 S7B-C). This result implies that vPCs are more likely to be vPCs in the subsequent
 272 sessions than NvPCs are, and that such a stabilization process plays a major role in the
 273 increase in vPCs. Together, these results demonstrate that training induces expansion of
 274 the vPC population by shifting the balance of a dynamic exchange between vPCs and
 275 NvPCs toward the direction of vPC stabilization and formation.

276 Next, to clarify the contributions of the formation, recruitment and consolidation of
 277 vPCs in the establishment of salience maps (Figures 1A-C and 5A-C, see also
 278 Introduction), we further subclassified the vPC population into RW, GT and NRNG
 279 according to their virtual place field positions and analyzed the transitions between them
 280 for each vPC subcategory in the second analysis. The formation of vPCs from NvPCs
 281 exhibited significant biases toward RWs (Figure 5D). However, a subpopulation of vPCs
 282 that became NvPCs in subsequent sessions also exhibited a similarly biased distribution
 283 (Figure 5E). The net effect, calculated as their difference, resulted in no significant biases
 284 (Figure 5F), implying that the disproportionate formation of vPCs predicted by the “direct
 285 formation model” (Figure 1A) exists but is counteracted by a similar bias in the
 286 disappearance of part of the vPCs. In addition, the distribution of vPCs derived from former
 287 NRNGs appeared not to be biased toward RWs or GTs but was rather uniform (Figure
 288 5G), providing evidence against the “lateral recruitment model” (Figure 1B). In contrast, the
 289 distribution of vPCs derived from former GTs or RWs was substantially biased toward the
 290 location by which each of the two vPC subcategories was defined, as predicted by the
 291 “selective consolidation model” (Figures 1C and 5H-I). These results further confirm that
 292 RWs and GTs have a marked tendency to persistently encode the same locations and
 293 strongly support the idea that selective consolidation of relevant spatial representations is
 294 the primary mechanism underlying the establishment of hippocampal salience maps.

295

296 **Robust map reorganization by conjunction of salience**

297 Our findings for experience-dependent map establishment thus far imply that the
 298 stability of spatial representations in the hippocampal map can be controlled by the
 299 presence of salient features. This result raises a further question concerning how this
 300 process is engaged in the plasticity of pre-established maps, which was addressed by
 301 imaging the salience maps during re-training in the reward rearrangement task. In this
 302 task, mice trained on the standard virtual linear track (i.e., the track on which visual
 303 landmark and reward delivery were associated with two distinct locations) for 15 sessions
 304 were further trained for the following 5 sessions in the same linear track, except that the
 305 location of reward delivery was shifted to the landmark location (Figure 6A). This task
 306 allowed us to examine the changes in the map that occurred when mice re-experienced
 307 the environment in which two separately located salient features were now jointly
 308 presented. We divided the entire re-training period into early (session 1-2) and late
 309 (session 3-5) sessions. Distance traveled, running speed and fraction of time spent
 310 running were not significantly changed between before and after the reward
 311 rearrangement (Figure S8A-B). However, this manipulation triggered robust reorganization
 312 of the salience maps (Figure 6B). Notably, the fraction of vPCs decreased immediately
 313 after reward rearrangement but recovered as the re-training proceeded (Figures 6B and
 314 S8C). Furthermore, the over-representation of the previous reward location disappeared
 315 suddenly as early as the first rearrangement sessions, whereas over-representation of the
 316 location associated with the conjunction of the pre-existing landmark and the relocated
 317 reward was markedly enhanced over the course of re-training (Figure 6C-E). The effect of
 318 this enhanced over-representation appeared rather additive (normalized fractions of vPCs,
 319 Pre GT, 1.14 ± 0.10 ; Pre RW, 1.65 ± 0.09 ; Late GT+RW, 1.89 ± 0.18), supporting the view
 320 that the magnitude of increases in place cell numbers represents the degree of salience in

the hippocampus map. Interestingly, the reward rearrangement also enhanced the representation of the location associated with the wall pattern transition (Figure 6C-E), demonstrating that a salience conjunction can also be formed broadly with a nearby less-salient visual cue.

Cell-by-cell comparisons of the maps imaged at adjacent sessions further revealed salience-dependent, location-specific regulation of hippocampal map stability (Figure 7A-D). The reward rearrangement triggered a significant reduction in stability for the representation of the previous reward location and the non-salient locations in the early phase of re-training, while the stability of the location with conjunctive salience was essentially maintained (Figure 7B and D). The stability of the non-salient locations recovered in the late phase, but that for the previous reward location remained low (Figure 7C-D). These results demonstrate that the hippocampal map plasticity accompanies a redistribution of vPC stability through a short period of heightened map instability. The presence and absence of salience thus govern the hippocampal map representations through a dynamic regulation of place field stability.

Cellular dynamics for map reorganization

Finally, to elucidate the cellular mechanism underlying the salience rearrangement-induced map plasticity, we analyzed the contribution of direct formation, lateral recruitment and selective consolidation of vPCs during this process (Figures 1A-C and 8). For simplicity of terminology, cell categories are labeled based on the condition before reward rearrangement. More specifically, GT and RW cells refer to the vPCs that encode the landmark and reward locations at the time before reward rearrangement, and their field positions maintain the same designations so that they become associated with the landmark plus reward and no salience after reward rearrangement, respectively. The reward rearrangement elicited a rapid and sustained reduction in *de novo* formation of RW

from NvPCs in the early and late phases and a delayed increase in *de novo* GT formation in the late phase of re-training (Figure 8A-i and B-i). The elimination of RW into NvPCs subsided in the late phase (Figure 8A-ii and B-ii), likely reflecting the substantial reduction of the RW formation in the preceding early phase. Consequently, the net effect gave rise to a remarkable transient decrease in RW formation in the early phase and a delayed increase in GT formation (Figure 8A-iii and B-iii). These results demonstrate that the reward rearrangement and the formation of conjunctive salience markedly influence the net vPC formation and that this salience-dependent down- and up-regulation of vPC formation underlies the rapid decrease in the vPC fraction and disappearance of the over-representation of the previous RW location in the early phase and the recovery of the vPC fraction and enhancement of the over-representation of the GT location in the late phase of plasticity.

The major mechanistic differences between map establishment and reorganization are not limited to the regulation of vPC formation. The reward rearrangement significantly increased recruitment of GT from NRNGs toward the late phase of re-training (Figure 8A-iv and B-iv). Furthermore, the recruitment of GT from RW also exhibited a significant transient increase in the early phase of re-training (Figure 8A-vi and B-vi). These results indicate that a conjunction of salience formed by reward rearrangement augments the recruitment of vPCs that encode a previously salient location as well as non-salient locations. The density of stable RW cells exhibited a sudden and prolonged drop in the early and late phases (Figure 8A-vi and B-vi), whereas that of GT remained high throughout the re-training period (Figure 8A-v and B-v), consistent with earlier findings (Figure 7). In summary, the salience rearrangement-induced map reorganization is mediated by a rapid disappearance of the over-representation of the previously salient location followed by a gradual enhancement of the over-representation of the location associated with a newly formed strong conjunction of salience. In contrast to the primary

role of selective place field stabilization in initial map establishment, the adaptive change of the hippocampal salience map is cooperatively achieved by multiple forms of cellular dynamics that involve not only stabilization but also direct formation and lateral recruitment of place cells (Figure 8C, see Discussion for details). Notably, GT cells immediately after reward rearrangement were derived more from RW cells than from NRNG cells (46 RW-to-GT cells and 36 NRNG-to-GT cells of 107 total GT cells from 7 mice compared with 22 and 70 cells, which were expected from the uniform distribution across positions, $P < 0.0001$, Chi-square test). This suggests that RW cells form a unique subpopulation in CA1 and may encode information associated with the reward itself (Figure S9).

Discussion

Theories of hippocampus-dependent memory and navigation are based on the premise that spatial and non-spatial information are jointly represented in the hippocampus (Knierim et al., 2006; Eichenbaum and Cohen, 2014). Accumulating evidence demonstrates that reward or geometric cues associated with particular locations are represented by an increased density of relevant place cells in hippocampal neural maps, particularly those in the CA1 deep sublayer (O'Keefe and Conway, 1978; Wiener et al., 1989; Hetherington and Shapiro, 1997; Hollup et al., 2001; Dombeck et al., 2010; Dupret et al., 2010; Danielson et al., 2016). By tracking the activity of individual neurons in G-CaMP7 transgenic mice trained in a spatial task in virtual reality, we investigated the cellular principles underlying the formation and plasticity of these maps. We observed that over-representation of salient locations occurred with different time courses and degrees depending on the nature and extent of salience, which resulted in more precise population coding for these locations. We also showed that hippocampal maps were consolidated by experience-dependent stabilization that was correlated with the behavioral performance of the animals. Moreover, stabilization of spatial representations for salient locations but not

399 *de novo* formation and lateral recruitment supported the establishment of the over-
 400 represented maps, providing evidence that place field stability is fine-tuned by experience
 401 and salience. Finally, we revealed that reorganization of pre-formed maps was mediated
 402 by a coordinated interplay of *de novo* formation, lateral shifts and selective consolidation.
 403 These findings provide a comprehensive framework for cellular mechanisms underlying
 404 the formation and plasticity of hippocampal functional maps and explain how salient
 405 experience can form lasting yet adaptable memory traces.

