Supporting Information

SI Materials and Methods

Electrode Turning. One tetrode remained in the cortex and was used as a reference for all recordings. Another tetrode was lowered to the stratum lacunae-nucleare to record hippocampal local field potentials. Of the remaining 12 tetrodes, approximately half were advanced toward the CA1 cell layer and the other half toward the CA3 cell layer over a period of at least 2 wk after surgery. The depth profile of the local field potential on different tetrodes (1) was used as a guide for electrode movement. When the tetrodes approached the cell layers, further movement of tetrodes was done in small increments over several days. Once the tetrodes were adjacent to the cell layer, as indicated by the presence of low-amplitude multunit activity, tetrodes were not turned again and were allowed to settle into the cell layer for stable recordings over a period of many days.

Recording Procedures. For recording spikes and local field potentials, the electrode assembly was connected to a multichannel head-mounted preamplifier. The x–y position of light-emitting diodes on the preamplifier was tracked at 30 Hz by processing video images. Unit activity was amplified and band-pass filtered at 600 Hz to 6 kHz. Spike waveforms above a trigger threshold (40–60 μV) were time-stamped and recorded at 32 kHz for 1 ms.

Spike Sorting. Spike sorting was performed offline using the graphical cluster-cutting software MClust (2). Clustering was performed manually in 2D projections of the multichannel parameter space (consisting of waveform amplitudes, the difference between peak and trough of the waveform, and waveform energies). Autocorrelation and cross-correlation functions were used as additional separation criteria. Putative excitatory cells were distinguished from putative interneurons by spike width and average rate. Only putative excitatory cells were included in analysis. Recording stability within a day and across days was confirmed as described in Fig. S1. In particular, sleep sessions before and after the behavioral sessions were used in addition to the recordings in behavior to assess recording stability, and a modified version of MClust was used that allows for the confirmation of the cluster boundaries of each cell in each 10-min recording session.

Cluster Quality Metrics. For each cluster, we calculated the L-ratio and isolation distance as described (3). When calculating the Mahalanobis distance, we included the features that we used for clustering (see above). We calculated the cluster quality for each experimental block by including all putative spikes that were detected during all behavioral sessions of the block. Full-day cluster quality was reported as the mean cluster quality between the two blocks of a given day. Cluster quality was compared across hippocampal subregions using a two-sample Kolmogorov–Smirnov (KS) test.

Spatial Maps. Spatial firing-rate distributions for each well-isolated neuron were constructed in the standard manner, by summing the total number of spikes that occurred in a given location bin (5 × 5 cm), dividing by the amount of time that the animal spent in that location, and smoothing with a Gaussian centered on each bin (4).

Spatial Correlations. Spatial firing patterns of individual cells were compared between sessions with a spatial correlation procedure (4, 5). The Pearson correlation coefficient for firing rates in corresponding pixels was calculated for pairs of spatial maps of each cell. Pixels visited less than 150 ms in either session were excluded to avoid artifacts in the correlation measure.

Place Field Boundaries. Standard practice has been to define boundaries by first finding the pixel with the maximum firing rate and then iteratively extending the field to any adjacent pixels that exceed a predefined threshold (4, 6–8). In our data, we found that a subset of fields had clearly separated peak values that were nonetheless joined by adjacent pixels and could thus not be separated by this method. We therefore developed a contour-based analytical method to separate fields (Fig. S11).

Individual Passes Through Place Fields. For each place field and each experimental session, the path segments inside of field boundaries were identified. Time periods when the path segments were continuously inside the field boundary for at least 200 ms were considered a pass through the field if the path crossed inside of the contour at 60% of the field’s peak value. The average firing rate for a pass was calculated as the number of spikes during the pass divided by the duration of the pass. In addition, we calculated the average velocity during the pass and the minimum distance to the center of the field (Fig. S6). For any analyses reported by fields, we discarded fields through which the rat passed fewer than five times in any individual session.

Rate Changes Within Sessions. For each place field recorded on single shape, day 1, the rate change per session was calculated by fitting a regression line to the rates during individual passes through the field and by taking the difference of the y-values of the line between the end and start of the session. If rates fluctuated randomly in time, the average difference over repeated sessions should be close to zero. To measure whether a place field showed consistent rate changes, a Student t test was performed on the set of eight rate changes (one from each of the eight sessions within the day). If the mean rate change was found to be different from zero at the 0.05 level, that field was considered to change its rate consistently.

