Supplementary Figure 1 The recording apparatus. (a) Photograph of the pegboard, on which a tethered rat is exploring. (b) Photograph of the helical track. (c) Sections of a typical path on the pegboard showing that the rats tended to run diagonally, but not in a regular and stereotyped way. The sections of this run have been selected to start (green arrow) and stop (red arrow) at the point where the rat turned around on the apparatus. The small red dots show spikes from a grid cell. The complete trial (in this case, shorter than usual, hence the bias to one side) is shown on the right.
**Supplementary Figure 2** (3 pages) Firing characteristics of the complete set of place cells recorded on the pegboard arena. Each cell’s data are plotted as follows: Left top: Spike plots for place cells on the open field, showing position (black lines) with spikes superimposed (blue squares). Left bottom: Same data re-plotted as firing rate maps, depicted as described in the Fig. 1 legend. Middle column: Spike plots for place cells on the pegboard arena, showing position (black lines) with spikes superimposed (blue squares). Right column: Same data re-plotted as firing rate maps.
Supplementary Figure 3 Firing rate with respect to distance from the field peak (in layers, for the pegboard, or coils, for the helix). For the helical track, the dummy cell data are also shown. Note that these plots exclude the peak itself, which by definition had the highest firing rate; nevertheless, firing continued to fall steadily with distance, rather than fluctuating randomly, suggesting a systematic modulation of firing rate by height cues.
Supplementary Figure 4 (4 pages) Data for the complete set of grid cells recorded on the flat arenas (a-d) or the pegboard (e-h). For the spike plots (a and e), spikes are shown in red and the path of the rat is shown in black. Rate maps (b and f) are smoothed, contour-plotted version of the spike plots, showing hot-spots of activity corresponding to the firing fields. Peak rates (Hz) are shown in white text. Spatial autocorrelograms of the firing fields (c and g) show a predominantly hexagonal close-packed pattern for recordings in the flat arenas (c), and a striped pattern for recordings on the pegboard (g). Symmetry plots (d and h; see Supplementary methods for details) showed a predominantly 6-peaked pattern for the flat arenas (d) and a two-peaked pattern on the pegboard (h).
Supplementary Figure 5 Symmetry analyses for grid cells on flat arenas vs. the pegboard. (a) Rotational symmetry analysis. From top to bottom are: raw data from 2 cells, corresponding firing rate contour plots, spatial autocorrelation maps (black Xs mark peaks, red Xs mark peaks for the corresponding flat trial, for comparison) and rotational autocorrelation plots. Lowermost plots show population rotational symmetry plots (black line = mean, green = 95% confidence limits). (b) Mean (+/− s.e.m.) symmetry plot peaks for grid cells recorded on the flat arenas (light red) or pegboard (dark red), calculated for a range of peak-trough thresholds. (c) Histogram of translational symmetry (means +/- s.e.m).
Supplementary Figure 6 Spike plots (left panels) and firing rate maps (right panels), for four grid cells recorded on the pegboard when it was laid in a horizontal position, showing a clear grid structure.
**Supplementary Figure 7** Firing rate maps from the 17 pegboard grid cells, decomposed into up-going, down-going, and left- and right-going trial segments. The stripe pattern persisted regardless of travel direction.
Supplementary Figure 8 Comparison of firing fields on the flat arenas (left of each pair) vs. pegboard for the 16 grid cells that were recorded on both types of apparatus. Note that in no cases is the stripe width on the pegboard less than the inter-field distance on the flat arena.
Supplementary Figure 9  Procedure for analyzing spatial firing on the helical track. (a) Each run was divided into its component 5 (in this case) or 6 coils. This was done independently for upward (shown here; 5 runs from bottom to top in this example) and downward runs. (b) Each coil was unwound into a linear plot and divided into 64 bins, and firing was plotted as firing rate histograms (i.e., number of spikes in each bin normalized by dwell time), stacked on top of each other. Numbers below each firing rate histogram indicate the coil number, with coil 1 at the bottom and coil 5 (in this case) or 6 at the top. The linearized plots in (b) were labelled according to distance from the peak rate coil: the peak rate coil was coil 0, and coils below were incremented positively, in either direction.
**Supplementary Figure 10** (2 pages) The complete set of place fields recorded on the helical track. Data are separated into up-going and down-going runs. Note that firing tended to be localized in a particular region of each segment, and furthermore, that this region was the same on successive coils. Thus, there is no evidence of positional remapping. However, a number of cells (outlined in bold) showed rate modulation, inasmuch as the firing rate declined across coils at every step of the way.
Supplementary Figure 11 Distribution of firing field peaks on the helical track. Upper graphs show place cells recorded on either the 5-coil or 6-coil configuration of the track, separated into up-going and down-going directions. Lower graphs show primary or secondary grid cell fields, also separated into up-going or down-going runs. There is no consistent tendency for field peaks to cluster at any particular level of the helix, in either the up-going or down-going directions.
Supplementary Figure 12 The complete set of grid fields recorded on the helical track. Data are depicted as shown in Supplementary figure 2 and Supplementary figure 9. Note that as with place cells, firing tended to be localized in a particular region of each segment, but unlike the place cells there was usually more than one firing location on a given coil. Firing locations were generally the same on successive coils. Some cells (outlined in bold for primary fields only) showed rate modulation, where firing rate declined across coils at every step of the way, but this occurred far less often than with the place cells (18/79 (23%) of the grid fields, including the secondary fields which have not been highlighted in the figure, compared with 53/103 (51%) of the place fields.
Supplementary Figure 13 (2 pages) Histological sections from 7 of the 10 rats in which grid cells were obtained (in the remaining 3 rats, the electrode track could not be identified). Each set shows (left) a low-power view of the section in which the electrode track was found (red dot indicates estimated site of electrode tip, rectangle shows area that was magnified) and (right) a high power view of the electrode track area. Estimated electrode tip sites: 216 and 245 = Deep layers of either medial entorhinal cortex (MEC) or parasubiculum (PaS), 255 = MEC, 262 = border of PaS and MEC, 323 = layer II of MEC and layer V/VI of MEC/PaS (two tracks), 352 = layer III of either MEC or PaS, 363 = subiculum/dentate molecular layer.
**Supplementary Methods**