406

407 **Cellular mechanisms for formation and plasticity of hippocampal maps**

408 The observed contrasting cellular dynamics between map formation and plasticity
 409 suggests that hippocampal circuits transit between two distinct modes during different
 410 experiences. Based on our observations, we propose the following model — during initial
 411 map formation, *de novo* place cell formation biased toward salient locations is
 412 counteracted by similarly biased place cell removal, whereas over-representations are
 413 developed and maintained by a selective consolidation and elevated equilibrium state of
 414 salient place cells with little input from neutral place cells by lateral recruitment. This
 415 “encoding” mode is dynamically shifted to “updating” mode upon rearrangement of salient
 416 features. Thus, the balanced formation and disappearance of place cells is transiently
 417 disequilibrated during the early phase of map plasticity to achieve a rapid shift in the peak
 418 place cell density. In parallel, a subset of cells that encoded previously salient locations
 419 rapidly move their fields to locations with relocated features during the early phase,
 420 followed by further recruitment of neutral place cells during the late phase. These
 421 processes are accompanied by transient weakening of place cell stability during the early
 422 phase and its recovery during the late phase. Thus, our findings indicate that a simple
 423 stabilization principle, which allows for encoding salient locations as more persistent traces
 424 and allocating more cellular resources to them, governs map formation, whereas parallel

and coordinated engagement of multiple processes, which supports the rapid updating of stored information, controls the adaptive reorganization of pre-established maps.

The model assumes that neural signals conveying information about the presence of salience modulate the above processes. For example, salience signals may modulate place cell stability via a dopamine-dependent mechanism (Kentros et al., 2004) and place field formation and disappearance at CA1 pyramidal cell dendrites and EC3 inputs (Bittner et al., 2015; Sheffield et al., 2015). The salience-chasing property observed in this study (i.e., from RW to RW+GT) may arise within or outside the hippocampus (Weible et al., 2009; Deshmukh and Knierim, 2011; Tsao et al., 2013). Remarkably, the lateral recruitment of neutral place cells identified in the late phase of plasticity may be mediated by a mechanism different from salience chasing that likely reflects the binding of cells to a reference frame of salience (Gothard et al., 1996) and may require stronger signals derived from salience conjunction to occur. Such powerful signals may effectively permit “overwriting” of pre-existing neutral place fields with those for salient locations, whereas modest salience signals only facilitate *de novo* place field formation from non-place cells.

Salience coding in the deep CA1 sublayer

The finding that the time course and magnitude of increases in place cell density vary depending on the nature and extent of salient features advances the idea that the hippocampal deep CA1 sublayer is specialized for salience mapping and proposes a map-based hippocampal salience code that is different from the known firing rate-based coding scheme (Leutgeb 2005b; Komorowski et al., 2009). Unlike previous studies, we observed that the over-representation of salient locations arose in a task that involved learning of salience-place associations through repetitive experience rather than explicit goal-directed spatial navigation (Dupret et al., 2010; Danielson et al., 2016). This observation favors the notion that hippocampal encoding of salience occurs rather automatically without effortful

goal-driven learning. Rapid mapping of motivational salience is consistent with its strong behavioral relevance as the source of positive reinforcement, whereas gradual mapping of environmental salience presumably reflects experience-dependent learning of the environment that could contribute to landmark-based navigation (Sato et al., 2017). The extra quantity and stability as well as the idiosyncratic salience-chasing property of place cells for salient locations imply that such “complex” place cells may constitute subpopulations distinct from “simple” place cells for neutral locations in the CA1 deep sublayer (Gauthier and Tank, 2017; Geiller et al., 2017).

The present findings raise a crucial question as to how the presence of salience is signaled to the CA1 deep sublayer. The emergence of reward and landmark over-representations with different time courses suggest that signals for each salience arise from different sources, the same source with different patterns and intensities, or perhaps a combination of these. Recent studies demonstrate that reward-responsive ventral tegmental area (VTA) neurons are reactivated during rest periods (Gomperts et al., 2015; Valdés et al., 2015) and optogenetic activation of dopaminergic VTA inputs to CA1 sustains newly acquired hippocampal spatial representations and memory (McNamara et al., 2014), pointing to VTA dopaminergic input as a potential signal for motivational salience. The hippocampus integrates spatial information from the medial entorhinal cortex (MEC) with non-spatial information from LEC to represent objects and events within a spatial context (Knierim et al., 2006; Eichenbaum and Cohen, 2014). LEC neurons start to fire in the vicinity of objects and reward when these features are introduced to the environment (Deshmukh and Knierim, 2011; Tsao et al., 2013). Notably, long-range inhibitory projections from LEC to the dorsal CA1 area respond to diverse salient stimuli, including aversive, motivational and other sensory cues (Basu et al., 2016), making them a candidate for the putative salience signals. Although salience signals may further arise from brain areas other than VTA and LEC, these signals may act to stabilize

477 representations of salient locations in the CA1 deep sublayer, potentially through
 478 enhanced reactivation of relevant experience (Singer and Frank, 2009). The deep
 479 sublayer-specific CA2 input and mutual suppression between the two sublayers (Kohara et
 480 al., 2014; Lee et al., 2014) may help to associate the deep sublayer map more with those
 481 salience signals.

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497

498 **Author Contributions**

499 M.S. and Y.H. designed the study. M.S. and M.K generated the Thy1-G-CaMP7 transgenic
500 mice. M.S. and T.I. built the virtual reality set-up. M.S., K.M., M.K., D.G.-D. and K.K.
501 performed the imaging experiments. M.S., K.M., T.I. and T.T. analyzed the data. M.O. and
502 J.N. made the G-CaMP7-T2A-DsRed2 transgene. T.T., M.S., H.Y. and T.F. developed the
503 image analysis software. M.S. and Y.H. wrote the paper.

504

505 **Declaration of interests**

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507

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640 **Figure legends**

641 **Figure 1. Models, transgenic mice and behavioral task.** (A-C) Models that can account
642 for the formation of hippocampal over-representation of salient locations. (A) Direct
643 formation model. (B) Lateral recruitment model. (C) Selective consolidation model. See
644 Introduction for details. (D) Transgene construct for Thy1-G-CaMP7 mice (top) and
645 expression of G-CaMP7 (bottom left, green) and DsRed2 (bottom right, red) in a
646 parasagittal section of a mouse at 6 months of age. Scale bar = 2 mm. (E)
647 Characterization of the G-CaMP7-expressing cell population in the dorsal CA1
648 hippocampus of Thy1-G-CaMP7 transgenic mice. Sections of mice at 2-3 months of age
649 were immunolabeled with anti-calbindin (Calb, left) or anti-glutamic acid decarboxylase
650 65/67 (GAD, right) antibodies (magenta). Arrowheads indicate examples of calbindin-
651 positive, G-CaMP7-negative cells. SO, *stratum oriens*; SP, *stratum pyramidale*; SR,
652 *stratum radiatum*. Scale bar = 20 μ m. (F) A schematic representation of the two-photon
653 microscope and virtual reality setup used in this study. (G) Virtual endless linear track task.
654 The linear track segment contained a green gate as a visual landmark and a reward
655 delivery point at two distinct locations. When the mouse's virtual position reached the point
656 indicated by the red dotted line in the middle, it returned to the origin, so the same track
657 segment was presented repeatedly. The bottom panel shows a camera view of the track
658 displayed on the LCD monitor. (H) Example behavioral data from a single 10-min session.
659 From top to bottom, the mouse's virtual position on the linear track, running speed, timing
660 of reward delivery, and behavioral state during which a period of running is represented in
661 blue. (I) Behavioral changes induced by repeated training. Total distance traveled
662 (Distance, upper left), the fraction of time spent running (Run period, upper right) and
663 running speed (Speed, lower left) are shown.

664

Figure 2. Establishment of salience maps in the hippocampal CA1 deep sublayer.