Shape Preference Score. To measure whether neuronal firing within a place field occurred preferentially in one enclosure shape, we calculated a shape preference score. For each field, the rates from the individual passes were combined to yield a distribution of firing rates for the square enclosure and a distribution of firing rates for the circular enclosure. The degree to which these distributions overlapped was calculated as the area under the receiver operating characteristic curve for the distribution in the square compared with the distribution in the circle. If all firing rates in the square enclosure were smaller than in the circle enclosure, the area under the curve was 1. If all firing rates in the square enclosure were smaller than in the circle enclosure, the area was 0. If the distributions were identical, the area was 0.5. We then did a linear transformation on this measure (subtracting 0.5 and multiplying by 2), so that the shape preference score would vary between −1 and 1, with 0 indicating no shape preference. The signed value of this score provides a nonparametric estimate of preferred firing in the square enclosure shape compared with the circle enclosure shape. The absolute value of the score gives an indication of the extent of firing preference for either shape.

Rate and Shape Preference Variability. To compare the variability in firing rate and in shape preference between CA1 and CA3 across time intervals, we calculated the average firing rate of each cell.
and the shape preference score of each place field at different time points. Morning firing rates were compared with afternoon firing rates, morning preference scores were compared with afternoon preference scores, and day 1 preference scores were compared with day 2 preference scores. The values for the two different time points were plotted against each other. If cells showed the same firing rate or the same degree of shape coding at the two time points, the data points would fall on the identity line.

We used an F-test to determine whether the variance around the identity line differed between the CA1 and CA3 subregions. Because the average shape preference could systematically change over time, resulting in a best-fit line that is different from the identity line, we repeated our analysis with a standard linear regression line. The results with the regression line replicated the findings with the identity line. To evaluate whether the variability in shape preference could be related to cluster quality, we repeated F-tests within each brain region for place fields with clusters in the highest quartile of cluster quality compared with place fields with clusters in the lowest quartile of cluster quality.

**Population Vector Correlations.** For each behavioral session, rate vectors were constructed by arranging the spatial maps of all cells recorded from all animals in an x-y-z stack, where x and y represent the two spatial dimensions in 5 × 5 cm bins and z represents the cell-identity index (9, 10). CA1 and CA3 cells were analyzed in separate stacks. For multiple-day analysis, only cells that were tracked from the beginning of the first day through the end of the last day were included in the population vectors. The distribution of mean rates along the z axis for a given x-y location represents the composite population vector for that spatial bin and hippocampal subregion. For each pair of sessions, the Pearson correlation coefficient was calculated for spatial bins at corresponding locations. Cells with firing below 1 Hz in all bins of the two sessions were excluded from the analysis. To allow for comparisons between the square and circle enclosure shape, the analysis was restricted to the 16 × 16 cm bins that were common to both shapes, yielding 256 correlation estimates for a pair of sessions. The correlation coefficients of all spatial bins were averaged to estimate the average population vector correlation for a pair of sessions. All average correlations for a particular time interval (e.g., <1 h, 6 h) were compared using Student t tests or, for experiments with more than two time points, using ANOVA with time interval and enclosure shape as factors or, in Fig. S5, with time interval and number of enclosure shapes as factors. Bonferroni corrections were applied to correct t tests and post hoc analysis for multiple comparisons. To create the cumulative density plots, the population vectors of all spatial bins from pairs of maps with the same time lag were combined, and the functions were plotted for each time lag. The complete matrix of population vector correlations between all 10-min sessions in which cells were recorded over 2 d are shown in Fig. S9.

**Histology and Three-Dimensional Reconstruction of the Tetrode Array in Serial Sections.** Rats received an overdose of sodium pentobarbital and were perfused intracardially with saline and 4% formaldehyde. The brains were extracted and stored in formaldehyde. Frozen coronal sections (40 μm) were cut and stained with cresyl violet. Each section through the segments of the hippocampus with electrode tracks was collected for analysis. All tetrodes of the 14-tetrode bundle were identified by finding their electrode tracks across sections. A small angular deviation between the plane of sectioning and the electrode tracks resulted in an apparent downward shift of the tissue damage. The electrode tip was considered to be located in the section where the tissue damage became negligible (Fig. S7E). Recordings from a tetrode were included in the data analysis if the tetrode’s deepest position was in the CA1 or CA3 pyramidal cell layer.