**Subjects**

Subjects were 22 male Lister-hooded rats initially weighing 250g, of which 12 were hippocampally implanted and 9 entorhinally implanted. In addition, one grid cell was obtained from a rat with electrodes aimed at postsubiculum, which histology revealed to have ended up in the dentate molecular layer.

Rats were maintained on an 11:11h light/dark cycle, where lights were dimmed to half their strength between 7 pm and 8 pm (simulating dusk) and 7 am to 8 am (simulating dawn). The rats were given sufficient food to maintain 90% of their free-feeding weight and were allowed unlimited access to water. Rats were rewarded throughout the spatial foraging tasks with honeyed rice or sweetened cereal. Initially rats were housed in groups of four; after surgery they were housed individually to prevent damage to their implants. The handling and care of animals were conducted under Home Office licensing according to the guidelines in the Animals (Scientific Procedures) Act of 1986.

Some rats participated in only the pegboard arena or only the helical track experiment and some participated in both. Additionally, at around the same time some rats also took part in other experiments involving environments of different kinds. No differences were seen in the recordings from rats experiencing multiple environments vs. those experiencing only one, so the data were pooled.
**Apparatus**

**Pegboard arena**

The Pegboard (Supplementary figure 1a) consisted of a wooden board 121x121 cm square, in which were drilled evenly-spaced holes of 9 mm diameter. Wooden dowels (17cm x 9 mm) were plugged into the holes at 10-20 cm intervals to provide footholds for the rats to climb on. The pegboard was usually placed in a vertical position during training and recording, but was occasionally laid down horizontally, for comparative recordings. The camera viewed the pegboard from a horizontal position about 2 m away.

**Helical track**

The helical track (Supplementary figure 1b) was constructed entirely of Plexiglas and consisted of triangular steps winding in a right-handed spiral around a central core. The perimeter of the core consisted of 24 rods, onto which were threaded the steps, and which thus formed the inner wall of the staircase (there was no outer wall). The core diameter was 33 cm, and the outside diameter of the staircase was 52 cm. Each step was 27 cm wide, 11 cm deep at its outer extent and 1.2 cm high, and each complete coil comprised 12 steps, making the entire height of a coil 14.4 cm. The entire track was 7.5 coils high, of which either 5 or 6 were available to the rat (the topmost and bottom-most coils were barricaded with cardboard). The 5-coil helix provided 72 cm of vertical travel to the rat and the 6-coil helix provided 86.4 cm.

The recording system and PC were placed on a shelf running along one wall of the room and the cable was hung from the ceiling over the helical track. A
monochrome video camera was mounted in the ceiling over the centre of the helix. Room lights were dimmed slightly to prevent erroneous tracking of internal reflections from the Plexiglas steps.