(A) *In vivo* two-photon imaging of G-CaMP7-expressing CA1 pyramidal neurons through a hippocampal imaging window. A stainless cylindrical imaging window with a glass coverslip attached to the bottom was implanted above the dorsal CA1 (dCA1) area of the hippocampus (HP) after aspiration of the overlying cortical tissue (left). A representative fluorescence image of G-CaMP7-expressing pyramidal neurons in the dorsal CA1 hippocampus (right). Active cells are shown as bright cells in this grayscale image. Scale bar = 100 μ m. A, anterior; L, lateral. (B) Examples of virtual place cell (vPC) maps imaged in the same animal at the early (session 1), middle (session 7) and late (session 14) phases of training on the virtual endless linear track task (top). vPCs and non-vPCs (NvPC) are represented by filled circles of various colors and gray dots, respectively. The different colors of the filled circles represent different locations of the virtual place fields. Heat maps shown below are distributions of virtual place fields of the corresponding sessions ordered by their positions (bottom). (C) The fractions of vPCs relative to the number of total identified cells imaged at each session. (D) Example scatter plots showing the relationship between the fraction of vPCs and the fraction of time spent running for early (session 4), middle (session 9) and late (session 15) phases of the training. The red line in each panel represents linear regression. The value shown at top right indicates the vPC formation factor, which is defined as the slope of the regression line. (E) Changes of vPC formation factors during training. (F) Averages of vPC formation factors for early (sessions 1 - 5), middle (sessions 6 - 10) and late (sessions 11 - 15) phases of training. *P = 0.030, ANOVA with post hoc test for linear trend, n = 5 sessions each. (G) Histograms indicating the distribution of vPCs with respect to the track position for early (session 1), middle (session 6) and late (session 12) phases of the training. The average data from 7 mice are shown. For comparison, the histogram of the early phase was scaled by its maximum value to that of the late phase and is plotted in light blue on its right Y-axis. The

691 green, red and magenta dashed lines delineate the positions of the landmark, reward
 692 delivery and boundary of different wall patterns, respectively. The areas shown in green,
 693 red and magenta indicate those that define gate, reward and wall cells, respectively. (H)
 694 Hippocampal spatial representations as expressed by the fractions of gate cells (GT,
 695 green), non-reward, non-gate vPCs (NRNG, blue), and reward cells (RW, red) relative to
 696 the number of total vPCs identified in each session. Values were normalized to that
 697 obtained in the case of uniform distribution (i.e., 0.0125/bin), and values greater than 1
 698 indicate that the locations are over-represented. (I) Average normalized fractions of GT
 699 (green), NRNG (blue) and RW (red) for the early, middle and late phases of the training.
 700 ^{*a}, $P = 0.017$, $F_{(2,12)} = 4.91$; ^{*b}, $P = 0.017$, $F_{(2,12)} = 4.79$; n.s.^c, $P = 0.78$, $F_{(2,12)} = 0.247$; one-
 701 way ANOVA, $n = 5$ sessions each; ^{#d}, $P = 0.011$ vs. NRNG Early, $F_{(1.101, 4.405)} = 8.40$; ^{###e},
 702 $P = 0.0006$ vs. NRNG Middle; ^{##f}, $P = 0.0076$ vs. NRNG Middle, $F_{(1.138, 4.550)} = 26.5$; ^{###g},
 703 $P = 0.0006$ vs. NRNG Late; ^{###h}, $P = 0.0006$ vs. NRNG Late, $F_{(1.449, 5.795)} = 73.5$; one-way
 704 ANOVA; $n = 5$ sessions each.

705

706 **Figure 3. Precise estimation of salient locations by over-represented CA1 salience**
 707 **maps.** (A). Examples of trajectories estimated by the Bayesian decoder for early (top) and
 708 late (bottom) phases of training. The results of the entire 90-second test periods from the
 709 same animal are shown. Blue and red lines represent real and estimated trajectory,
 710 respectively. Periods of immobility that separated continuous running were excluded from
 711 decoding and their positions are indicated by vertical dashed lines. (B) Average median
 712 errors for the early, middle and late phases of training. ^{*} $P = 0.018$, $F_{(2,54)} = 3.65$, one-way
 713 ANOVA, $n = 15$, 19 and 23 sessions. (C). Relationship between absolute vPC numbers
 714 and average median estimation errors across the locations. The symbols filled with pink,
 715 red and dark red indicate sessions in the early, middle and late phases, respectively. The
 716 dashed line represents the threshold for well-decoded sessions. Only the sessions with

717 running times ≥ 240 s were analyzed (n= 57 sessions). (D) Average median errors across
 718 all well-decoded sessions plotted against the position (left) and those for the locations
 719 encoded by GT, NRNG, and RW (right). * $P = 0.026$, *** $P < 0.0001$, $F_{(1.312, 31.49)} = 8.14$; one-
 720 way ANOVA, n = 25 sessions.

721

722 **Figure 4. Experience-dependent consolidation of salience maps.** (A) Example
 723 hippocampal CA1 vPC maps imaged in two consecutive sessions in the early phase of the
 724 training. Maps shown on top, middle and bottom present cells identified in common in both
 725 sessions (Common cells), cells identified as vPCs in both sessions (Common vPCs), and
 726 cells identified as vPCs with stable (< 10 cm difference) virtual place fields in both
 727 sessions (Stable vPCs), respectively. vPCs and non-vPCs are represented by filled circles
 728 of various colors and gray dots, respectively. The different colors of the filled circles
 729 represent different locations of the virtual place fields. The histogram shown at the bottom
 730 indicates the distributions of the stable vPCs against the track position. The green and red
 731 dashed lines delineate the positions of the landmark and reward delivery, respectively.
 732 The same convention applies to B. (B) vPC maps imaged in the late phase of training in
 733 the same animal as presented in A. (C) The fraction of common vPCs relative to the
 734 number of common cells identified in the two consecutive sessions that were compared.
 735 The X-axis indicates the earlier of the two sessions that were compared. (D) vPC stability
 736 calculated as the fraction of stable vPCs relative to the number of common vPCs identified
 737 in the two consecutive sessions that were compared. (E) Average vPC stability for the
 738 early (sessions 1 - 4, which indicates the earlier of the two sessions that were compared),
 739 middle (sessions 5 - 10) and late (sessions 11 - 14) phases of training. * $P = 0.029$ vs.
 740 Early, ** $P = 0.0048$ vs. Early, $F_{(2,11)} = 7.90$, one-way ANOVA; n = 4, 6 and 4 session pairs.
 741 (F) The average fractions of stable vPCs relative to the number of common vPCs plotted
 742 against the track position. Values were calculated from data across all sessions and

averaged for 7 mice. The green and red dashed lines delineate the positions of the landmark and reward delivery, respectively. The areas shown in green and red indicate those that define gate and reward cells, respectively. (G) The average vPC stability for gate cells (GT), non-reward, non-gate vPCs (NRNG) and reward cells (RW). $^{\#}P = 0.029$ vs. NRNG, $^{##}P = 0.0018$ vs. NRNG, $F_{(1,202, 7.212)} = 13.9$, one-way ANOVA, $n = 7$ mice from 2 groups. (H) The relationship between vPC stability and task performance. The X-axis presents the task performance of each mouse measured by the difference in the fraction of time spent running between the early (average of sessions 1 - 5) and late (average of sessions 11 - 15) phases of the training. The Y-axis presents the difference in vPC stability between the early and late phases of training.

Figure 5. Formation, recruitment and stabilization of different vPC categories during map establishment. (A-C) Schematic diagrams of direct formation (A), lateral recruitment (B) and selective consolidation models (C). In each diagram, four functional cell categories, namely, reward cells (RWs, red), gate cells (GTs, green), non-reward, non-gate vPCs (NRNGs, blue) and non-vPCs (NvPCs, gray), and the transitions between them are defined. RWs, GTs and NRNGs constitute subcategories of vPCs and are shown enclosed by a dashed line. The transitions relevant to each model are highlighted in black. (D) Formation of different vPC categories from NvPCs. (left) A histogram showing the distribution of vPCs that were NvPCs in the previous sessions against the track position. The values were calculated from data across all sessions and averaged for 7 mice. For comparison, the histograms shown in D, E and G-I are plotted on the left Y-axes on the same scale. In addition, the histograms in G and I were scaled by their maximum values and plotted in a light color on the right Y-axes. (right) The average cell density of each vPC subcategory formed from NvPCs. $^{\#}P = 0.023$ vs. NRNG, $\chi^2_{(2)} = 8.00$; Friedman test, $n = 7$ mice from 2 groups. (E) Elimination of different vPC categories into NvPCs. (left) A

769 histogram showing the distribution of vPCs that became NvPCs in subsequent sessions
 770 against the track position. (right) The average cell density of each vPC subcategory that
 771 became NvPCs in the subsequent sessions. $^{##}P = 0.0099$ vs. NRNG, $\chi^2_{(2)} = 8.86$;
 772 Friedman test, $n = 7$ mice from 2 groups. (F) Net vPC formation. (left) A histogram of the
 773 difference obtained by subtracting the histogram in E from that in D. Note that this
 774 histogram only shows the distribution of locations for entry into and exit from the vPC
 775 populations and can have negative values for some locations. (right) The average cell
 776 density of the remaining vPCs classified by the vPC subcategories. (G) Transition and
 777 stability of the NRNGs. (left) A histogram showing the distribution of vPCs that were
 778 NRNGs in the previous sessions against the track position. (right) The average cell density
 779 of each vPC subcategory that was derived from NRNG. (H) Transition and stability of the
 780 GTs. (left) A histogram showing the distribution of vPCs that were GTs in the previous
 781 sessions against the track position. (right) The average cell density of each vPC
 782 subcategory that was derived from GTs. $^{\#}P = 0.023$ vs. NRNG, $\chi^2_{(2)} = 7.14$, Friedman test,
 783 $n = 7$ mice from 2 groups. (I) Transition and stability of the RWs. (left) A histogram
 784 showing the distribution of vPCs that were RWs in the previous sessions against the track
 785 position. (right) The average cell density of each vPC subcategory that was derived from
 786 RWs. $^{###}P = 0.0005$ vs. NRNG, $\chi^2_{(2)} = 14.0$, Friedman test, $n = 7$ mice from 2 groups.