Clusters of spikes from single cells were tracked across multiple days. (A) The behavioral design and the experimental timeline are shown for the 2-d, single-shape experiment including rest and behavioral sessions. The sessions that are shown in B and D are highlighted in gray. (B) Each panel shows the projection of the peak amplitude recorded on one channel of a tetrode located in the CA1 pyramidal layer compared with the peak amplitude recorded on another channel of the same tetrode. The same projection is shown for each cluster diagram, and different diagrams correspond to different time points in the experiment. Each dot represents one sampled spike. Colored dots of a single color represent spikes that were assigned to a single cell. After de-isolating clusters on a single day, the cluster boundaries were applied to data recorded from the same tetrode on subsequent days. If clusters persisted in the same region of parameter space where they had been identified on the previous day, boundaries were adjusted to assure that all spikes that were part of the cluster were included. **Fig. S1.**
included within the boundaries. If the spikes for a set of clusters on one tetrode could be included with minor adjustments of the boundaries from the day before, the clusters were considered to be the same set of cells as on the day before, and the cells were included in our analysis as trackable across days. Because we occasionally found cells that appeared to turn on or turn off during the day, it was imperative to confirm that these effects were not simply a consequence of a tetrode having moved into or out of the range where the cell could be recorded. We thus only included cells in the analysis that were active at the beginning of the day (in the first sleep session or the first behavioral session in either shape) and at the end of the day (during the last sleep session or the last behavioral session in either shape). The highlighted (blue) cluster depicts a cell that fired robustly during all behavioral sessions on the first day and in the morning of day 2; it then fired very few spikes during the second afternoon (PM3–4, day 2) before regaining robust activity during the PM Rest2 session, indicating that the average number of spikes recorded during the afternoon was not due to the cell having moved out of the assigned parameter range, but was rather due to a genuine decrease in firing rate. Note that the pattern of clustered spikes remained stable throughout the entire recording sequence, conforming to the criterion for assessing stability of the recording electrode as described above. (C) Average waveforms from four clusters shown in B; the color of the waveform corresponds to the color of the corresponding spike cluster. The average waveform ± SD is shown for the first behavioral session (Left) and the last behavioral session on the second day (Right). The average waveforms and the average amplitudes remained consistent throughout the experiment as demonstrated by the comparison between AM1, day 1 and PM4, day 2. (D) Spatial maps from the same four cells in C during the behavioral sessions in B. The average firing rate in each 5 × 5 cm pixel is color-coded with a color scale from 0 Hz (blue) to the maximum peak rate within the day (red). The peak firing rate for each cell on each day is indicated to the right of the PM4 map. Although spatial maps from tracked cells were not used to determine whether clusters were the same from one day to the next, we visually inspected spatial maps from tracked cells. The location of place fields was identified to be the same from one day to the next, confirming that our method for tracking cells was reliable. (E) For each cluster that had a place field, isolation distance was used as a metric of cluster quality. Higher isolation distance indicates better cluster quality. (Left) There was no difference in the distribution of cluster quality between CA1 and CA3 on either the single day (Upper, KS test statistic = 0.21, not significant (n.s.)) or the clusters that were tracked across 2 d (Lower, KS test statistic = 0.29, n.s.). (Middle) The variability in shape preference observed in CA1 was not related to cluster quality. The same data as presented in Fig. 4 are shown with dots color-coded by cluster-quality quartile. The clusters with highest quality are shown as blue circles, the clusters with lowest quality are shown as yellow squares, the middle 50% of clusters are in gray (clusters whose quality metric was undefined are omitted from the plot). The degree to which clusters vary with reference to the identity line is not different between the best and worst quartiles for either single day (Upper; F(20, 20) = 0.97, n.s.) or 2-d data (Lower; F(11, 13) = 1.37, n.s.) data. (Right) The same measures are shown for CA3 data. Variability was not different between the best and worst quartiles for single day (Upper; F(9, 9) = 2.79, n.s.) or 2-d data (Lower; F(6, 6) = 2.43, n.s.). (F) Cluster quality was assessed using a second metric, L-ratio. For this metric, lower scores indicate better clusters. Panels are laid out as described in E. There is no difference in the distribution of cluster quality between CA1 and CA3 for single-day data (KS test statistic = 0.24, n.s.). For clusters tracked across 2 d there is a small but statistically significant difference in distributions with CA1 clusters having lower scores than CA3 (P < 0.05, KS test statistic = 0.38), indicating that cluster quality in CA1 is slightly better than in CA3. There is no difference in variability between the clusters in the best quartile and worst quartile in either brain region [CA1, single day: F(20, 20) = 1.21, n.s.; CA1, 2 d: F(12, 12) = 1.21, n.s.; CA3 single day: F(9, 9) = 0.63, n.s.; CA3 2 d: F(6, 6) = 0.38, n.s.].

