

Distinct Ensemble Codes in Hippocampal Areas CA3 and CA1

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The hippocampus has differentiated into an extensively connected recurrent stage (CA3) followed by a feed-forward stage (CA1). We examined the function of this structural differentiation by determining how cell ensembles in CA3 and CA1 generate representations of rooms with common spatial elements. In CA3, distinct subsets of pyramidal cells were activated in each room, regardless of the similarity of the testing enclosure. In CA1, the activated populations overlapped, and the overlap increased in similar enclosures. After exposure to a novel room, ensemble activity developed slower in CA3 than CA1, suggesting that the representations emerged independently.

The hippocampus plays a fundamental role in encoding, consolidation, and retrieval of episodic and semantic memory (1–4). In mammals, the hippocampus has differentiated into a recurrent network of densely interconnected pyramidal cells (CA3) and a feed-forward network with almost no intrinsic excitatory connections (CA1) (5). These structural differences suggest distinct roles for CA3 and CA1 in hippocampal memory formation, but *which* functions are performed by the two subfields has remained elusive (6).

Firing properties of individual pyramidal cells in CA3 and CA1 offer limited clues about computational advantages of hippocampal differentiation. In both subfields, the majority of pyramidal cells have place-specific firing fields controlled by geometric relations in the animal's local environment (2, 7–10), and there are only small quantitative differences in their spatial firing characteristics (10). However, cell assemblies in CA3 and CA1 may contain additional information (11). A major function of such assemblies may be to augment differences between correlated input patterns (1, 8, 12–18), so as to minimize interference between stored information (19). To examine whether such an orthogonalization process (pattern separation; 20) is implemented differentially in CA3 and CA1, we compared ensemble firing in connected segments of these areas (5; Fig. 1A) while rats were chasing food in enclosures with varying geometric similarity (large square, small square, small circle) in three different rooms (A, B and C).

Individual place cells in CA3 and CA1 had similar firing characteristics (7, 10, 22; Fig. 2A; tables S1 and S2). The most striking difference was the significantly lower proportion of active neurons in CA3 (10). With a rate threshold of 0.25 Hz, the proportion of active cells ranged from 0.17 (B) to 0.32 (A) in CA3 and from 0.48 (C) to 0.66 (A) in CA1 (Z values from 4.1 in C to 8.0 in A, $P < 0.001$; Fig. 1B–D and fig. S1). The sparser firing of CA3 may indeed favour more orthogonal representations, but it cannot be established from discharge profiles in a single condition whether active orthogonalization takes place. To examine orthogonalization processes more directly, we quantified the extent to which the active set of neurons overlapped between two familiar rooms (A and B), using a measure which takes into account the 'passive' effect of different sparsity in CA3 and CA1 (Fig. 2B).

The analyses indicated that ensemble codes in CA3 and CA1 are different (Fig. 2B). In CA3, representations for A and B were nearly independent. The measured overlap ranged from 0.11 to 0.14 for the four possible comparisons of A and B (AB, AB', A'B, and A'B'), whereas the expected overlap for independent firing ranged from 0.12 to 0.14, depending on exact firing rate distributions between A, B, B' and A' (Fig. 2B; t between 0.1 and 1.2, n.s.). The distribution of CA3 population-vectors was highly informative about which room the rat was in (0.54 ± 0.06 bits at $\tau = 150$; mean \pm S.E.M.). In CA1, however, there was significant overlap between the representations for A and B. Observed values ranged from 0.36 to 0.42, whereas expected values were between 0.26 and 0.28 (t between 3.7 and 5.2, all $P < 0.001$). Population vectors differed less than in CA3 (0.30 ± 0.05 bits for CA1 at $\tau = 150$; $t(17) = 3.2$, $P < 0.005$).

