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Competing interests statement

The authors declare that they have no competing financial interests.

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Long-term plasticity in hippocampal place-cell representation of environmental geometry

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The hippocampus is widely believed to be involved in the storage or consolidation of long-term memories[1–4]. Several reports have shown short-term changes in single hippocampal unit activity during memory and plasticity experiments[5–12], but there has been no experimental demonstration of long-term persistent changes in neuronal activity in any region except primary cortical areas[13–16]. Here we report that, in rats repeatedly exposed to two differently shaped environments, the hippocampal-place-cell representations of those environments gradually and incrementally diverge; this divergence is specific to environmental shape, occurs independently of explicit reward, persists for periods of at least one month, and transfers to new enclosures of the same shape. These results indicate that place cells may be a neural substrate for long-term incidental learning, and demonstrate the long-term stability of an experience-dependent firing pattern in the hippocampal formation.

In rats, hippocampal lesions cause deficits in spatial behaviour[2,17–20]. One of the major behavioural correlates of the firing of hippocampal pyramidal cells is the animal's location. Previous experimental and theoretical work suggests that the major determinant of the location and shape of place-cell firing fields is the distance from two or more walls in particular directions[21,22]. This theory predicts that these cells will have related patterns of firing in enclosures of different shape. We tested this prediction (see Methods 'preliminary experiment' and Supplementary Information) and found, in each of seven rats, that place fields were

very similar on initial exposure to square and circular boxes (Fig. 1a). 73% of the cells (48/66) had 'homotopic' fields in both shapes (that is, in the same location, see Methods for definition of 'homotopic'). Other environmental manipulations, such as translation (Fig. 1b), removal (Fig. 1c), and reconfiguration of the box into shapes other than squares and circles (not shown), showed that firing patterns relate to the box walls and not to other cues in the testing arena. This finding of similarity across shapes conflicts with earlier experiments[7,23], performed on animals that had had considerable experience of the testing enclosures. We asked whether experience was the critical factor in producing neuronal discrimination between different shapes.

We recorded hippocampal CA1 cells from a new group of three animals during repeated exposures to different shapes of enclosure (see Methods 'main experiment'). Recording was performed during the animals' entire experience (up to three weeks) of these enclosures: unlike previous studies (for example, refs 5,6,7,8,9,10,11,12, 21, 23,24,25,26,27,28,29,30), there was no unrecorded training phase. Four boxes were used: two identical circular-walled, and two identical square-walled boxes (hereafter simply 'circle' and 'square'), all made of the same materials so that discrimination on the basis of geometry could be separated from discrimination on the basis of other differences between boxes.

We recorded on successive days monitoring the activity of the same group of neurons where possible, in some cases following individual cells for over a week. In other cases we obtained different samples from the same overall population. Either way, on the first day the firing patterns in the two shapes were similar, replicating our previous work; on later trials, the patterns diverged while those in
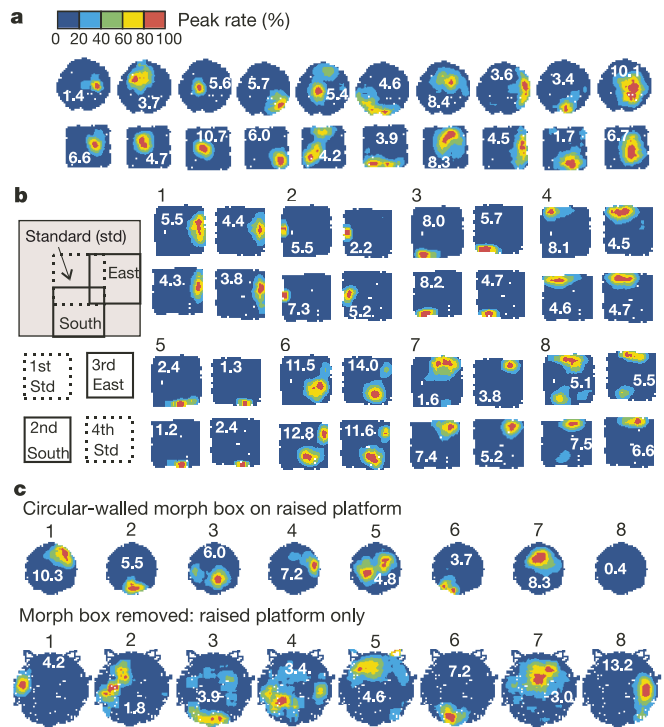