**Fig. S2.** The similarity in the activity patterns of the CA1 neural network continued to decrease on a third recording day. (A) To assess differences in CA1 network activity patterns across time intervals of up to 60 h, we extended the hippocampal recordings across 3 d in three animals. On the third day, behavioral sessions were shifted by 6 h, providing additional comparisons for the population of cells that was tracked across all 3 d (n = 30 CA1 cells). (B) Population vector correlations between pairs of recordings in the same enclosure shape are shown as dots. The black error bars correspond to the mean ± SEM for recordings at a particular time interval. The correlation coefficients for the CA1 population activity decreased as a function of elapsed time between recording sessions [F(8) = 78.8, P < 0.001]. At 24- and 48-h intervals, which represent recordings at matching times of day, are indicated in gray. Repeated CA1 recordings at the same time of day on two consecutive days show a smaller correlation than repeated recordings at shorter intervals, but at a different time of day (P < 0.001 for the post hoc comparison). The decrease in the population vector correlation therefore does not show a circadian pattern. (C) Cumulative distribution functions for population vector correlations between pairs of recordings in the same enclosure shape.
**Fig. S3.** The activation of hippocampal neurons during intervening behavioral testing is unrelated to the pattern of decorrelation with extended time. (**A**) Population vector (PV) correlations for comparisons between recordings in the same enclosure shape at intervals of 24 h (Left) or 30 h (Right). Each comparison between two sessions is shown as a dot, and the black bars are the mean ± SEM for a time interval. Differences in the number of intervening blocks did not result in differences in the degree of decorrelation (comparisons between 24-h intervals: t = −0.64, n.s.; comparisons between 30-h intervals: t = −1.82, n.s.). (**B**) PV correlations between pairs of recordings in the same enclosure shape without intervening blocks. The 24-h comparisons are from recordings without an intervening AM block (Fig. S2). Each comparison between two sessions is shown as a dot, and the black bars are the mean ± SEM for a time interval. A decorrelation of the CA1 population activity with time was observed [F(3) = 41.6, P values for all post hoc comparisons <0.001 except that the comparison between the 18- and 24-h interval is n.s.].