We hypothesized that the correlated activity in CA1 depended on the distinctness of A and B and thus compared the overlap obtained with similar and different enclosures in these rooms (Fig. 2C and figs. S2 and S3). Shared features had no effect in CA3 (Fig. 2C). With identical square boxes in A and B (high similarity), the overlap in CA1 was almost as large as with repeated testing in the same room (0.55 for AB vs. 0.63 for AA' and 0.66 for BB'). With squares of different size (medium similarity) the overlap decreased to 0.43. With

boxes that differed both in size and shape (low similarity) the overlap was no longer distinguishable from the expected value obtained with random permutations (0.32 vs. 0.28). These effects were statistically significant ($F(2,140) = 6.0$, $P < 0.005$) and consistent across animals (high-similarity: 0.56, 0.54; medium-similarity: 0.53, 0.45, and 0.32; low-similarity: 0.41, 0.34 and 0.27). These effects were also seen in spatial correlations between pairs of firing fields in A and B (Fig. 2D) and in the temporal structure of the ensemble activity (Fig. 2E) (supporting online text).

Place fields develop as animals explore novel environments (2, 9). We asked whether differences between CA3 and CA1 emerge gradually or are present from the beginning in rats exposed to a novel room (room C; Fig. 3). In CA3, the overlap between C and A or B fluctuated around expected values (0.16 vs 0.14) ($t(83) = 0.79$, n.s), implying that new representations were decorrelated already on the first trial (Fig. 3B). In CA1, the new representations correlated significantly with those in A and B (overlap 0.38; expected value: 0.24; $t(104) = 4.6$, $P < 0.001$). This overlap in CA1 was not influenced by the geometry of the enclosures (Fig. 3B; $t(63) = 0.0$ for medium vs. low similarity).

To determine whether the ensemble codes in CA1 derive from those in CA3, we next compared their time courses. In CA3, the new spatial map stabilized only after 20–30 min in C (Fig. 3A). The overlap between the first and the last 10-min blocks (CC'') was low compared to the overlap between repeated trials in the familiar rooms (AA' and BB') (Fig. 3C vs. Fig. 2B). The distribution of population vectors was more different between the first and the last 10 min (CC'') than between the first and the middle 10 min (CC') (Fig. 3D, E; supporting online text), suggesting that the stabilization of the ensemble structure in CA3 took 20 min or more. In simultaneously recorded CA1 cells, reliable place fields were mostly apparent already during the first minutes (9). The overlap between the first and last 10 min was higher than for CA3 ($t(91) = 5.1$, $P < 0.001$; Fig. 3C), and the population-vector distributions were more similar (Fig. 3D, E) and not significantly different from those obtained between repeated tests in A or B (supporting online text). The faster manifestation of an ensemble code in CA1 suggests that representations in CA3 and CA1 arise independently and that the latter may emerge through direct input from the entorhinal cortex (21).

The functional differences between CA3 and CA1 suggest a rationale for the differentiation of their intrinsic structure (6). With its orthogonalized activity patterns, CA3 may store accurate representations of context, one in one with the representation of local position by individual neurons. This provides a neuronal substrate for the observation that animals with hippocampal lesions show increased susceptibility to interference (19) and fail to discriminate between contexts

with different conditioning histories, particularly when the contexts differ only minimally (23, 24). As input similarity increases, the CA3 network may eventually switch from pattern separation to pattern completion (1, 12, 25, 26), resulting in larger overlap in CA3 than in CA1 (26) and suggesting that CA3 contains coherent but flexible population codes that can be used both to disambiguate and to identify contexts (27). Taken together, the findings imply that encoding of context, defined as the relationship between stimuli and the time and place in which events occur, may be a major function of CA3 (2–4, 28, 29). While pattern separation and pattern completion probably arise within the dentate/CA3 complex (13, 15), the exact contribution of the dentate to ensemble activity in CA3 remains to be elucidated.

In contrast to CA3, population codes in CA1 responded to common features of the rooms. When rats were tested with identical boxes in two different rooms, representations in CA1 were only weakly more different than during repeated recording in the same room. This suggests that place cells in CA1 can respond to individual landmark configurations independently of background context, as observed after cue misalignments in CA1 (26, 30, 31; supporting online text). However, orthogonalized codes from CA3 may be reflected in equally orthogonal representations in CA1 under certain conditions, such as with low sensory input and during memory-based behaviour. The orthogonalized codes may then be exported to other brain structures (32, 33), associating a contextual tag to information stored there.