Figure 1 Similarity of spatial firing during early exposure to circular- and square-walled enclosures. **a**, Similar place fields of 10 representative simultaneously recorded CA1 neurons in circle and square. Probe trials on two different groups of cells (**b**, **c**) show that this similarity is determined by box walls rather than similar sets of background cues in the testing arena in both circle and square conditions. Box-wall translation by 40 cm (**b**) eastwards (upper right) or southwards (lower left) does not affect firing fields relative to the box frame, while box-wall removal (**c**) induces remapping. Fields with less than a 1.0-Hz peak rate are not shown.

the same shape remained similar. Figure 2 summarizes this process and also shows that the divergence endures after a delay. Across all three animals, 14/17 cells (82%) had homotopic fields on day 1 (Fig. 2a), after which remapping proceeded, but not at the same rate in all animals. Rat 1 (data shown by an asterisk) had three identical fields in the two shapes on day 1 but had completely remapped by day 5 (showing no overlap between six fields). After similar data was obtained on day 6, time-series recording from this animal was terminated. Rats 2 and 3 took considerably longer to remap with 12/14 place cells displaying 'similar' fields on day 1, 7/13 on day 5, 7/32 on day 21 (see Methods for numerical quantification of 'similar'). Examination of the animals' behaviour during these days did not reveal any systematic differences that would explain these changes

(see Supplementary Information).

The firing patterns in the square and circle on day 21 (Fig. 2d) differ either because the cell fires in one shape and not the other (top row), or because it fires in two different places in the two shapes (bottom row). To quantify these changes, we transformed the circle topologically into a square of the same area as the square box and used these transformed data to compute three different measures of disparity (see Methods). The first, rate divergence (RD), is sensitive to the difference in firing rates between boxes of different shape compared to boxes of the same shape; the second, peak divergence (PD), is sensitive to the difference between the location of firing field peaks in the two shapes compared to boxes of the same shape, and the third, field divergence (FD), is the proportion of the cells with
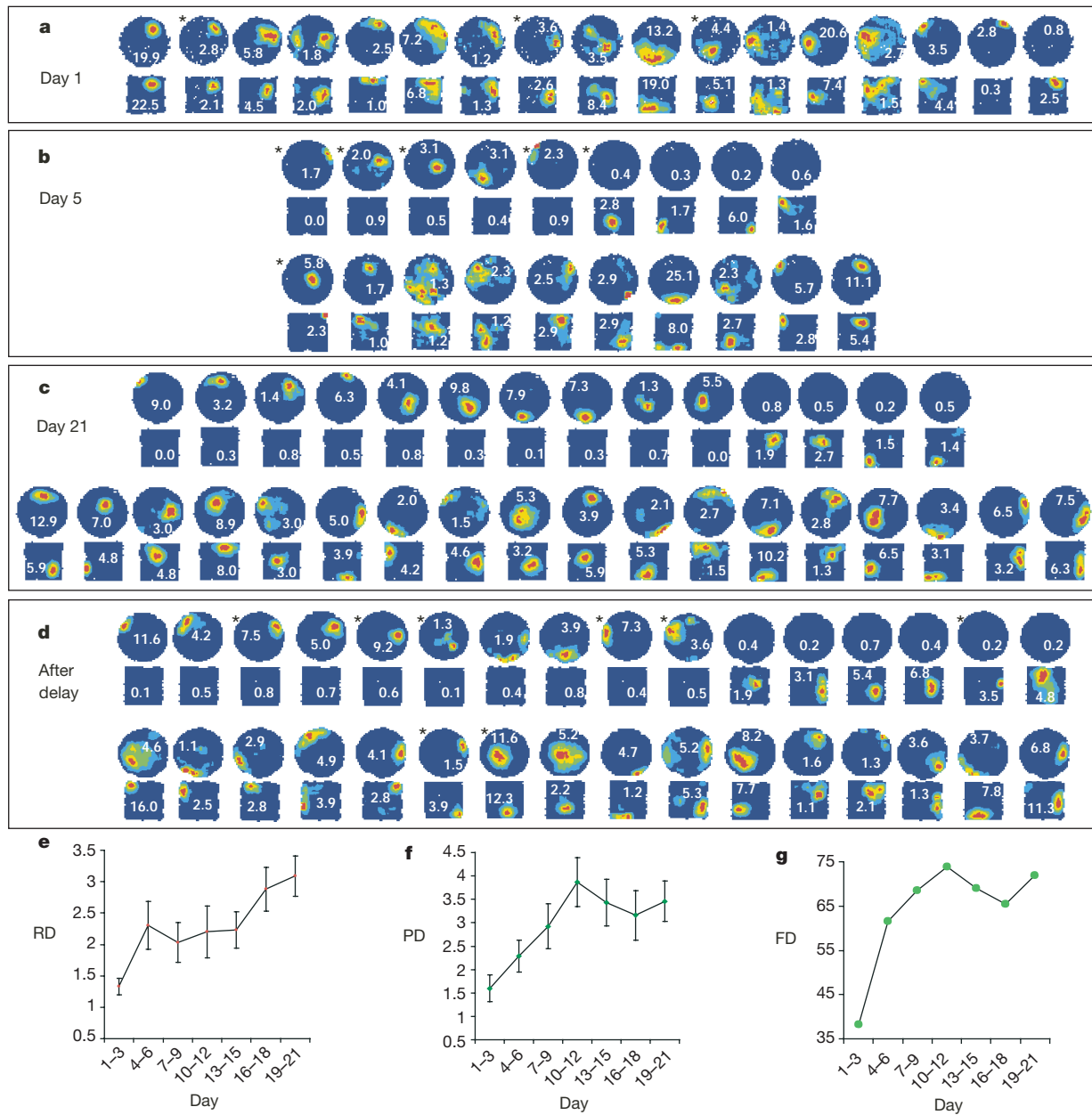