**Open field**

Several two-dimensional horizontal environments consisting of square or circular arenas ranging from 60-200 cm across, were used to gather comparative data concerning place field size, grid field size and spacing.

**Electrode implantation**

Four tetrode bundles (HM-L insulated platinum-iridium wire, 25 µm in diameter, California Fine Wire Company, CA, USA) mounted in a 16-channel microdrive (Axona Ltd, Herts, UK) were used. The microdrive enabled advancement of the tetrodes in small steps (25-200 µm at a time) through the brain. For hippocampal electrodes, the tetrodes were implanted in the neocortex 3.8-4 mm posterior to bregma, 2.2-2.5 mm lateral to the midline, and 1.5 mm ventral to the brain surface. For entorhinal electrodes, the tetrodes were angled 6-10 degrees in the parasagittal plane and implanted at 0.1-0.3 mm anterior to the transverse sinus (approx. 1.2 mm posterior to lambda), 4.0-4.5 mm laterally, and 1.5-2.0 mm deep. For one rat, a grid cell was also obtained from an implant in which the tetrodes were aimed at postsubiculum (co-ordinates AP 6.7 mm, ML 2.8 mm, DV 1.6 mm).

At the end of surgery, rats were given an intramuscular injection of buprenorphine (Vetergesic; 0.1 ml at 0.3 mg/ml) for postoperative analgesia, and returned to their home cages where they were henceforth housed singly. They were allowed at least one week to recover, during which time food
deprivation was discontinued. Following surgery rats were given two days of oral analgesia in flavored jelly in the form of either buprenorphine (Vetergesic 0.3 mg/ml; 0.25 ml per jelly cube) or meloxicam (Metacam 1.5 mg/ml; 0.05 ml per jelly cube). Additionally their drinking water was laced with enrofloxacin (Baytril 0.25 mg/ml; 0.4 ml per 100ml water) to provide antibiotic prophylaxis for the next five days.

**Recording**

Recording began at least a week after surgery, usually longer, and took place using a 16-channel Axona recording system (DacqUSB, Axona Ltd, Herts, UK). The system comprised a headstage that connected to the microdrive, a lightweight recording cable to transfer the signal from the animal, a preamplifier and system unit to process the signal, and a desktop PC for further processing and storage of the data. Position was monitored via a video tracking system (Axona Ltd, Herts, UK) that detected an LED mounted on the headstage, and extracted the x-y co-ordinate (with respect to the camera) at 50 Hz.

Animals were connected to the recording equipment via the headstage that was plugged into their microdrive. Spike signals were amplified (7 000–40 000 times) and bandpass filtered (500 Hz to 9 kHz). Tetrodes were gradually advanced over days until hippocampal or entorhinal neuronal activity was detected, at which point recording trials began.

**Pegboard arena**

In this apparatus rats foraged over the pegboard, by clambering over the pegs (Supplementary figure 1c) searching for honey-flavoured rice that the
experimenter distributed on the pegs. The pegs were cleaned and interchanged every day to decrease the influence of local olfactory cues. The pegboard was placed in the centre of a well-lit recording room, surrounded by general laboratory equipment which provided external visual cues for the animal. Recordings were made for 10-30 minutes, until reasonable coverage of the area had been obtained. Occasionally, pegs were moved between two successive recordings in order to allow the rat to enter previously unvisited areas, to obtain a more complete coverage of the arena.

**Helical track**

Rats were pre-trained to repeatedly run from the bottom to the top and back again, initially collecting honeyed rice all along the route, until they were familiar with the apparatus, after which food (honeyed rice or chocolate-coated cereal) was placed only at the bottom and top ends of the track. Cardboard ‘walls’ were used to prevent animals from reaching the very top of the track and to prevent them jumping off the bottom. Four rats with hippocampal implants and 6 rats with entorhinal implants were allowed access to five coils, and two rats with hippocampal implants to six coils. Because rats’ behaviour was different on reward steps, at the extreme top and bottom of the helix, these were excluded from later analysis. The criterion for the end of pre-training was at least 14 smooth runs (seven upward and seven downward) within 20 minutes on the helical track, after which recording trials began.

For the recording trials, rats were connected to the headstage and placed just in front of the card at the bottom of the helical track. Some trials were run with two people, in which case the experimenters stood on opposite sides of the
helix and passed the recording cable around from one to the other. On trials run by a single person, the experimenter moved around the helix with the rat, holding the cable clear of the track. After either 20 minutes or 14 runs, whichever occurred first, the rats were taken off at the bottom step and returned to the holding box. The helical track was then wiped clean to remove odour cues.

