Extended experience reveals distinct representational dynamics governing the CA1 spatial code

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Abstract

Hippocampal subregion CA1 is thought to support episodic memory through reinstatement of a stable spatial code. However, recent calcium imaging experiments demonstrate that the majority of this code changes rapidly, thus challenging this presumed function. Here we show that the mouse CA1 spatial code contains distinct representational components mediated by separate neural ensembles with different long-term dynamics, including a persistent representation of spatial geometry and prior experience.

Main Text

Hippocampal subregion CA1 represents a mixture of external and internal cues – including the shape of the environment[1], visual landmarks[2], objects[3], task-relevant information[4], and past experience[5,6] – through changes to the spatial tuning properties of its principal cells[7]. This code is hypothesized to support episodic memory by later reinstating its content. However, recent longitudinal calcium imaging experiments in mice have challenged this view. Such experiments have revealed that the bulk of these cells change their spatial tuning properties more rapidly than expected (on the order of days) with only a subset of these cells (15-25%) providing a persistent spatial representation on this timescale[8]. This rapid turnover thus challenges the idea that the CA1 spatial code provides the stable representation of content necessary to support episodic memory, while the presence of a persistent subpopulation raises unanswered questions about whether this subpopulation simply occupies the lower tail of a drift distribution or instead conveys unique content governed by unique dynamics.

Such long-term representational dynamics may reflect two possible processes. On one hand, these dynamics may reflect population-wide representational drift, perhaps a product of plasticity within the hippocampal circuit[9]. In this case, we would expect changes to be independent of the content of the representation, and thus pose a strong challenge to the persistent reinstatement hypothesis. On the other hand, these dynamics may reflect a time-varying representational component within an otherwise stable code, perhaps a product of a specific time-varying input. In this case, we would expect these dynamics to be restricted to a distinct component of the representation at the level of the population, and possibly at the level of individual neurons.

To adjudicate between these possibilities, we recorded daily from CA1 with calcium imaging (Fig. 1a) in mice freely exploring open environments for 32 days in an extended version of a geometric morph paradigm (5 mice, 160 sessions total; Fig. 1b; Fig. S1). In this paradigm, mice were first familiarized with two geometrically-distinct environments, and later tested in deformed (morphed) versions of these environments spanning the shapespace between the two familiar environments. Previous acute versions of this paradigm have demonstrated that a subset of CA1 cells exhibit experience-dependent attractor-like properties in their spatial tuning, with an abrupt transition in their preferred firing locations near the midpoint of the morph sequence, while other cells fired at geometrically similar locations across the morph sequence[10,11]. By employing more geometrically-dissimilar familiar environments and extending this paradigm across a 32 day period, we can thus assay three potential components of the CA1 representation – a representation of the spatial geometry (*geometry*), a representation of experience-dependent attractor dynamics (*attractor*), and a representation which varies with time (*time*).

We began by first characterizing these data within the morph sequence akin to previous acute reports. Following motion correction[12], cells were segmented and calcium traces were extracted via constrained nonnegative matrix factorization[13,14] (Fig. S1). The likelihood of spiking events (henceforth *firing rate*) was inferred from deconvolution of the filtered calcium traces via a second-order autoregressive model[15]. Cell identity was tracked across recording sessions on the basis of extracted spatial footprints (n = 2745)[16]. We found that individual cells exhibited a variety of dynamics, including abrupt transitions in their preferred locations near the midpoint of the morph sequence, persistent firing at geometrically-similar locations across the morph sequence, and the appearance of punctate spatial tuning in a subset of environments (Fig. 1c). Across the population, the morph sequence produced sigmoidal transitions between the (partially-correlated) familiar environment rate maps (Fig. 1d; Fig. S2). Fitting these transitions with sigmoidal functions allowed us to quantify dynamic properties across sequences within

each mouse, revealing qualitatively distinct patterns within each mouse and a trend toward increased decorrelation of maps across sequences (Fig. S2).