Figure 2 Repeated exposure to circle and square enclosures increases place field divergence. Similar patterns on day 1 (**a**) are followed by more divergent patterns on day 5 (**b**), and still more by day 21 (**c**). Pattern divergence is maintained after long delays (**d**). Various measures of firing pattern divergence from the slower-learning rats 2 and 3 show experience induces increases in: **e**, rate divergence (RD: estimated range 1.0 to 5.3);

f, peak divergence (PD: estimated range 1.0 to 5.3); and **g**, proportion of cells with divergent fields (FD: estimated range 29% to 90%). Graphs show mean values (error bars in **e**, **f** are ±s.e.m.) over 3-day blocks. See Supplementary Information for details. Asterisks indicate data from faster-learning Rat 1.

fields in both shapes that are not homotopic. Figure 2e–g depicts these measures in rats 2 and 3, averaged in 3-day blocks. All three measures show an increase over time (correlation between time and mean RD: $r = 0.89$, $P < 0.004$; mean PD: $r = 0.78$, $P < 0.025$; mean FD: $r = 0.70$, $P < 0.05$). The cells of rat 1 remapped primarily by rate divergence (mean RD correlation with time: $r = 0.91$, $P < 0.01$). Reflecting these trends, the firing patterns at the end of the time series ('dayend': day 6 for rat 1, day 21 for rats 2 and 3) were significantly more divergent than on day 1: mean RD increased from 1.32 ± 0.22 to 3.59 ± 0.69 ($P < 0.002$); mean PD from 1.15 ± 0.19 to 4.00 ± 0.59 ($P < 0.0001$); FD from 18% (3/17) to 76% (16/21) ($x^2, P < 0.001$).

The gradual shift in the population statistics (averaged across cells and animals) could be due to abrupt transitions at different times in different cells, or to gradual changes within each cell. We were able to track the activity of several individual cells throughout their transition period and found that most diverged in an incremental fashion. Figure 3a shows three different ways in which neurons with initially homotopic fields in both boxes were observed to differentiate between them. Figure 3b–d shows one example of each. The cell in Fig. 3b develops a new subfield whose rate progressively increases over the trial while that in the old field decreases; this process repeats on the subsequent trial and the new field remains thereafter. The cell in Fig. 3c illustrates the progressive translation of field position in one box over time. The cell in Fig. 3d is an example of a common change, a progressive reduction of firing rate to below the 1-Hz threshold in one shape together with the maintenance of firing rate in the other. In each case, the divergence process can be seen to take place over several trials or days.