The x-y position was determined via the tracking system, whereas the z dimension was extracted later by the experimenter, by replaying the stored positional data second by second and recording the times at which rats started and ended each coil.

Open field

Usually, preliminary recordings were made as rats foraged in the environments for 5-30 minutes, to ensure the spatial nature of the neuronal activity.

Data analysis

Rate maps

For each cell, firing rate maps were constructed by dividing the entire camera viewing area into 64 x 64 bins, each approximately 2 x 2 cm, and then dividing the total number of spikes in each bin by the dwell time to determine the firing rate in each bin. The resulting maps were then smoothed with a boxcar algorithm (width 5 bins on the helix or 7 bins on the open field and pegboard). The fields were then extracted by a thresholding process in which patches of at least 5 consecutive bins with firing greater than 15% of the peak rate were
considered part of the field and the rest were set to zero.

**Open field and pegboard analyses**

The place and grid field data were analysed to obtain values of field length in two dimensions, aspect ratio, orientation and – for grid cells – inter-peak spacing (for multiple fields) and symmetry order. Smoothed rate maps were generated with the Tint analysis program as described above, and peak firing rates were identified with Matlab and defined as the center of the main field.

**Spatial analysis**

For the pegboard (described below) we implemented an algorithm to coalesce fields that had been fragmented by the more interrupted trajectory of rats on that apparatus. To make sure that this did not introduce any disparities in field properties compared with the open field, we thus used the same algorithm for the open field data. The algorithm was as follows: Within the main field, each bin in turn was tested to check if, within a 5 x 5 bin range, there were any outlying bins containing above-threshold firing from a different but nearby field. If this was so, it was assumed this bin belonged to the main field, despite not being contiguous with it, and it was thus included in the main field. This procedure was repeated twice. Because the procedure was originally designed to extract fields in environments which were less completely sampled than open field arenas, problems occurred when extracting grid fields from cells with a small grid spacing in an open-field environment. Thus, if more than one peak was selected as the main field, these data were not included in the data set for analysis of field size and orientation (described below), but were still analysed for symmetry.
Visited bins within the main field were then used to calculate field properties. The major and minor axes of the fields were extracted using Matlab and then used to calculate aspect ratio (i.e. ratio of major to minor field size) and major-axis orientation of the main fields.

For the grid cells, two-dimensional autocorrelation maps were generated by taking the smoothed firing rate map and repeatedly correlating it with itself after it was shifted in successive 1-bin increments in x and y directions.

Formally, the spatial autocorrelogram was defined as:

\[ r_{x, y} = \frac{\eta \sum_{x, y} \lambda(x, y) \delta(x, y) - \delta(x, y)}{\sqrt{\eta \sum_{x, y} \lambda(x, y)^2 - \sum_{x, y} \lambda(x, y)^2} \sqrt{\eta \sum_{x, y} \lambda(x, y)^2 - \sum_{x, y} \lambda(x, y)^2}} \]

Where \( r_{x, y} \) is the autocorrelation between bins with an offset of \( \delta_x \) and \( \delta_y \), \( \lambda(x, y) \) is the firing rate in the bin located at \( (x, y) \) and \( \eta \) is the number of bins over which the estimate was made. For periodic patterns, the autocorrelograms are also periodic.

The spatial autocorrelograms were used to calculate both field spacing and field width for open field trials (note that an additional field width was also used for one analysis, see below). Field spacing was calculated as the mean distance from the central point in the spatial autocorrelogram to the six nearest peaks. Field width was calculated as the diameter of the largest circle that contained all pixels in the central region above a threshold of 0.15.

Vertical rate analysis

To determine whether the observed vertical stretching on the pegboard was rate modulated or not, the pegboard was divided into five horizontal layers of
equal size, thus matching the helical track. For the main field, the layer on
which a given cell fired maximally was given a value of 0, while layers above
and below it were successively numbered positively (therefore not
distinguishing direction). The resulting scale thus ranged from 1 to 4. Peak
firing rates on all layers were converted to percentages by setting the peak
rate of the layer with maximal firing to 100% and then expressing the peak
rates of the remaining layers as percentages of this maximal rate. In order to
determine whether firing rates declined steadily with increasing distance from
the peak (as expected if modulated by height) or fluctuated randomly (as
expected if not height-modulated) we statistically compared firing rates by
conducting a regression analysis of firing rate against distance (in layers) from
the peak. Because the peak coil is by definition at 100%, we excluded it from
the analysis.