**Fig. S4.** Changes in network activity patterns at shorter timescales. (**A**) The change in firing rate within a session is shown for individual fields in CA3 and CA1, recorded across eight sessions in the same shape (four during the AM and four during the PM block). To obtain estimates for the firing rate at the beginning and at the end of each session, a regression line was fitted to the firing rates during single passes through the place field. For each field, the difference in firing rate between the beginning and the end of each of the eight 10-min sessions is shown as a dot. Colored dots depict rate differences when the mean rate change was significantly different (at the 0.05 level) from zero, which is the expected value in the case of random fluctuation in firing rate. The black bars show the mean ± SEM for each field. The fields of each hippocampal subregion are sorted in the order of their mean firing rate difference. Many CA3 cells consistently changed their firing rates within a series of 10-min recording sessions, often resetting toward the previous start-value. Together with the finding that CA3 changed to a small degree over extended time periods, these results show that changes in firing within a session do not result in corresponding changes in population activity over long time intervals. (**B**) Representative CA3 and CA1 place fields for the effects described in **A**. Place cells were repeatedly recorded within the same shape. The regression line for firing rate vs. time in each session is shown in black.
Fig. 55. The difference between population vector correlations in CA1 and CA3 is consistent across experimental paradigms. (A and B) For comparison, data from Fig. 3C (A) and Fig. 5B (B) are redrawn. In A, the PV correlation is plotted for the two-shape experiment, in which animals were run in two enclosure shapes during each recording block. In B, the same analysis is shown for the single-shape experiment, in which animals were exposed to only one shape for the entire 30-h experiment. (C) The difference between CA1 and CA3 was larger in the two-shape condition compared with the single-shape condition, but increased in parallel over time for the two conditions [main effects for time and paradigm: F(4) = 22.3, P < 0.001 and F(1) = 97.4, P < 0.001; no significant interactions]. This suggests that the decorrelation due to time may be added in CA1 to a small decorrelation that occurs in both subregions in response to other aspects of the experience, such as the ongoing changes in the shape of the recording enclosure.
Fig. S6. Method for calculating firing rates during individual passes through a place field. (A) The spatial maps from a representative cell are shown for the four behavioral sessions in the morning (AM sessions 1–4) and for the four sessions in the afternoon (PM sessions 1–4). The boundary of the place field is outlined in white (see Fig. S11 for the method that is used for defining boundaries). (B) For each 10-min behavioral session, the path of the rat is shown in gray. The spikes fired by the cell are superimposed on the path in red. As expected, most spikes occur within the place field boundary, shown in black. (C) Each of the four panels shows spikes of an individual pass through the place field during a 10-min session (AM3 on the Left, PM3 on the Right). The path of the rat from the entire 10-min session is shown, and single passes (from crossing the boundary into the field until exiting the field) are highlighted in color. Spikes that occurred during the pass through the field are superimposed on the path as dots. (D) For each pass through the field, we calculated the average firing rate as the number of spikes that occurred during the pass divided by the duration of the pass. We also calculated the average velocity at which the rat traveled. The average firing rate for each pass is shown as a colored bar (purple for foraging sessions in the circle), with firing rate on the y-axis and the time within the behavioral session on the x-axis. The average velocity of each pass is represented as a bar plotted downward from the time axis (in blue). The representative passes shown in C are marked with arrows. (E) Firing rates for all passes although the place field throughout the entire recording day.
Firing rates of complete cell samples recorded simultaneously on the same tetrode as the representative examples shown in Fig. 2. (A) CA1 place fields recorded simultaneously on the same tetrode as field 1 in Fig. 2A. (B) CA1 place fields recorded simultaneously on the same tetrode as field 2 in Fig. 2A. (C) CA3 place fields recorded simultaneously on the same tetrode as field 3 in Fig. 2B. (D) CA3 place fields recorded simultaneously on the same tetrode as field 4 in Fig. 2B. For each place field in A–D, all 10-min recording sessions throughout the AM and PM are shown. The order of the enclosure shapes is indicated above the plot (Upper Left). Each bar represents the firing rate of the cell during a pass of the animal through the place field (Fig. 56). Time within the recording session is shown on the x-axis. The bars are colored green for square enclosures