We asked whether this remapping was permanent. Each animal was returned to its home cage and not replaced in either environment for a period of several weeks (28, 17 and 39 days in rats 1–3 respectively) after its last exposure there. After this delay, the animals were retested in the circle and square. In all cases the majority of cells differentiated between the two boxes (see Fig. 2d), suggesting that the learning process was a permanent one. None of the 8 cells recorded from rat 1 (asterisks) have similar fields, and only 5 of the 24 cells from the slower-learning rats 2 and 3 have similar fields. The overall pattern remains highly divergent with no significant differences in our three measures between the last training day and the day of the delay test (all $P > 0.05$). In a further (additional 29 days) delay test on rat 2 after that shown in Fig. 2d, only 3/10 cells had similar firing patterns (see Supplementary Information).

It is unlikely that this shape-specific divergence in place fields could be due to the passage of time alone rather than to the rats' experience of the enclosures. Some corroboration of this was obtained in a delay test on one of the animals from the preliminary experiment. On its first day of exposure to the two boxes, 9/10 cells had similar fields (mean across-shape distance 7.2 ± 1.0 cm). This rat then had six further days exposure to the two shapes, followed by a series of probe trials in the square box only, after which there were minimal signs of remapping, 5/7 cells being similar (mean across-shape distance 7.2 ± 1.7 cm). Following a delay of 32 days without further experience of the boxes, 5/5 cells (see Supplementary Information) were similar (mean across-shape distance 5.5 ± 1.7 cm). We conclude that the post-delay pattern divergence seen in rats 1, 2 and 3 indicates long-term storage and retrieval of an experience-dependent learned pattern.

What is the perceptual basis for the acquired environmental discrimination, and is it transferable? Two wooden squares and two wooden circles were used during the learning phase, and all measures compared inter-shape with intra-shape changes, so the cells must become tuned to geometrical features. We wondered whether geometrical tuning was strong enough to override changes in non-geometrical features in a transfer test. Rats 1 to 3, trained in the wooden boxes, were now tested in the 'morph' circle and 'morph' square, two environments created from the same deformable structure (see Methods). In addition, two new rats (4, 5) were trained in the morph circle and morph square and then tested in the wooden circle and wooden square boxes. We recorded 65 cells in total (41 in the wooden-to-morph box transfer study, 24 in the
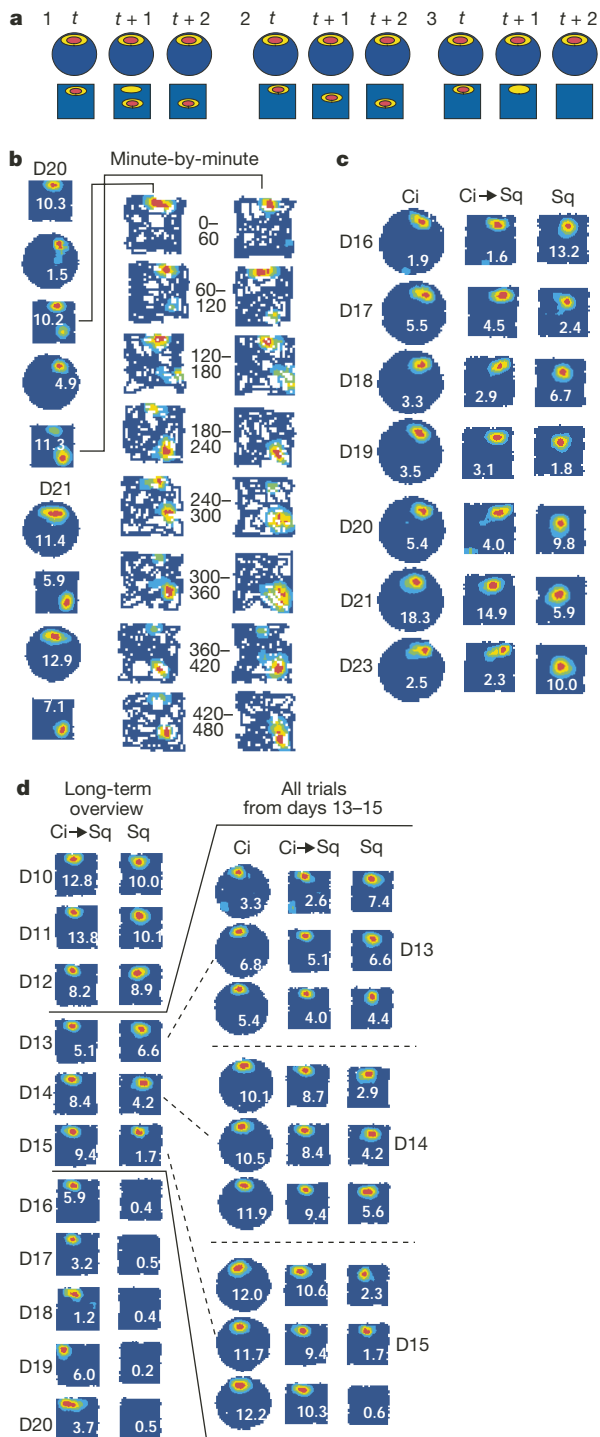