Spatial information

Skaggs’ spatial information is a measure of the degree to which the firing of
an individual cell can be used to predict the location of the animal$^4$.
Information content is defined as follows;

$$IC = \sum_i p \left( \frac{r_i}{R} \right) \log \left( \frac{r_i}{R} \right)$$

Where $p$ is the probability of being in pixel $i$, $r_i$ is the rate in pixel $i$ and $R$ is the
overall rate.

This measure was employed in three ways. First, a spatial information score
for the two-dimensional smoothed firing rate map for each cell was obtained.
Second, the smoothed firing rate map was collapsed horizontally such that
each bin of the resulting linearized rate map was a mean of the firing rate of the bins collapsed into that bin. A separate spatial information score was then obtained from this linearized rate map. Finally the same process was employed except that rate maps were collapsed vertically instead of horizontally.

Symmetry analysis

To determine the symmetries of grid cell firing for horizontal and vertical environments, two symmetry analyses of grid cell firing patterns were undertaken, one rotational and one translational. These comprised a double autocorrelation procedure in which each autocorrelation map was rotated or translated and then re-correlated with the original, as follows.

Rotational symmetry was determined by adapting the method of Sargolini et al.\textsuperscript{5}, cropping the autocorrelation maps to remove the central peak and the area outside the outermost of the six central peaks, and then rotating the map and re-correlating it with the original, in 1-degree increments up to the full 360 degrees. The Pearson product moment correlation at each step was plotted on a graph of correlation against angle and zero-lag-averaged (window size = 30 bins) to remove minor irregularities. The smoothed plot was then differentiated to identify peaks/troughs (where gradient = 0). The vertical distance between each peak and its adjacent troughs was calculated, and the number of times in a given plot this difference exceeded a given threshold was counted, for all thresholds ranging from 0-0.3. This provided a variable plot of peak number against threshold, where the definition of “peak” varied from small irregularities in the symmetry plot (when threshold was close to
zero, and peak number thus high) to large fluctuations (where threshold was high, and excluded all but the tallest peaks). The plots thus obtained were compared between flat arena and pegboard conditions.

Translational symmetry was determined by sliding each autocorrelation map in 1-bin increments and re-correlating the translated map with the original at each step. The collected values for a given translation direction (horizontal and vertical, for the pegboard, and the arbitrarily chosen “horizontal” and “vertical”, for the horizontal maps) were then averaged to produce a mean +/- s.e.m. for each translation direction. In practical terms, this amounted to taking the central vertical and central horizontal columns of the re-correlated autocorrelation maps and collapsing each to a single, averaged value. For grid cells recorded on the pegboard the vertical autocorrelation should have a constantly high value because every step of the autocorrelation procedure maps a stripe onto itself. Conversely, the horizontal value should be much lower as the stripe(s) progressively map onto the inter-stripe space. The values for grid cells recorded in horizontal environments should also be much lower.

Stripe width and stripe spacing

For grid cells, we obtained stripe width by collapsing the smoothed data from the pegboard into one level, or row, of firing rates. This ‘flat-rate’ was then thresholded at 15% peak rate, as described previously. Stripe width was calculated for the main subfield as the number of bins on which there was firing multiplied by the bin size. In order to provide precise comparison of stripe width with the size of the fields produced by cells on the flat arenas, we
isolated the best horizontal grid field and subjected it to the same flattening and thresholding process as the stripe.

We calculated stripe spacing for all grid cells expressing more than one stripe by producing a linear spatial autocorrelation on the smoothed (but not thresholded) flat-rate, made in 1-bin steps. The stripe spacing was the distance between the main correlation peak and the next positive peak. To compare with the fields on the flat arenas, this distance was compared with the distance as obtained from the autocorrelograms.

**Helical track**

The helical track data were first collapsed onto one coil, to determine overall firing field parameters. Thereafter, the helix was divided into its individual coils and the remaining analyses, conducted from a lateral perspective, took into account z (vertical) position, as described below.

The helical track was unwound into its component five or six coils, and each coil was linearized as shown in Supplementary figure 2 and divided into 64 bins per coil. The firing rate per bin was calculated by dividing the number of spikes fired in that bin by the dwell time. These data were then boxcar-smoothed with a boxcar width of five bins. Next, the field was defined as those patches of firing that consisted of at least 5 consecutive bins where firing was at or above a threshold of 15% of the overall peak rate. Firing rates in all other bins were replaced with zeros. The data thus extracted were then subjected to a number of analyses in order to determine horizontal location (with respect to the linearized coil), relationship of firing location between coils (including the peak location in the z dimension), field size, aspect ratio, wavelength (for grid
cells), directionality and stability, as described below.