787

788 **Figure 6. Robust reorganization of salience maps induced by a conjunction of**
 789 **different kinds of salience.** (A) Design of the reward rearrangement task. Mice were first
 790 trained in the standard linear track that included a visual landmark (GT) and reward
 791 delivery (RW) at separate locations for 15 sessions (Pre, left). Once training was complete,
 792 the location of reward delivery was shifted to match the location of the visual landmark
 793 (GT+RW), and the mice were re-trained in this new arrangement for the following 5
 794 sessions (Rearr 1-5, right). (B) Examples of vPC maps imaged in the same animal in the

pre (session Pre -1), early (session Rearr 1) and late (session Rearr 5) phases of the reward rearrangement task (top). vPCs and non-vPCs (NvPC) are represented by filled circles of various colors and gray dots, respectively. The different colors of the filled circles represent different locations of the virtual place fields. Heat maps shown below the vPC maps are the distributions of virtual place fields of the corresponding sessions ordered by their positions (bottom). (C) Histograms indicating the distribution of vPCs with respect to the track position for pre (session Pre -2), early (session Rearr 1) and late (session Rearr 5) phases of the rearrangement task. The average data from 7 mice are shown. For comparison, the histograms of the pre and early sessions were scaled by their maximum values to that of the late session and are plotted in light blue on their right Y-axes. The green, red and magenta dashed lines delineate the positions of the landmark, reward delivery and boundary of different wall patterns, respectively. The areas shown in green, red and magenta indicate those that define gate, reward and wall cells, respectively. (D) Hippocampal spatial representations as expressed by the fractions of gate cells (GT, green), non-reward, non-gate vPCs (NRNG, blue), reward cells (RW, red) and wall cells (WL, magenta) relative to the number of total vPCs identified in each session. Values were normalized to that obtained in the case of uniform distribution (i.e., 0.0125/bin), and values greater than 1 indicate that the locations are over-represented. (E) Average normalized fractions of GT (green), WL (magenta), NRNG (blue) and RW (red) for pre, early and late phases of the reward rearrangement (Ra) task. #^a, P = 0.044 vs. NRNG Pre, ##^b, P = 0.0022 vs. NRNG Pre, F_(1.433, 8.597) = 13.4; #^c, P = 0.026 vs. NRNG Early, F_(2.019, 12.11) = 10.8; ##^d, P = 0.0087 vs. NRNG Late, ##^e, P = 0.0095 vs. NRNG Late, ##^f, P = 0.0095 vs. NRNG Late, F_(1.785, 10.71) = 18.3; *g, P = 0.011, *h, P = 0.011, F_(1.511, 9.067) = 10.5; **i, P = 0.0099, **j, P = 0.0041, F_(1.672, 10.03) = 11.6; ***k, P = 0.0008, ***l, P = 0.0008, F_(1.408, 8.446) = 38.8; one-way ANOVA, n= 7 mice from 2 groups.

Figure 7. vPC stability during reward rearrangement task. (A) Example hippocampal CA1 vPC maps imaged in two consecutive sessions in the pre phase of the reward rearrangement task. Maps shown on top, middle and bottom present cells identified in common in both sessions (Common cells), cells identified as vPCs in both sessions (Common vPCs), and cells identified as vPCs with stable (< 10 cm difference) virtual place fields in both sessions (Stable vPCs), respectively. vPCs and non-vPCs are represented by filled circles of various colors and gray dots, respectively. The different colors of the filled circles represent different locations of the virtual place fields. The histogram shown at the bottom indicates the distributions of the stable vPCs against the track position. The green and red dashed lines delineate the positions of the landmark and reward delivery, respectively. The same convention applies to B and C. (B-C) Hippocampal CA1 vPC maps imaged in the early (B) and late (C) phase of training in the same animal as presented in A. (D) The average vPC stability for GT, NRNG and RW for pre, early and late phases of the reward rearrangement task. #^a, $P = 0.049$ vs. NRNG Pre, $\chi^2_{(2)} = 6.00$; #^b, $P = 0.033$ vs. RW Pre, ##^c, $P = 0.0063$ vs. RW Pre, $\chi^2_{(2)} = 11.2$; **d, $P = 0.0040$, *e, $P = 0.049$, $\chi^2_{(2)} = 11.1$; **f, $P = 0.0040$, *g, $P = 0.049$, $\chi^2_{(2)} = 11.1$; Friedman test, $n = 7$ mice from 2 groups.

Figure 8. Map plasticity is mediated by a dynamic interplay of stabilization, formation and recruitment of vPCs. (A-i) Formation of different vPC categories from NvPCs. The histograms show the distributions of vPCs that were NvPCs in the previous sessions against the track position, and those for pre, early and late phases of the reward rearrangement task are shown from top to bottom. The values were calculated from data across all relevant sessions and averaged for 7 mice. The green, red and magenta dashed lines delineate the positions of the landmark, reward delivery and boundary of different wall patterns, respectively. The areas shown in green, red and magenta indicate those that define gate, reward and mid cells, respectively. (A-ii) Elimination of different vPC

categories into NvPCs. The histograms show the distributions of vPCs that became NvPCs in the subsequent sessions against the track position. (A-iii) Net vPC formation. The histograms of the differences were obtained by subtracting the histograms in A-ii from the corresponding ones in A-i. (A-iv) Transition and stability of the NRNGs. The histograms show the distributions of vPCs that were NRNGs in the previous sessions against the track position. (A-v) Transition and stability of the GTs. The histograms show the distributions of vPCs that were GTs in the previous sessions against the track position. (A-vi) Transition and stability of the RWs. The histograms show the distributions of vPCs that were RWs in the previous sessions against the track position. (B-i) Formation of different vPC categories from NvPCs. The average cell density of each vPC subcategory formed from NvPCs are shown for pre, early and late phases of the reward rearrangement task. The periods of reward rearrangement are shaded in gray. ***a, $P = 0.0002$ vs. RW Pre, **b, $P = 0.0015$ vs. RW Pre, ***c, $P < 0.0001$ vs. GT Pre, ##d, $P = 0.0055$, ###e, $P = 0.0005$, ###f, $P < 0.0001$, ###g, $P < 0.0001$, $F_{(2,12)} = 14.5$, two-way ANOVA, $n = 7$ mice from 2 groups. (B-ii) Elimination of different vPC categories into NvPCs.***h, $P = 0.0007$ vs. RW Pre, ###i, $P < 0.0001$, $F_{(2,12)} = 8.01$, two-way ANOVA, $n = 7$ mice from 2 groups. (B-iii) Net vPC formation. The difference between the formation and elimination of each vPC subcategory are shown. **j, $P = 0.0019$ vs. RW Pre, *k, $P = 0.032$ vs. GT Early, #l, $P = 0.011$, $F_{(2,12)} = 16.4$, two-way ANOVA, $n = 7$ mice from 2 groups. (B-iv) Transition and stability of the NRNGs. **m, $P = 0.0029$ vs GT early, ###n, $P < 0.0001$, $F_{(2,12)} = 5.22$, two-way ANOVA, $n = 7$ mice from 2 groups. (B-v) Transition and stability of the GTs. **o, $P = 0.0042$ vs. GT Early, ###p, $P = 0.0005$, $F_{(2,12)} = 7.18$, two-way ANOVA, $n = 7$ mice from 2 groups. (B-vi) Transition and stability of the RWs.***q, $P < 0.0001$ vs. RW Pre, ***r, $P < 0.0001$ vs. RW Pre, ###s, $P < 0.0001$, #t, $P = 0.036$, $F_{(2,12)} = 4.71$, two-way ANOVA, $n = 7$ mice from 2 groups. (C) A model for reorganization of the hippocampal salience map elicited by the rearrangement of reward. The key processes in each phase are shown in red.

873 Augmentation and reduction of the processes are shown with thicker and dashed lines,
874 respectively. Selective consolidation of GT and RW plays a dominant role in establishment
875 and maintenance of the salience map during training before the rearrangement (Pre, left).
876 Immediately after reward arrangement, the fraction of vPCs decreases because of
877 decreased RW formation and stabilization, and a subpopulation of RW move their fields to
878 the new reward location (Early, middle). After a few sessions, the stability and formation of
879 GT as well as recruitment from NRNG to GT increase (Late, right).