Figure 3 Schematic (**a**) and observed (**b**, **c**, **d**) incremental development of divergent firing in individual place cells. 'Ci ⟶ Sq' denotes transformed-circle firing-rate maps (see Methods). D*n* denotes recording day. **b**, Cell develops a new subfield in square. **c**, Cell shifts its field in square relative to circle. **d**, Cell reduces its firing rate in square, relative to circle. Peak rates in square are about equal to those in circle on day 13, about 50% on day 14, less than 25% on day 15, and below the 1-Hz threshold thereafter.
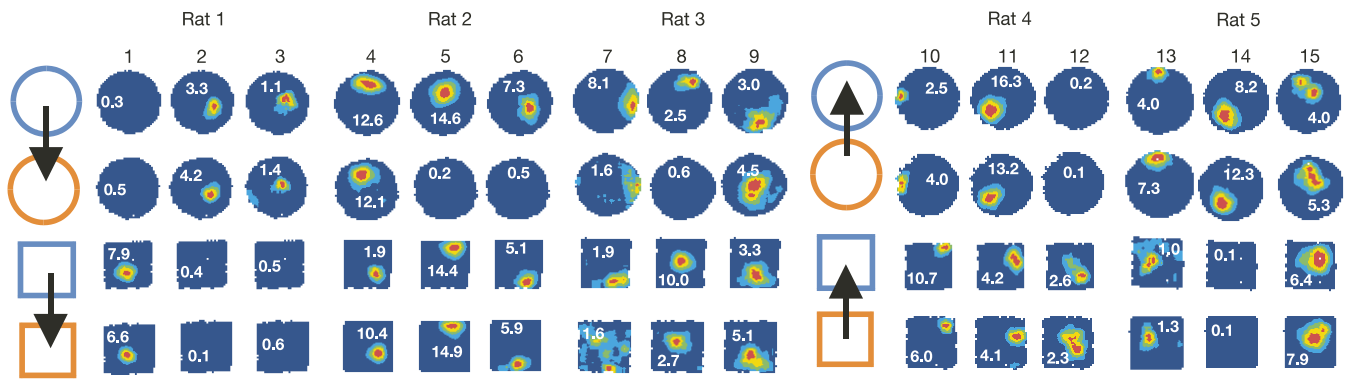
Figure 4 Shape-specific firing patterns learned in one type of enclosure were reproduced in different enclosures of similar shape. Diagrams indicate type and shape of enclosure (blue, wooden box; orange, 'morph' box). Firing-rate maps from three representative cells in each of five animals are shown in rows corresponding (top to bottom) to wooden circle, morph circle, wooden square, morph square. Firing-rate maps of rats originally trained in the wooden boxes (rats 1, 2, 3) are shown on the left, and those of rats originally trained in the morph box (rats 4, 5) on the right.

morph-to-wooden box transfer study). Figure 4 shows three representative cells for each rat. Overall we found firing patterns to be strongly influenced by geometrical shape, despite the change in box construction and material. The mean distance between the place field peaks of cells firing in boxes of the same shape but different material was only 7.0 ± 0.6 cm ($n = 51$, $P \ll 1 \times 10^{-10}$, see the Monte Carlo simulation in Methods). The differences in cells' firing rates between trials in boxes of different material were not significantly different to the differences in firing rates between trials in the boxes of the same material ($P > 0.05$, paired t-test, see Supplementary Information). In all five rats, the firing patterns in the training square were reproduced in the test square (Fig. 4, square trials), while the circle-to-circle transfer results were more mixed (with some cells in rats 2 and 3 ceasing firing in the morph circle, see Fig. 4 cells 5, 6, 8, and a few firing in unrelated locations, see Fig. 4 cell 9). In summary, the five rats showed considerable tendency to generalize their geometrically tuned responses across perceptually different environments.