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and purple for circular enclosures. For each pass, the corresponding running speed of the animal is plotted downward below the x-axis (blue bars). A pass during which the cell does not fire is indicated by the presence of a velocity bar for that pass without a corresponding rate bar. A regression line for firing rate vs. time is fitted to each session (shown in black). (E) Cresyl violet-stained section showing recording sites in the hippocampus. Final recording sites in CA3 are outlined in blue, for CA1 in red. (Scale bar, 500 μm.)
Fig. S8. Firing rates during individual passes through place fields were weakly correlated with the animal’s velocity and with the minimum distance to the center of the place field. (A) For each field, we calculated the correlation (Spearman’s rank correlation coefficient) between the animal’s average velocity and the cell’s firing rate on individual passes through the field. All passes in all behavioral sessions from a single enclosure shape (square or circle) were combined. Fields that were silent in a particular configuration were excluded from analysis, as were fields through which the rat passed fewer than five times in any 10-min session. The distribution of correlation values is shown for CA1 (Upper, median = 0.16) and CA3 (Lower, median = 0.23). Examples of representative fields in (B) CA1 or (C) CA3. Each panel shows a scatter plot of rate vs. velocity for all passes through the place field. Correlation coefficients are noted for each field, and coefficients that are significantly larger than zero are marked in blue. (D–F) Panels are laid out as in A–C, but here the correlation between firing rate and the minimum distance of the path to the center of the place field is reported [CA1, median = −0.18; CA3, median = −0.23; in E and F, fields with a correlation coefficient significantly less than zero (at the 0.05 level) are marked in pink]. We expect a negative correlation because the center of the place field is defined as the place at which the cell’s peak firing occurred. Traveling closer to the center should thus lead to increased spiking. Though there is a moderate correlation in firing rate with both velocity and distance to the center of the place field, this correlation is not sufficiently high to explain the variability in our data. Of particular relevance to our data, rats did not show a consistent trend over extended time in either their velocity profile or in the average distance to the center of the place field. The difference in the mean velocity between morning and afternoon sessions never exceeded 2 cm/s and, on average, was less than 0.5 cm/s.
Fig. 59. Correlation matrices for repeated recordings in the same enclosure shape. (A) Each AM and PM recording block during two recording days (day 1, day 2) included two sessions in the square enclosure and two sessions in the circle enclosure (Fig. 3). Over 2 d, eight recording sessions were therefore performed in enclosures of the same shape. The correlation matrices depict all possible comparisons between repeated recordings in the same shape at different time points. The mean population vector correlation (i.e., the average across the spatial bins of the recording enclosure) of each pairwise comparison between identical enclosures is shown. The scale bar to the right of the matrices shows the color code for the correlation coefficients. All four matrices use the same color scale. Comparisons between the same sessions are shown along the diagonal, and their correlation coefficient is, by definition, 1. Comparisons at 24-h intervals are highlighted by black boxes. The matrices are symmetrical along the diagonal and the same values are thus shown in the Upper Right and Lower Left. In CA1, the highest correlation coefficients were measured at the time intervals closest to the diagonal and decreased correlation coefficients at longer time intervals. A small decorrelation was also observed in CA3, but the effects are minor compared with CA1 (see Fig. 3 for statistics). (B) For the single-shape control days, each AM and PM block during two consecutive recording days included four recording sessions in the same shape. The correlation matrices therefore include comparisons between each of the 16 sessions. As observed in A, population vectors in CA1 (Left) became substantially decorrelated with increasing time intervals. In contrast, population vectors in CA3 remained highly correlated for intervals of up to 30 h (see Fig. 5 for statistics).
Fig. S10. CA1 place fields that were recorded simultaneously on the same tetrode as those in Fig. 4B. The firing fields that are identical to those in Fig. 4B are highlighted in gray. For each place field, firing correlates are shown for all 10-min recording sessions within the AM and PM for each of the two recording days, with time within each recording session on the x-axis. The order of the enclosure shapes throughout the sessions is shown above the plot (Top). Each bar represents the firing rate of the cell during a pass of the animal through the place field as described in Fig. S6.
Fig. S11. Illustration of the method for calculating boundaries of place fields. To illustrate that the analysis procedure separates adjacent fields, a cell with multiple fields is shown as an example. (A) Spatial maps are first calculated for each of the eight behavioral sessions in a single day. The firing rates within each map are color-coded according to the scale bar on the right, and the shape of the box is superimposed on each map. (B) A reference map was calculated as the average of the eight maps in A. Note that the place fields in the reference maps are overlapping, but that the maps of individual sessions suggest that the firing rates in different areas appear to be modulated independently. (C) Contours were calculated at 20 levels between zero and the peak rate. The contours are shown superimposed on the reference map. (D) Peak contours (shown in red, green, and blue in the three panels) were found. Surrounding contours are in gray. The three fields share many of the contours at lower levels. (E) Each shared contour is divided into segments at inflection points of the contour, and each segment is assigned to the nearest field. Each segment is illustrated in the color of the field to which it is assigned. (F) The end points of the segments and the outside contours of different colors in E are combined into outer boundaries of the fields. (G) The maps in A are redrawn with the boundaries of the three identified fields superimposed. (H) Two additional examples of cells with multiple, adjacent fields. The large panels (left) depict the reference map, and the smaller panels show the eight individual sessions with the field boundaries superimposed (arranged as in A). (I) The method also identifies the boundaries of single fields. Three examples of cells with single fields are shown. (J) Histograms of the number of fields of CA1 cells (upper) and CA3 cells (lower) in the single-shape condition. (K) Histograms of the number of fields of each cell in the two-shape condition. The shift toward a higher number of fields per cell is a result of recording in two box shapes, because fields occasionally appear in one box shape but not in the other.