New representations formed at a slower rate in CA3 than in CA1. The slower manifestation of a stable map in CA3 may, perhaps, reflect the predominantly recurrent nature of interactions in this network, which may require that a stable orthogonalized representation of a new context is reached iteratively. In contrast, the population activity in the predominantly feedforward network of CA1 may be established at the very beginning of the trial, or even be available beforehand, hardwired in the circuit (27). The faster appearance of the CA1 ensemble code suggests that it emerges independently of the CA3 representation, probably via the direct projections from entorhinal cortex (21). However, representations in CA1 may still evolve further. The relation between geometric similarity and overlap was not expressed on day 1 in the novel room, suggesting that the more limited disambiguation of differences in CA1 is a later refinement, like other delayed changes of ensemble activity in this subfield (34, 35). Whether these slower processes reflect the integration of inputs from CA3, conveying orthogonalized memory representations, with processed sensory information carried to CA1 by direct inputs from entorhinal cortex, remains to be determined.

References and Notes

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20. Orthogonalization (pattern separation) refers to the tendency to decorrelate representations, assessed relative to the baseline expected for unrelated conditions. Orthogonalization is expressed as a difference in both the subset of active cells and the relative firing locations of cells that were active in each of the conditions (supporting online text). Remapping is a special case limited to the spatial domain.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/1100265/DC1

Materials and Methods

SOM Text

Figs. S1 to S3

Tables S1 and S2

References and Notes

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Fig. 1. Sparser representations in CA3 than CA1. **(A)** Representative electrode locations (arrows) in CA3 and CA1 (same rat). **(B)** Cumulative frequency diagram showing lower proportion of active cells in CA3 than CA1 in room A (all cells; see also fig. S1). **(C,D)** Relation between firing rates on repeated tests in the same room **(C)** or in different rooms **(D)**. Each point corresponds to one cell.

Fig. 2. Orthogonalized representations in CA3 but not CA1. **(A)** Colour-coded rate maps showing place fields in identical enclosures but different rooms (day 10). Top panel indicates recording sequence, geometry of test boxes, and orientation of cue card. Spikes were recorded simultaneously from CA3 and CA1. Rows show cells; columns show trials in rooms A and B (not chronologically ordered). Plots are scaled to indicated maximum rates (red, maximum; blue, silent; white,

not visited). **(B)** Overlap between active populations in A and B (mean \pm S.E.M.). Overlap was measured by averaging across cells the ratio between the lower and higher rates in a pair of trials (top inset). Red lines indicate overlaps expected by assuming independent firing in the two trials (bottom inset). **(C)** Overlap as a function of geometric similarity of the enclosures in A and B. **(D)** Mean spatial correlation (\pm S.E.M.) of place fields in A and B (squares only; supporting online text). **(E)** Effect of geometric similarity on ensemble coactivity in CA1 but not CA3 in 2 rats with >8 simultaneously recorded cells in each area (brown: low similarity session; green: high similarity session). High information corresponds to distinct distributions of population vectors in A and B.

Fig. 3. Emergence of new ensembles in CA3 and CA1. **(A)** Development of place fields in CA3 and CA1 neurons in a novel room C (3 blocks of 10 min; same cells as in Fig. 2A). **(B)** Overlap of activity between A (familiar) and C (novel). Boxes had different size or both different size and different shape. Arrows indicate expected values (purple, CA3; blue, CA1). **(C)** Overlap between pairs of 10-min blocks in C (C, 0–10 min; C', 10–20 min; C'', 20–30 min). **(DE)** Development of ensemble structure in CA3 and CA1 **(D,** same experiment as in A; **E,** whole sample). The difference in neuronal activity between early and late blocks of the trial was assessed by measuring how much information the distribution of population vectors provided about which part of the trial was being recorded. **(F)** Horizontal speed during exploration of room C (mean \pm S.E.M.).