Horizontal analysis

In order to determine whether the repeated firing of a cell on multiple coils represented the “same” field repeatedly expressed, as opposed to new and unrelated fields on each coil, we undertook two comparisons. First, we calculated the bin-by-bin correlation between the fields on different coils, which should be high if the fields occupied the same relative horizontal location and low if they were unrelated. Pearson’s R was computed for each pair, and then averaged across all pairs.

Second, we employed a lateral shift analysis where we computed the distance by which the firing map from one coil would have to be shifted with respect to the firing map of another to maximise this correlation. If the fields were in the same place this shift should approach zero, and if they were unrelated it should be large. To prevent the available to-be-compared bins from decreasing as the two coils were progressively shifted apart, we wrapped each coil around on itself, so that the helix effectively became a stack of circular tracks. Left shifts were not distinguished from right shifts, so the maximum possible shift was 32 bins (half the coil). For both analyses, the actual data set was compared against a dummy data set (“dummy cells”), which we constructed by selecting the field for each coil at random from the complete data set, so that there would be (on average) a low correlation between coils. Cells recorded on more than one trial were averaged over trials to produce one value per cell.
Horizontal field size and field spacing

To obtain horizontal field size, we collapsed the helix into one coil by adding together the non-smoothed rates on all coils and dividing by the total number of coils. This ‘flat-rate’ was then smoothed and thresholded above 15% of the peak rate, as described previously. For place cells, the horizontal field size was the number of bins on which there was a field multiplied by the bin size. For grid cells, we averaged all subfield sizes to produce the average horizontal field size for each cell.

For all grid cells firing in more than one horizontal location on the helix, we calculated the spacing between the peaks (in the frame of reference of the linearized helix: i.e., the traversed inter-peak distance) by producing a linear spatial autocorrelation on the smoothed (but not thresholded) flat-rate, made in 1-bin steps. The field spacing was the distance between the main peak and the next peak whose correlation value was above zero.

Vertical analysis

Vertical field analysis determined three properties of firing fields: (i) The vertical location of the field peaks, (ii) the vertical extent (i.e., height) of the fields, and (iii) the vertical rate analysis. For grid cells, we extracted the primary field (defined as the subfield with the highest firing rate) and if possible the secondary field (the subfield with the next-highest firing rate) in order to assess these properties.

(i) Vertical location: Chi-square tests were used to determine whether fields were equally distributed along the levels of the helix. The comparison was between the actual distribution of field numbers and a hypothetical uniform
distribution.

(ii) Vertical extent: the vertical field extent, for both place and grid cells, was calculated as the number of coils over which a field was expressed multiplied by coil height (14.4 cm). Additionally, for cells recorded on the 5-coil helix, the numbers of fields spanning 1, 2, 3, 4 or all 5 coils were counted for place vs. primary grid cells, and fields spanning all 5 coils vs 1-4 coils were compared with a Chi-square test.

(iii) Vertical rate analysis: Relative firing rates across coils were analysed in the same way as for the vertical rate analysis of cells on the pegboard, to determine whether firing rates on different coils were independent or not. The coil on which a given cell fired maximally was given a value of 0, and coils above and below it were numbered according to their distance from coil 0. For place cells, in which some rats were run over 6 coils instead of 5, data from the 5-coil and the 6-coil version were combined. The resulting scale thus ranged from 1 to 5 for place cells (given the maximum of 6 coils on the helix) and 1 to 4 for grid cells.

The peak firing rates on all coils were rescaled by setting the peak rate of the coil with maximal firing at 100% and then converting the peak rates of the remaining coils to percentages of the maximal rate.

A regression analysis was performed in the same way as for the pegboard, testing the data against the hypothesis that firing rates fall away steadily with distance from the peak. The peak itself, set to 100%, was excluded from the analysis. This was also performed on dummy cells, for comparison with the real data set.
Directionality

In two-dimensional open-field environments, the internal representation of the animal’s position typically does not change according to the direction of travel (that is, place and grid cells are non-directional). However, in environments where repetitive actions occur, for instance shuttling between two ends of a linear track for food reward, most place and grid cells become directional, firing in a particular location only when travelling in one direction\(^6\). We assessed directionality by calculating coil-by-coil and overall correlations between upward and downward runs, and averaging these. Cells having correlations below a user-defined correlation value of 0.4 were considered directional.