That environmental experience leads CA1 place cells to discriminate between environments on the basis of geometrical features, in the absence of differential reinforcement, and to maintain this discrimination for a long time, is consistent with the cognitive map theory of hippocampal function[2]. (We note that rapid learning also plays an important role in this theory.) Further, the learning site(s) are most probably hippocampal, because cells in the superficial layers of entorhinal cortex, which comprise the dominant projection to the hippocampus, show inter-shape pattern similarity in exactly those circumstances in which hippocampal cells show divergence[23]. We have captured hippocampal firing patterns throughout the animals' entire experience in two geometrically distinct enclosures, from initial entry, through to discrimination, transfer and delay stages. Our results identify a potential neural basis for hippocampal long-term memory, and may provide a model by which to test deficits in neuronal encoding, storage and retrieval, such as those induced by Alzheimer's disease. □

Methods

Twelve Lister Hooded rats maintained on a 12:12 hour light:dark schedule, weighing 280–425g at time of surgery, were used as subjects: 7 in the preliminary experiment, 5 in the main experiment (numbered 1 to 5 in the text). Methods were similar in both experiments. We describe the main experiment, pointing out differences where appropriate. See Supplementary Information for more details.

Testing procedures

Experiments were conducted in a black-curtained, circular testing arena 2.3 m in diameter. Rats were kept on a holding platform outside the arena before and after every trial. Two identical circular-walled (78 cm diameter), and two identical square-walled (59 cm sides), smooth, light-grey wooden boxes (all 50 cm high) were used alternately, placed on a platform 27 cm above the floor. This platform was cleaned before every trial. The centre of

each box always had the same location in the arena. A white cue card (58 cm wide, 50 cm high) placed at the north portion of the internal wall, and standardized procedures for placing the rat into the box, provided directional constancy. During trials the rat searched for grains of sweetened rice randomly thrown into the box about every 30 seconds. In the time-series component of the experiment, rats had alternating trials, circle first, in each shape for consecutive days. On days 1 and 2, rats had four trials, from day 3 onwards, six trials per day, and each rat had four trials for delay testing later on. Transfer trials used a 'morph' box made of 32 pieces of rectangular cross-section plastic tubing, 50 cm high, held together by packing tape and covered by masking tape on the inner surface. It could be configured as a 78-cm-diameter circle or a 62-cm-sided square. Rats 4 and 5, and the 7 rats of the preliminary experiment, were trained in the morph square and morph circle. For these animals an external cue card (102 cm high, 77 cm wide) suspended 25 cm inside the black-curtain rail provided directional constancy.

Monte Carlo simulation

Field peaks in the square or transformed circle are simulated as points drawn from a uniform distribution within a square box of side L. Points are binned, like the data, using 25×25 bins. The distances between one million pairs of points were found. The mean separation was $0.522L$ (s.d $\pm 0.249L$), 5% had a separation $\leq 0.127L$, and 8.3% had separation $\leq 0.17L$.

Cell recording and data analysis

Methods of extracellular tetrode recording and firing-rate map construction were as previously reported[21,30]. Rats in the main experiment were first run in the enclosures 3 to 8 weeks after surgical implantation of microdrives, once recording stability had been assessed on the holding platform outside the curtained testing arena. Place units were isolated offline and firing-rate maps were constructed by dividing the number of spikes by the rat's dwell time in a given location bin using customized software (Axona). The circle was transformed into a square using a standard algorithm (see Supplementary Information). Data from the square, circle, and transformed-circle were divided into 2.4-cm-sided square bins. The firing rate in each bin was smoothed using a boxcar average over the 5×5 surrounding bins centred on that bin. The five colours of firing-rate maps are each autoscaled to represent 20% of the peak rate (red to dark blue). Unvisited bins are shown in white.

Firing-rate maps from all cells and trials were inspected. We found no evidence of episode-specific, box-specific cell firing or any privileged time points at which remapping occurred. A few cells firing in only one trial per day were excluded as unreliable. In the main experiment, 30 cells from rat 1 (mean 5.0 per day), 264 cells from rat 2 (mean 12.6 per day), and 205 cells from rat 3 (mean 9.8 per day) contributed to the time-series data, from day 1 to 'dayend' (day 6 for rat 1, day 21 for rats 2 and 3). In all three rats, we recorded more cells later in the time series than early on.