880

881 **Figure S1. Transgene expression in Thy1-G-CaMP7 transgenic mice.** (A) G-CaMP7
882 expression (green) overlaid with parvalbumin (PV, left) or somatostatin (SOM, right)
883 immunofluorescence (magenta) in the dorsal hippocampal CA1 area at 4 months of age.
884 SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum; Scale bar = 20 μ m. (B)
885 Expression of G-CaMP7 (left) and DsRed2 (right) in a parasagittal section of the
886 hippocampus at 1 month of age. DG, dentate gyrus; CA1, CA1 area of the hippocampus;
887 CA3, CA3 area of the hippocampus; S, subiculum. Scale bar = 500 μ m. (C) Low
888 magnification images of G-CaMP7 (left) and DsRed2 (right) expression in the visual cortex
889 at 3 months of age. Scale bar = 200 μ m. (D) High magnification images of G-CaMP7
890 expression in layer 2/3 (L2/3, left) and layer 5 (L5, right) in the visual cortex at 3 months of
891 age. Scale bar = 20 μ m. (E-N) G-CaMP7 expression at 1 month of age in the olfactory
892 bulb (E), amygdala (F), thalamus (G), midbrain (H, I), cerebellum (J, K), pons (L) and
893 medulla (M, N). EP, external plexiform layer; Gl, glomerular layer; GrO, granule cell layer
894 of the olfactory bulb; IP, internal plexiform layer; M, mitral cell layer; BLA, basolateral
895 amygdala; LA, lateral amygdala; dLGN; dorsal lateral geniculate nucleus; SN, substantia
896 nigra; IG, intermediate gray layer of the superior colliculus; SG; superficial gray layer of the
897 superior colliculus; GrC, granule cell layer of the cerebellum; Mo, molecular layer; PC,
898 Purkinje cell layer; WM, white matter; DCN, dorsal cochlear nucleus; IN, interposed

cerebellar nucleus; LN, lateral cerebellar nucleus; PN, pontine nucleus; MVN, medial vestibular nucleus; 4V, fourth ventricle; SpV, spinal trigeminal nucleus. Scale bar = 200 μ m (left panels of E, F, G and H as well as I, L, M and N) or 100 μ m (right panels of E, F, G and H as well as J and K).

Figure S2. Spatiotemporal deconvolution of calcium imaging data using a non-negative matrix factorization algorithm. (A) A background-subtracted maximum intensity projection image that represents active cells in this example session. (B) Cell identification using the algorithm. In this session, 942 cells were identified. The contours of individual cells are shown by lines of different colors. (C) Time traces of fluorescence intensity (v, top) and inferred spike trains (u, bottom) of an example cell. The anatomical position of the cell is indicated in the inset by a red arrow. (D) Example of vPCs. Shown from top to bottom are a time series of the mouse's virtual position with the timing of cellular activity indicated by red dots (top), a time series of fluorescence intensity and inferred cellular activity (middle), a histogram of cellular activity plotted against the position in the virtual linear track (bottom left) and the anatomical position of the cell (bottom right). (E) Another example of vPCs. This cell was imaged in a different part of the same field of view as D and activated at a different location in the virtual linear track.

Figure S3. Experience enhances more effective formation of vPCs. (A) The fractions of vPCs were plotted against the corresponding fractions of time spent running for all sessions ($n = 105$ sessions). The red line represents linear regression ($r = 0.59$). (B, C) Bar graphs indicating mean fractions of time spent running and those of vPCs in the early, middle and late phases of training. The sessions in which mice ran 30-50% (B) and 50-70% (C) of the time were analyzed. $^{\#}P = 0.047$, $F_{(2,29)} = 4.07$, $n = 7, 12$ and 13 sessions,

one-way ANOVA. The comparison between Early vPC and Late vPC in 50-70% running exhibited a near-significant trend ($P = 0.071$, $F_{(2,19)} = 2.48$, $n = 6, 8$ and 8 sessions).

Figure S4. A delayed increase in vPCs that encode a location of a boundary of different wall patterns. (A) The fractions of wall cells (magenta) in each session are expressed as normalized fractions relative to the uniform distribution of vPCs. The data on NRNG in Figure 2H are presented again in this plot for comparison (blue). (B) Average normalized fractions of wall cells (WL) for the early, middle and late phases of the training. The data on NRNG in Figure 2I are presented again in this graph for comparison (blue). * $P = 0.037$, $F_{(2,12)} = 4.79$, $n = 5, 5$ and 5 sessions; one-way ANOVA, $^{##}P = 0.0079$, $U_{(5,5)} = 0$, vs. Late NRNG, $n = 5$ and 5 sessions, two-tailed Mann-Whitney test.

Figure S5. Alignment of cells across sessions. (A) Image registration with or without the displacement estimated by two-dimensional correlation coefficients. A DsRed2 reference image of the target session (top left) was overlaid with that of the source session (top center) without image displacement (top right). This placement resulted in global misalignment of cell positions between the target (red) and source (green) images. To correct this misalignment, two-dimensional correlation coefficients between the two images were calculated within a range of $\pm 25 \times \pm 25$ pixel displacements (middle). The DsRed2 image of the same target session (bottom left) was then overlaid with that of the source session (bottom center) shifted by the amount of displacement that provided the maximum correlation coefficient (bottom right). This procedure improved global image alignment, as shown by an increase in well-aligned pixels, represented in yellow in the overlaid image. (B) Cells overlaid without correcting image displacement. Cells in the target and source sessions are presented as red dots and blue circles, respectively. (C) Cell aligned after the correction of image displacement. Many cells in both images are now properly aligned,

and their local anatomical arrangements are mostly preserved. (D) Cell pairs that were considered to be the same cells are shown (see Methods for detailed criteria). (E) The average fractions of cells aligned between two consecutive sessions. Values are expressed relative to the number of total cells identified in the target sessions. The X-axis indicates the earlier of the two sessions that were compared. (F) The average two-dimensional correlation coefficients plotted against the session intervals between the two images compared. Data are presented as the mean $\pm$ SD ($n = 7 - 98$ session pairs).

Figure S6. The experience-dependent increase in vPC stability is not due to the increased number of vPCs. (A) Average fractions of common vPCs relative to the number of total vPCs for the early, middle and late phases of training. $\#^a$, $P = 0.029$ vs. Early, $\#\#^b$, $P = 0.0075$ vs. Early, $F_{(2,11)} = 7.08$; one-way ANOVA, $n = 4, 6$ and 4 session pairs. (B) Average fractions of stable vPCs relative to the number of total vPCs. $\#^a$, $P = 0.015$ vs. Early, $\#\#^b$, $P = 0.0037$ vs. Early, $F_{(2,11)} = 8.90$; one-way ANOVA, $n = 4, 6$ and 4 session pairs.

Figure S7. Stability and transition of vPCs and NvPCs across sessions. (A) Two functional cell categories, virtual place cells (vPCs) and non-virtual place cells (NvPCs), and the stability and transition of each category between sessions of interest and their immediately successive sessions are shown in this model. $P_{\text{vPC-vPC}}$ represents the probability that a vPC in one session remains a vPC in the next session, and $P_{\text{vPC-NvPC}}$ represents the probability that a vPC in one session becomes an NvPC in the next session. Similar conventions apply to $P_{\text{NvPC-vPC}}$ and $P_{\text{NvPC-NvPC}}$. (B) Stability and transition of vPC and NvPC. The X-axis indicates the earlier of the two sessions that were compared. (C) The average probabilities of vPC stability ($P_{\text{vPC-vPC}}$) and vPC formation ($P_{\text{NvPC-vPC}}$) for the early and late phases of training. **a , $P = 0.0046$, $t_{(6)} = 4.40$; **b , $P =$

976 0.0086, $t_{(6)} = 3.84$; #^c, $P = 0.044$, $t_{(6)} = 2.55$; #^d, $P = 0.014$, $t_{(6)} = 3.45$; unpaired two-tailed t-
977 test, $n = 4$ session pairs each.

978

979 **Figure S8. Behavior and vPC fractions during the reward rearrangement task. (A)**

980 Total distance traveled (Distance, left) and running speed (Speed, right). (B) The fraction
981 of time spent running (Run period). (C) The fraction of vPCs. The periods during which the
982 reward delivery was relocated are shaded in gray. In B and C, plots indicating average
983 values for pre, early and late phases of re-training are shown on the right. ** $P = 0.0018$ vs.
984 Pre, $F_{(2,12)} = 12.6$, one-way ANOVA, $n = 7$ mice from 2 groups.

985

986 **Figure S9. A subpopulation of cells that persistently encode the reward locations.**

987 The positions of virtual place fields of common vPCs in two consecutive sessions during
988 the pre-training (A, Pre; $n = 1106$ cells), before and after the reward rearrangement (B,
989 Rearr; $n = 462$ cells), and during the late phase of the reward rearrangement task (C, Late;
990 $n = 1281$ cells) are displayed as two-dimensional plots. The X-axis and Y-axis indicate the
991 positions of the earlier and later of the two sessions, respectively. Each dot represents the
992 position of a cell's virtual place field, and cells from all mice and relevant sessions are
993 shown. The red arrow in B indicates a cluster of cells that were active at the reward
994 locations regardless of their absolute positions. The green and red lines delineate the
995 positions of the landmark and reward delivery before the reward rearrangement,
996 respectively. The blue diagonal line and the two flanking black dotted lines represent the
997 range that defines stable vPCs.