Stability

Finally, because stability of the spatial representation is an important feature for any navigational system, we looked at whether the three-dimensional place and grid fields were stable over more than one recording session. All pairs of sessions were correlated in two ways; on a per-coil basis, the values of which were averaged to produce one value per cell, and on the entire rate array. Except for a few cases, correlation values produced by these two methods were very similar. These were averaged to produce a more objective value, which was used to assess stability. A user-defined cut-off correlation value of 0.4 distinguished unstable cells (below 0.4) from stable cells (above 0.4).

In addition, we looked to see whether the location of the peaks was stable across trials. The coil on which the peak of a cell’s activity occurred was
determined for each cell for each day. Pairwise comparisons between the peak location for a given trial and the peak locations for all the other trials were computed for each cell and then averaged. The real data were compared against simulations constructed using the same number of cells and same number of trials, but with peak locations randomly assigned.

**Histology**

All rats received an intraperitoneal injection of sodium pentobarbital and were transcardially perfused with phosphate buffered saline followed by a 4% paraformaldehyde (PFA) solution. The brains were removed and, in most cases, cryo-protected in a 20% sucrose/4% PFA solution overnight or until the brain had sunk, after which they were transferred to a 4% PFA solution until sectioning. In all cases 40 µm sections were taken; for implants aimed at the hippocampus and postsubiculum, coronal sections were taken, and sagittal sections were taken for all medial entorhinal implanted animals. Sections were cut on a freezing microtome, mounted on gelatine-coated glass slides and stained with cresyl violet. High-power images were acquired using an Olympus microscope at 2.5x magnification using an Xli digital camera (XL Imagining Ltd.) and Xli-Cap image capture software. The low-power magnification images were acquired on a CanoScan LiDE 210 flat-bed document scanner (Canon (UK) Ltd). Electrode tracks and the final positions of the electrodes were estimated from the high-power light-microscope images.

**Supplementary results**

We recorded 53 place cells and 34 grid cells on flat arenas, 40 place cells and
17 grid cells on the pegboard maze, and 61 place cells and 27 grid cells on the helical track. The complete place cell data set on the pegboard is shown in supplementary figure 3, and for the grid cells in supplementary figure 4. For the data described below, means +/- s.e.m. are reported throughout, except where otherwise specified.

**Pegboard**

Most of the pegboard analyses are detailed in the main text. However, we also performed an additional analysis, of “symmetry class”, to further characterize the differences between horizontal and vertical firing field properties.

**Grid field symmetry class**

We used two symmetry analyses, one translational and one rotational. Each analysis comprised a double autocorrelation procedure, adapted from the methods of Hafting et al.\textsuperscript{2} and Sargolini et al.\textsuperscript{5}, in which a spatial autocorrelogram, generated from the firing rate map, was rotated or translated (with no wraparound) and re-correlated with the original autocorrelogram at each step. For the rotational analysis the resulting values were plotted and the major peaks determined by counting peaks above a threshold peak-trough correlation difference, for thresholds ranging from 0.0 to 0.3 (Supplementary figure 5). The horizontal ratemaps generally produced six-peaked symmetry plots (mean peak number ranging from 5.82 +/- 0.13 to 5.29 +/- 0.22) whereas the pegboard plots were usually two-peaked, reflecting the two-fold symmetry of the stripes, (mean peak number ranging from 3.68 +/- 0.35 to 2.12 +/- 0.12). Chi-square analysis of plots having 2, 4 and 6 peaks for the flat vs. pegboard conditions was highly significant both for threshold=0.0 ($\chi^2(2, N=50)=29.6$,
Thus, firing patterns on the flat arenas had a different order of rotational symmetry from firing patterns on the pegboard.

For the translational analysis, the values generated by the re-correlations were averaged, for translations in orthogonal directions (horizontal and vertical for the pegboard maps, arbitrarily chosen “horizontal” and “vertical” for the flat maps). The flat maps produced low correlations in both translation directions, of 0.032+/-0.011 and 0.022+/-0.012 for “horizontal” and “vertical”, respectively. On the pegboard, horizontal translation also produced a low correlation of 0.044+/-0.027. By contrast, vertical translation correlations averaged 0.304+/-0.057. A two-factor ANOVA of environment type against translation direction showed a significant effect of environment \(F(1,64)=21.3, p<0.001\), a significant effect of translation direction \(F(1,64)=13.7, p<0.001\) and a significant interaction \(F(1,64)=18.2, p<0.001\). Post-hoc Tukey’s analysis confirmed that the vertical correlation mean on the pegboard was higher than the horizontal \(t(32)=4.15, p<0.001\), higher than the “vertical” mean in the flat environments \(t(32)=5.07, p<0.0001\) and higher than the “horizontal” mean on the flat \(t(32)=4.61, p<0.0001\). These findings arise because vertical translation mapped the stripes onto themselves, resulting in high mean correlation, whereas in all other conditions the translations progressively decorrelated the firing maps.