Definitions of terms

The peak firing rate and its (x, y) location in the square or transformed-circle firing-rate map for each cell in each trial were used in the quantitative measures defined below for each cell on each day (detailed description in Supplementary Information). Rate divergence (RD): the ratio of the average absolute difference in peak firing rate between trials in different shapes over the average absolute difference in peak firing rate between trials in the same shape. Peak divergence (PD): ratio of the average distance between peak locations in different shapes over the average distance between peak locations in trials in the same shape. Cells with fields in both shapes of enclosure are classified as 'homotopic' according to the distance between the mean location of peak firing in the square and the mean location of peak firing in the transformed circle. A cell is 'homotopic' if this distance is less than expected at $P < 0.05$ for random fields, that is, $0.127L$ in the Monte Carlo simulation. We combined data from more than one animal to calculate field divergence (FD): the number of non-homotopic cells as a percentage of the total number of cells with fields in enclosures of both shape. Numbers quoted for 'similar'-looking fields correspond to fields with peak locations within $0.17L$ ($P < 0.083$ in the Monte Carlo simulations),

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that is, it is a less strict criterion than homotopic. For analysis of the transfer test data, see Supplementary Information.

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Competing interests statement

The authors declare that they have no competing financial interests.

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Balanced responsiveness to chemoattractants from adjacent zones determines B-cell position

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B lymphocytes re-circulate between B-cell-rich compartments (follicles or B zones) in secondary lymphoid organs, surveying for antigen. After antigen binding, B cells move to the boundary of B and T zones to interact with T-helper cells[1–3]. Despite the importance of B–T-cell interactions for the induction of antibody responses, the mechanism causing B-cell movement to the T zone has not been defined. Here we show that antigen-engaged B cells have increased expression of CCR7, the receptor for the T-zone chemokines[4,5] CCL19 and CCL21, and that they exhibit increased responsiveness to both chemoattractants. In mice lacking lymphoid CCL19 and CCL21 chemokines, or with B cells that lack CCR7, antigen engagement fails to cause movement to the T zone. Using retroviral-mediated gene transfer we demonstrate that increased expression of CCR7 is sufficient to direct B cells to the T zone. Reciprocally, over-expression of CXCR5, the receptor for the B-zone chemokine CXCL13, is sufficient to overcome antigen-induced B-cell movement to the T zone. These findings define the mechanism of B-cell relocalization in response to antigen, and establish that cell position *in vivo* can be determined by the balance of responsiveness to chemoattractants made in separate but adjacent zones.

Using B cells from mice carrying immunoglobulin transgenes specific for the antigen hen-egg lysozyme (Ig^{HEL})[6] in a time course analysis, we found that movement of antigen-engaged B cells to the B-zone/T-zone (B/T) boundary is complete within 6 h of antigen exposure (Fig. 1a). Antigen-engaged B cells can remain at this location for at least two days[2], and interactions between T-helper cells and B cells are first observed in this zone[3]. Genetic studies have established that CCR7, and its ligands CCL19 and CCL21, are important for T-cell and dendritic cell localization in T-cell zones[7,8]. To test whether movement of B cells to the T zone might occur owing to increased expression of CCR7, we tested for levels of CCL19-Fc (Fc, fragment crystallizable) binding to antigen-stimulated B cells (Fig. 1b). Six hours after exposure to HEL antigen *in vivo*, Ig^{HEL}-transgenic B cells showed a two–threefold increase in binding of CCL19-Fc, and little or no change in CXCR5 expression (Fig. 1b). CCL19-Fc binding to CCR7-deficient B cells was minimal, establishing that CCL19-Fc staining served as a faithful reporter of CCR7 levels (see Supplementary Information). Efforts to measure how the increase in CCR7 affected chemotactic responsiveness of the cells were confounded, as B cells that had bound large amounts of HEL antigen attached irreversibly to transwell filters (data not shown), consistent with reports that HEL binds strongly to many surfaces[9].

We therefore developed a second approach to study antigen-engaged B-cell redistribution, incubating B cells from IgM^a con-