998 **Methods**

999

1000 **Ethics statement**

1001 All experiments were conducted in accordance with institutional guidelines and
1002 protocols approved by the RIKEN Animal Experiments Committee.

1003

1004 **Generation of Thy1-G-CaMP7 transgenic mice**

1005 The cDNA encoding G-CaMP7 (Ohkura et al., 2012) ligated to the coding sequence
1006 of DsRed2 via a *Thomosa asigna* virus-derived 2A peptide sequence (Sato et al., 2015) was
1007 subcloned into the Xho I site of the modified mouse Thy-1.2 promoter vector (Feng et al.,
1008 2000). The 8.7-kb DNA fragment was prepared by digestion with Not I and Pvu I restriction
1009 enzymes and subsequent gel purification and injected into the pronuclei of 466 fertilized
1010 eggs of C57BL/6J mice. From 32 offspring, 9 mice were identified as transgene positive,
1011 and 6 exhibited transgene expression in the brain. One founder mouse that expressed the
1012 transgene at a high level in the hippocampus was used for this study. Mice were
1013 genotyped by PCR using the primers 5'-CTGCTGCCCCGACAACCA-3' and 5'-
1014 GTCGTCCTTGAAGAAGATGG-3', which provided a 465-bp product of the G-CaMP7
1015 coding sequence from tail DNA samples of transgene-positive mice.

1016

1017 **Analysis of transgene expression**

1018 Thy1-G-CaMP7 transgenic mice were anesthetized deeply with Avertin and
1019 perfused transcardially with phosphate-buffered saline (PBS), followed by 4%
1020 paraformaldehyde (PFA) in PBS. Brains were removed and further fixed in 4% PFA at 4°C
1021 overnight. Parasagittal sections were cut on a vibratome to a thickness of 100 µm. Low-
1022 magnification fluorescent images of G-CaMP7 and DsRed2 (Figure 1D) were acquired
1023 with a Keyence BZ-9000 epi-fluorescence microscope equipped with a 4x objective. For

immunofluorescence labeling, coronal sections were cut on a vibratome to a thickness of 50 μ m and incubated with rabbit anti-calbindin D-28K (1:500, AB1778, Millipore, Billerica, MA), rabbit anti-GAD65/67 (1:500, AB1511, Millipore), mouse anti-parvalbumin (1:1000, clone PARV-19, P3088, Sigma, St. Louis, MO) or mouse anti-somatostatin (1:200, clone SOM-018, GTX71935, Gene Tex, Irvine, CA) antibody diluted in PBS containing 2% normal goat serum, 1% BSA, and 0.1% Triton X-100 at 4°C overnight, followed by Alexa 647-labeled goat anti-rabbit or anti-mouse IgG antibody (1:700, A-21245 or A-21236, Thermo Fisher Scientific, Waltham, MA) diluted in the same buffer at room temperature for 1 h. Fluorescence images were obtained using an Olympus FV1000 or FV1200 laser-scanning confocal microscope (Olympus, Tokyo, Japan) equipped with a 20x dry or a 60x water immersion objective lens. The reproducibility of labeling patterns was confirmed in two independent experiments.

Surgery

Adult male Thy1-G-CaMP7 transgenic mice, at least 12 weeks old and weighing 28-30 g at the beginning of surgery, were used for the experiments. The mice were anesthetized with isoflurane in ambient air (3% induction, 1.5% maintenance) and placed in a custom-made stereotaxic frame. To reduce secretions and brain edema, atropine (0.3 mg/kg, s.c.) and dexamethazone (2 mg/kg, s.c.) were administered prior to anesthesia. A circular piece of scalp was removed, and the underlying bone was cleaned and dried. Three small screws were then placed in the skull (two at the suture of the interparietal and occipital bones and one on the right frontal bone) to provide anchors for the head plate. A thin layer of cyanoacrylate was applied to provide a substrate to which the dental acrylic could adhere.

A stainless steel head plate (25 mm length, 4 mm width, 1 mm thickness) with a wide circular opening (7 mm inner diameter and 10 mm outer diameter, the center is 2.5

mm off relative to the middle of the long side of the plate) was affixed to the skull using dental cement. The center of the opening was targeted at 2 mm posterior to the bregma and 2 mm lateral to the midline in the left hemisphere. The cement was mixed with black ink to block light entry from the LCD monitor into the microscope and placed onto the skull such that it covered the entire skull, including the anchor screws, except for the area of skull inside the opening of the head plate.

Optical window preparation was performed as described previously with modifications (Sato et al., 2016). A few days after the head plate surgery, a 2.5-mm-diameter circular craniotomy was created on the skull overlying the dorsal hippocampus. The dura was removed with forceps, and the overlying cortex was aspirated in a small amount at a time using a blunted 25-gauge needle connected to a vacuum pump. This step was continued with occasional irrigation with cortex buffer (123 mM NaCl, 5 mM KCl, 10 mM glucose, 2 mM CaCl_2 , 2 mM MgCl_2 , 10 mM HEPES, pH 7.4) until the white matter, including the corpus callosum, was exposed. Then, the top-most layers of the white matter were gently peeled aside by holding with the vacuum-connected blunted needle such that its minimal thickness remained covering the dorsal surface of the hippocampus. To minimize bleeding, aspiration was initiated from a cortical area devoid of large vessels, and bleeding was treated immediately with a piece of gelatin sponge (Spongel, Astellas Pharma, Tokyo, Japan) wetted with cortex buffer. An imaging window was then inserted to mechanically support the cranial hole, its surrounding tissue and the hippocampal surface. The imaging window consisted of a stainless steel ring (2.5 mm outer diameter, 2.2 mm inner diameter and 1.0 mm height) with a round coverslip (2.5 mm diameter, 0.17 mm thickness, Matsunami Glass Ind., Osaka, Japan) attached to the bottom using a UV-curable adhesive (NOA81, Norland Products, Cranbury, NJ). To reduce brain movement during imaging, a small disk of medical grade clear silicone sheeting (0.13 mm thickness, 20-10685, Invotec International, Jacksonville, FL) was attached to the surface of the

coverslip facing the hippocampal tissue (Mower et al., 2011). When the window was positioned, the bottom coverslip was approximately parallel relative to the head plate, and the hippocampal surface was clearly visible through the bottom coverslip without any trace of bleeding. The upper rim was then cemented to the skull with dental acrylic.

After surgery, a metal cover (0.3 mm thickness) was screwed onto the upper surface of the head plate to protect the imaging window from dust. The mice were placed in a warmed chamber until they fully recovered from anesthesia and were then returned to their home cages. They were housed for at least 4 weeks of postoperative recovery before the start of handling.

Virtual reality (VR) set-up

A VR system with an air-supported spherical treadmill for head-fixed mice was constructed as described previously (Sato et al., 2017). A 20-cm-diameter Styrofoam ball placed inside the bowl provided a freely rotating surface on which the mouse stood. The mouse was positioned near the top of the ball with its head fixed via the steel head plate that was screwed into a rigid cross bar and posts. A single wide-screen 23" LCD display (Dell U2312, Round Rock, TX) placed 30 cm in front of the mice presented VR scenes rendered by OmegaSpace 3.1 (Solidray Co. Ltd., Yokohama, Japan) running on a Windows 7 computer in 81° horizontal and 51° vertical fields of view. The LCD monitor was large enough to cover the major part of the mouse's binocular and monocular visual fields (Sato and Stryker, 2008). The use of a single LCD monitor for VR presentation effectively elicits visual cue-based virtual navigation behavior in head-fixed mice (Youngstrom and Stowbridge, 2012; Sato et al., 2017).

The movement of the ball was measured with a USB optical computer mouse (G400, Logitech, Newark, CA) via custom driver and LabVIEW software (National Instruments, Austin, TX). The optical mouse was positioned in front of the mouse and at

the intersection of the mouse's sagittal plane and the equator of the ball. The signals along the horizontal axis (aligned parallel to the mouse's sagittal plane) generated by the running of the head-fixed mouse was used to compute rotational velocity in the forward-backward direction. This velocity signal was converted into analog control voltages (0-5 V) via a D/A converter and fed to a USB joystick controller (BU0836X, Leo Bodner, Northamptonshire, UK) connected to the OmegaSpace computer to move the mouse's position in VR.