**Helical track**

**Place cells**

We analysed the upward and downward runs of 61 well-isolated CA1 place
cells from 6 rats separately, totalling 103 place fields. The complete data set is shown in Supplementary figure 9 and Supplementary figure 11. From the real data we selected fields from coils at random to create 90 “dummy fields”, for comparative purposes.

Directionality
To the 42 cells that fired in both directions, we added another 17 for which one direction was excluded due to firing that was less than 50 spikes. These cells were considered to have rate remapped and were classified as directional. Out of 59 cells, then, 54 had a correlation between runs of less than 0.4 (or were considered rate remappers). Therefore, in line with previous work using linear tasks, the majority of cells in this study (92%) were directional.

Stability
Eight cells on upward runs and seven on downward runs from two rats were recorded over several days and analysed for stability. Inspection of the firing rate plots for these cells showed that, consistent with findings from studies of two-dimensional environments, the fields remained similar across trials. Statistical analysis confirmed that place fields on different trials were highly correlated with each other. The mean correlation (±s.e.m.) was 0.68 ± 0.06. Only 2 cells (13%) had correlation values below 0.4. Therefore, place cells on the helical track maintained stable fields over days. Actual peak locations were then compared using pairwise comparisons, which found an average inter-peak distance across repeated trials of 0.83 coils. The simulations (where peaks were assigned to randomly chosen coils) were repeated 10000 times, producing a mean of 1.60 and standard deviation of 0.25; a value lower
than 0.83 occurred only twice (probability of 0.0002). Therefore, peak locations in the real data were significantly more similar across trials than would be expected by chance, suggesting stability of the vertical place code.

**Grid cells**

We analysed the upward and downward runs of 27 grid cells separately, totalling 53 grid fields, from which we created 52 dummy grid fields.

**Directionality**

To assess directionality we compared the spatial and rate similarity between upward and downward runs. In total, we found 15 directional cells, 5 non-directional cells, and 6 cells that had a different classification on different trials. We then looked at cells recorded on one trial only, and found 15 directional and 3 non-directional cells. In both cases, the majority of cells are directional (58% vs. 83% respectively), in line with the place cells and with what has been reported previously for grid cells\(^{10}\). Note however that out of 7 cells recorded on multiple trials, the majority (71%) changed classification, from directional to non-directional, or vice versa. Examination of the place cell data found that out of 10 multiple-trial cells, none changed classification. This may reflect the generally higher variability of grid cell fields. This may be due to the greater number of subfields in grid cells; indeed, 4/6 grid cells displaying more than one subfield on the helix changed classification. The only single-field grid cell also changed classification, but only on the last two out of 11 trials. Three of the five grid cells that changed classification were also unstable over several days in at least one direction, and 2 of these unstable cells had more than one subfield. Both cells that did not change
classification were stable over days.

Stability

We next examined fields recorded on more than one trial. There were 10 fields in the upward direction and 8 in the downward direction. Again, the firing rate plots appear similar between trials. We calculated correlations between all pairs of trials and averaged them. The mean correlation was not as high as for place cells (0.50 ± 0.05 vs. 0.68 ± 0.06). However, the majority of fields were stable (67%). We found 6 fields below the user-defined cut-off point of 0.4 that were classified as non-stable across trials.

Actual peak locations were then compared using pairwise comparisons, which found an average inter-peak distance across repeated trials of 1.25 coils. The simulations (where peaks were assigned to randomly chosen coils) were repeated 10000 times, producing a mean of 1.60 and standard deviation of 0.19; a value lower than 1.25 occurred 275 times (probability of 0.03). Therefore, peak locations in the real data were significantly more similar across trials than would be expected by chance, suggesting, as for place cells (albeit more weakly), stability of the vertical place code.

Histology

The sites of the electrode tracks for the entorhinally implanted animals are plotted in Supplementary figure 13. Of the 10 implanted animals, 8 had electrode tracks terminating in dorso-medial entorhinal cortex or parasubiculum, while the tenth animal had electrodes apparently in the dentate molecular layer. Estimates sites from individual rats are detailed in the legend.
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