Water rewards (5 μ L/reward) were delivered by a microdispenser unit (O'Hara & Co., Ltd., Tokyo, Japan) attached to a water-feeding tube positioned directly in front of the mouse's mouth. The unit was triggered upon reward events in VR by 5 V TTL signals generated by an OmegaSpace script via a USB-connected D/A device (USB-6009, National Instruments). The behavioral parameters, such as the mouse's location in the virtual environment, the trigger signals for water rewards and the rotational velocity signals of the spherical treadmill, were recorded at 20-ms intervals using custom software in LabVIEW. The TTL signals for each frame sent by the microscope computer were recorded with the behavioral data to synchronize the imaging and behavioral data.

Behavior

At least 5 days before the start of behavioral training, mice implanted with the head plate and the imaging window were acclimated to handling and the Styrofoam ball. During this pre-training session, mice were handled by an experimenter for 5-10 min and then allowed to move freely on the top of the ball, which was rotated manually by the experimenter for another 5-10 min. The procedure was performed once a day and repeated for at least 3 days. The mice were then subjected to a water restriction schedule 2-3 days before start of the behavioral training. Body weight and general appearance were checked daily to ensure that the animals maintained at least ~85% of their pre-surgery body weight and exhibited no signs of abnormal behavior throughout the study. Mice were

housed in a group of one to four per cage in 12 h-12 h light-dark cycle (with lights on at 6 pm and off at 6 am on the next day). Experiments were performed during the dark phase of the cycle to enhance the locomotion of the mice.

The virtual endless linear track was created using an editor function of OmegaSpace. The mouse started at the origin of the virtual linear track segment and ran through the track unidirectionally with visual feedback rendered by OmegaSpace. The track segment was 100 cm long, measured as the number of rotations of the ball required to move from one end of the track to the other times the circumference of the ball. The mouse moved only one-dimensionally along the midline of the track with its view angle fixed toward the direction of moving. Different patterns were placed on the walls of each track subsegment as follows: vertical white and black stripes for 0-25 cm; horizontal white and black stripes for 25-50 cm; black dots on a white background for 50-100 cm. The floor was patterned with white grids on a black background. The space above the track was colored black. A green gate was placed as a salient landmark at 25 cm from the origin. Water rewards were delivered when the mouse reached a reward point located 75 cm from the origin. This reward point was located in the middle of a track zone with a certain wall pattern (i.e., black dots on white background) and not denoted with other salient visual cues. Upon reaching the other end of the segment, the mouse's virtual position was transferred back to the origin, and the same segment of the linear track was presented again. The approaching track segment next to the current one was always rendered on the monitor, so the mouse could see that the virtual linear track was infinitely long.

The mice underwent a total of 15 training sessions in the above task, with 1-2 sessions per day. Each session was 10 min long. When 2 sessions were performed in one day, within-day intervals were at least 4 h, and the mice were returned to their home cages between the sessions. The entire training period from the first to the last sessions was 225 ± 8 h (mean $\pm$ SD, $n = 7$ mice). The mouse was lightly anesthetized with isoflurane to

detach the metal window cover screwed onto the head plate and clean the imaging window before being placed into the VR apparatus. The head was then fixed to the crossbar above the ball via the head plate and we waited approximately 20 min until the mouse kept on the ball in darkness recovered fully from the anesthesia. During the behavioral session, the animal was allowed to behave freely in the head-fixed arrangement. G-CaMP7 fluorescence in hippocampal CA1 pyramidal neurons was simultaneously imaged as described below.

For the reward rearrangement task, mice first underwent 15 training sessions in the virtual linear track as described above. The mice were further trained for the following 5 sessions (Rearrangement 1-5) in the same virtual linear track except that the location of reward delivery (75 cm from the origin) was shifted to match the location of the landmark (25 cm from the origin). Data obtained from the last 4 sessions of the initial 15 training sessions before the shift (Sessions 12 through 15, also referred to as Pre -4 through -1) were analyzed as pre-rearrangement baseline sessions. The first rearrangement sessions were performed immediately after the last baseline sessions without releasing the mice from head fixation.

Imaging

Imaging was performed using a Nikon A1MP (Nikon, Tokyo, Japan) equipped with a 16x, NA 0.8 water immersion objective lens. The microscope was controlled with Nikon NIS-elements software. G-CaMP7 and DsRed2 were excited using a Ti-sapphire laser (MaiTai DeepSee eHP, Spectra-Physics, Santa Clara, CA) at 910 nm. Typical laser power was approximately 40 mW at the objective lens. G-CaMP7 fluorescence was separated using a 560-nm dichroic mirror and collected with an external GaAsP photomultiplier tube (10770PB-40, Hamamatsu Photonics, Hamamatsu, Japan) mounted immediately above the objective lens. The calcium-insensitive DsRed2 fluorescence, which helped to identify

1180 G-CaMP7-labeled pyramidal neurons, was simultaneously imaged and recorded using
1181 another GaAsP photomultiplier tube. The DsRed2 images were checked by the
1182 experimenter for the on-site assessment of the quality of image acquisition but not used for
1183 off-line quantitative image analysis, except for image alignment across sessions (Figure
1184 S5).

1185 To image G-CaMP7-labeled CA1 pyramidal neurons, the microscope was focused
1186 at a depth of approximately 150 μm from the hippocampal surface. To prevent the entry of
1187 light from the LCD monitor into the microscope, a small sheet of aluminum foil was
1188 wrapped around the objective lens, so the foil completely covered the space between the
1189 objective and the skull. Images of 512 x 512 pixels were acquired at a rate of 15 frames
1190 per second using a resonant-galvo scanner mounted on the microscope. Each imaging
1191 session was 10 min long. The size of the field of view was 532 x 532 μm . In repeated
1192 chronic imaging, previously imaged cell populations usually re-appeared at similar depths
1193 in new sessions. We took reference images of DsRed2 fluorescence at the beginning of
1194 each session to confirm that the reference image of the current session was very similar to
1195 that of the previous session by ensuring that blood vessels and neurons arranged in
1196 unique patterns appeared in the same parts of the two images.

1197

1198 **Image analysis**

1199 Each frame of a G-CaMP7 time-lapse movie was aligned to an average
1200 fluorescence image of the movie for motion correction using the TurboReg ImageJ plug-in.
1201 The registered movie was then denoised by a spatio-temporal median filter. This
1202 preprocessed movie $f(t,x)$ was reconstituted to the sum of fluorescence intensity of
1203 individual cells using a modified non-negative matrix factorization algorithm, as described
1204 in detail elsewhere (Vogelstein et al., 2010; Pnevmatikakis et al., 2016; Takekawa et al.,
1205 2017). Briefly, this algorithm assumes that the fluorescence intensity of each cell can be

deconvoluted to the spatial filter $a_c(x)$, which represents the position and shape of the cell, and the time variation $v_c(t)$ derived from spiking activities $u_c(t)$:

$$f(t, x) \sim N(a_0(x) + v_0(t) + \sum_c a_c(x)v_c(t), \sigma^2)$$

where a_0 , v_0 are baselines, and σ^2 is intensity of Gaussian noise. As is the case in cell identification using independent component analysis (Mukamel et al., 2009), this algorithm preferentially detects cells that change their fluorescence intensities over time (“active cells”) because cells that barely do so are regarded as being near baseline. Each spike derives the transient elevation of fluorescence intensity with a double-exponential shape:

$$v_c(t) = \sum_{\tau=1}^t \left(\exp\left(-\frac{t-t'}{\tau_1}\right) - \exp\left(-\frac{t-t'}{\tau_2}\right) \right) u_c(t')$$

The exponential rise and decay time constants $\tau_1 = 0.09$ and $\tau_2 = 0.261$, respectively, were obtained by curve fitting of actual traces of cellular calcium transients in G-CaMP7-expressing CA1 pyramidal neurons in Thy1-G-CaMP7 mice *in vivo*. Spatial filters and spike timings were estimated by two iterative steps. In the first step, we prepared tentative spatial filters and estimated spike trains corresponding to respective filters by a least-squares approach with a non-negative restraint condition. Subsequently, spatial filters were estimated using the least-squares method on the condition that the estimated spike

$$f(t, x) \sim N(a_0(x) + v_0(t) + \sum_c a_c(x)v_c(t), \sigma^2)$$

trains were feasible. In addition, we introduced L1 sparse regularization derived from priors that represented the typical cell size and spike frequency. To determine the mutual relationship between a and u , a regularized term was also introduced to the model. This condition guaranteed the uniqueness of the scale of a , u and v . As a consequence, a , u and v are presented in arbitrary units, while the product of a and v corresponds to the observed data.

In practice, 512 x 512 pixel image data were divided into 4 x 4 of 128 x 128 pixel subareas with 32-pixel overlap regions. Each subarea was analyzed with the above algorithm, and the results were combined to cover the whole image area. After the initial calculation, the morphology of each spatial filter was defined as the region above 0.2 times its peak value, and the position of the filter was defined by its weighted centroid. We then removed the following filters as those that did not represent complete single pyramidal cell morphology: (1) filters whose areas were smaller than 25 pixels, (2) filters whose areas were larger than 400 pixels, (3) filters located on the edge of the image, (4) filters whose heights or widths were greater than 64 pixels because they often contained structures of multiple cells, and (5) smaller filters in filter pairs whose distances were closer than 10 pixels (10.4 μ m) and whose temporal correlation coefficients of activities were greater than 0.3 because they were considered to be derived from the same cell.

After those non-cell filters were removed, we recalculated the activity time series for the new filter set. Visual inspection confirmed that nearly all active cells that were represented in a background-subtracted maximum-intensity projection image were identified with this procedure (Figure S2A-B). All images of the entire session, regardless of the mouse's behavioral state, were used for this image analysis. The average number of cells identified from a movie of a session was 900 ± 246 (mean $\pm$ SD, $n = 105$ sessions).

Analysis of virtual place fields

Place fields were calculated using cellular activity during movement periods. We defined these periods as the time when the mouse moved at a speed of > 0.5 cm/s continuously for a duration of > 2 s to reject irrelevant movements, such as grooming and jittering on the ball. We divided the entire virtual linear track segment into 80 bins (bin size = 1.25 cm) and created a histogram of neuronal activity versus track position for each cell. The activity events were defined by binarizing the time series of inferred spike activity u at

a threshold of 0.1, which was empirically determined to remove baseline noise. The counts of the histogram were then divided by the mouse's occupancy time at each bin, and the resultant place fields were Gaussian-smoothed (Gaussian window size = 6.25 cm) and normalized to the maximum values. To test the significance of virtual place-related activity, we calculated the mutual information content between neuronal activity and the mouse's virtual location for each cell (Markus et al., 1994; Ziv et al., 2013). We compared this value to a distribution of mutual information content calculated using 1000 randomly permuted data for the same cell. The permutation was conducted by rotating the activity event time series by a random amount relative to the time series of the mouse's virtual positions. Cells were considered to be virtual place cells (vPCs) if their overall activity rates within the session were no less than 0.1 events/s and their mutual information contents in the real data were greater than the 95th percentile of the values obtained from the randomly permuted data. We defined the position of the virtual place field of each vPC by the position of the peak of the field. A vPC was considered to be a "gate cell", "reward cell" or "wall cell" if its virtual place field position was 17.5-32.5, 75-95 or 47.5-55 cm from the origin of the track segment. vPCs with virtual place field positions outside the above zones were categorized as "non-reward, non-gate vPCs." The vPC formation factor was defined by the slope of a least-squares regression line fitted to a plot of the fraction of vPCs against the fraction of time spent running, which contained data points from all animals in the session of interest. The linear regression model included no constant term under the assumption that no vPCs were formed without running in each session. When calculating the fractions of gate cells, reward cells and non-reward non-gate vPCs relative to the number of total vPCs, data from sessions with at least 35 total vPCs ($n = 93$ sessions) were used to avoid the effects of improperly large or small fractions caused by small cell numbers.

Bayesian decoding

Bayesian probability-based reconstruction of the subject's trajectory from imaging data was conducted essentially as described by Zhang et al. (Zhang et al., 1998; Ziv et al., 2013). We computed $P(\mathbf{x}|\mathbf{n})$, the conditional probability for the subject to be at location $\mathbf{x}$ given the neuronal activity $\mathbf{n}$ occurred within a time window as follows. $P(\mathbf{x})$, the unconditional probability for the subject to be at position $\mathbf{x}$ was calculated from the dwell time distribution at each spatial bin (bin size = 1.25 cm). $P(\mathbf{n}|\mathbf{x})$, the conditional probability for neuronal activity $\mathbf{n}$ to occur given the subject is at position $\mathbf{x}$, was computed using $f_i(\mathbf{x})$, the spatial map of neuronal activity of cell i at position $\mathbf{x}$, under the assumption that occurrence of each Ca^{2+} transient is statistically independent as well as that vPCs are independent of each other (Zhang et al., 1998; Ziv et al., 2013). Time-varying activity traces containing Ca^{2+} transients were transformed into binarized vectors of the neuronal activity (time step = 67 ms), in which 1 and 0 represent the presence and absence of a Ca^{2+} transient, respectively, and these vectors were binned in 200 ms bins for probability calculations. $P(\mathbf{n})$, the probability for neuronal activity $\mathbf{n}$ to occur, was estimated by normalizing $P(\mathbf{x}|\mathbf{n})$ by the condition that the sum of $P(\mathbf{x}|\mathbf{n})$ along $\mathbf{x}$ is equal to 1 (Zhang et al., 1998). The peak position of the resultant probability distribution of $P(\mathbf{x}|\mathbf{n})$, computed using the above conditional and unconditional probabilities by Bayes' theorem, was regarded as the reconstructed position of the subject and the entire trajectory was obtained by sliding the time window ahead. Estimation error was calculated as the difference between the real and the reconstructed positions. Data from the sessions with a running time ≥ 240 s were used for the reconstruction ($n = 57$ sessions). We trained the decoder with the subject's observed virtual positions and activities of all vPCs during the first 150 s of the running period and estimated the trajectory for the following 90 s running time of the same sessions using the corresponding vPC activities. The sessions with average median errors across all positions less than 10 cm were classified as well-

decoded sessions (n= 25 sessions), and the errors of these sessions were then averaged separately for the locations encoded by GT, NRNG, and RW cells for comparison.

Alignment of cells across sessions and analysis of cell transitions

To find a population of the same cells in images that were acquired in two different sessions, we first estimated the extent of overall image displacement that existed between the two image datasets. We searched for a peak in the two-dimensional correlation coefficient calculated between the two DsRed2 reference images obtained at the beginning of each session within a range of a 25 x 25 pixel (26.0 x 26.0 μm) displacement in the x and y dimensions (Figure S5A). All compared image pairs displayed a peak within this range (average displacement in the x dimension, $5.9 \pm 4.5 \mu\text{m}$; average displacement in the y dimension, $4.7 \pm 4.2 \mu\text{m}$; average peak correlation coefficient 0.77 ± 0.09 , mean $\pm$ SD, n = 98 image pairs). During the calculation of two-dimensional correlation coefficients, the image of one session (the “source” session) was systematically shifted relative to that of the other session (the “target” session). The map of the coordinates of all cell positions in the target session was then overlaid with that of the source session, shifted by the amount of the estimated displacement (Figure S5A). The cell closest to each cell in the target session was searched in the displaced source session map, and the cell that was found was regarded provisionally to be the same cell if they were separated by 5 pixels (5.2 μm) or less. Cells that were unable to find the closest cells within this range were rejected from the subsequent analysis. After finding the provisional counterparts in the displaced source session map, the same procedure was repeated for the cells in the displaced source session map to conversely find their closest partners in the target session. This step helped remove cell pairs that were redundantly assigned (e.g., two different cells in one session falsely assigned to the same single cell in the other session) and the resultant cell pairs that had mutually unique correspondence were considered to

be the pairs that represented the same cells (termed hereafter “common cells”). When comparing vPC maps, common vPCs were defined as a subset of common cells that were identified as significant vPCs (see above) in both consecutive sessions. Stable vPCs were defined as a subset of common vPCs with virtual place field positions in the consecutive sessions that were close to each other (i.e., virtual place field distance < 10 cm). The stability and transition probabilities of vPCs and non-vPCs (NvPCs) (Figure S7) were calculated as follows. First, we identified a population of common cells that belonged to the cell category of interest in the reference session N and then examined the category into which each of them was classified in the subsequent session N+1. The probability of transition from category A in session N to category B in session N+1, P_{A-B} , was calculated by dividing the number of cells that belonged to category A in session N and became a cell of category B in session N+1 by the total number of cells that belonged to category A in session N. The probability of stability was calculated similarly by considering it as a process of transition to the same category. The analysis of formation, recruitment and stabilization of vPCs (Figures 5 and 8) was conducted similarly, except that cells were classified into four categories in the reference session N, and the position of the virtual place field of each cell was tracked in the subsequent session N+1. The results are expressed as the cell density rather than probabilities to show the relative contributions of the different cell categories.

Statistics

Data are expressed as means $\pm$ SEM unless stated otherwise. When only two groups were compared, two-sided Student’s t-tests were used if the variances of the two groups were similar. Otherwise, two-tailed Mann-Whitney tests were used. When more than two groups were compared, analysis of variance (ANOVA) was used if variances of the groups compared were similar. Otherwise, a non-parametric version of ANOVA

(Friedman test) was used. In both parametric and non-parametric ANOVA, P-values were adjusted for post hoc multiple comparisons. Exact P-values are shown unless $P < 0.0001$. Statistical tests were performed using GraphPad Prism Version 6.05 (GraphPad Software, Inc., La Jolla, CA).

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Figure 1
Sato et al.

Figure 2
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Figure 3
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Figure 4
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Figure 5
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Figure 6
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A

B

C

Figure 8
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Figure S1
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Figure S2
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Figure S3
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Figure S4
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Figure S5
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Figure S6
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Figure S7
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Figure S8
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A Pre

B Rearr

C Late

Figure S9
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