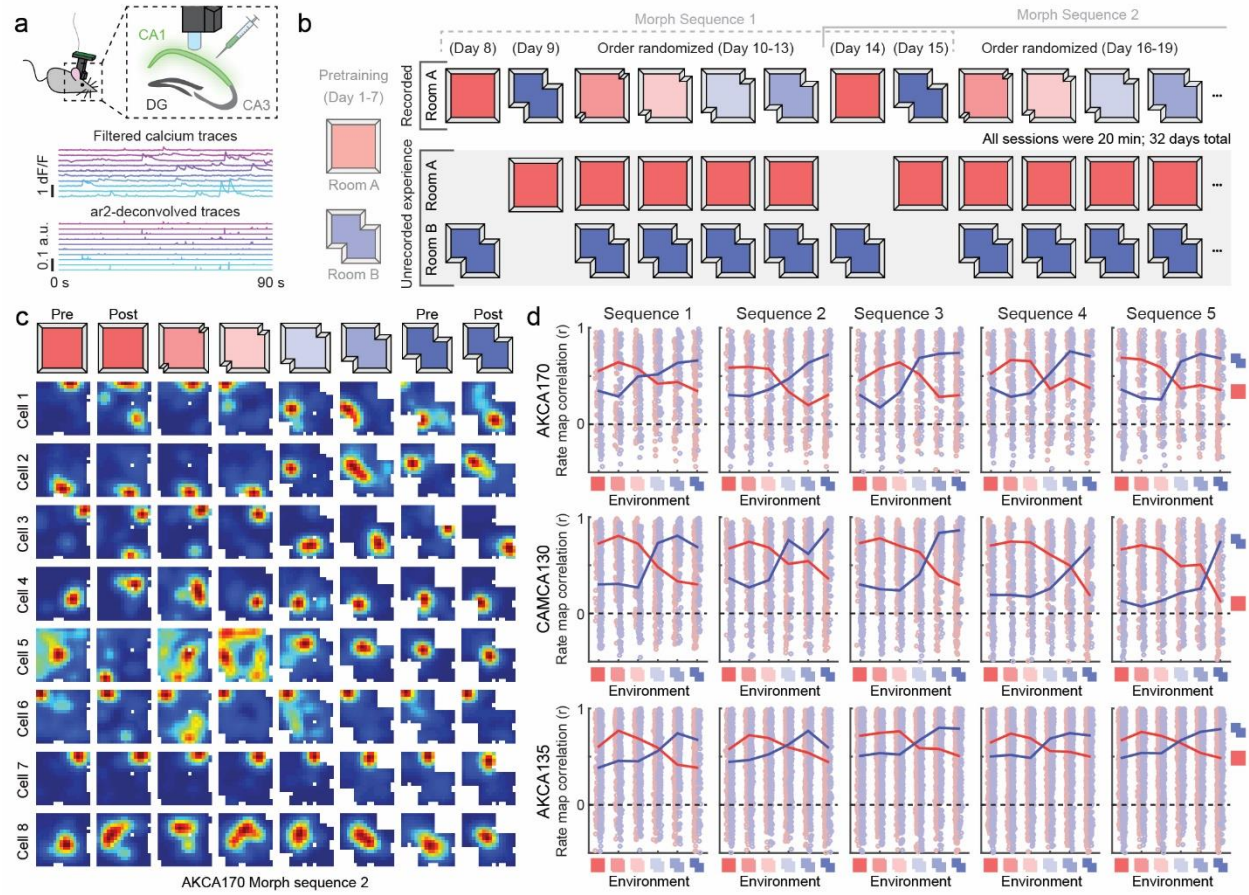

Figure 1. An adapted geometric morph paradigm characterizes the CA1 spatial code across extended experience. *a) Schematic of the miniscope recording procedure (top), as well as resulting calcium traces and firing rates inferred from second order autoregressive deconvolution (bottom). b) Schematic of the behavioral paradigm. Assignment of familiar environments to rooms and the order of familiar environment recordings were randomized across mice, and the order of unrecorded experience was randomized within mice. c) Example of eight simultaneously recorded cells tracked across one morph sequence exhibiting a diversity of dynamics. Rate maps normalized from zero (blue) to the peak (red) within each session. d) Rate map correlations between each environment and the familiar environments for each sequence. Bold lines indicate median across pairwise comparisons of cells (dots). Only comparisons between sessions where the cell had a within-session split-half rate map correlation exceeding the 99th percentile of the shuffled distribution in at least one session were included.*

Traditional within-morph sequence analyses only characterize the representational similarity between subsets of sessions. To take advantage of the full representational structure, we explored population-level representational similarity across all 32 days. To this end, we first computed the median rate map similarity across all tracked cells for each pairwise comparison of sessions (Fig. 2a). We then reduced this representation to two dimensions via nonmetric multidimensional scaling, an unsupervised technique for visualizing high-dimensional structure in a digestible low-dimensional space that preserves the representational similarity between

sessions as well as possible. The resultant embedding revealed two dimensions that strongly determined representational similarity in this paradigm: a spatial context component and a time-varying component (Fig. 2b). These components were pronounced in every mouse. Quantification of the embedded representations revealed that the contextual and time-varying components defined nearly orthogonal dimensions in this 2D subspace, with an absolute angular difference of 89.0° ± 5.8° (mean ± circular standard deviation) between context and time-varying dimensions (see *Methods*; all angles: [83.0°, 84.6°, 85.5°, 95.2°, 96.7°]; Rayleigh's test versus uniformity on the 0° to 180° range: p = 2.47e-3, z = 4.80). This reliable structure provides evidence that spatial context and time-varying components of the CA1 spatial code are representationally distinct at the level of the population.

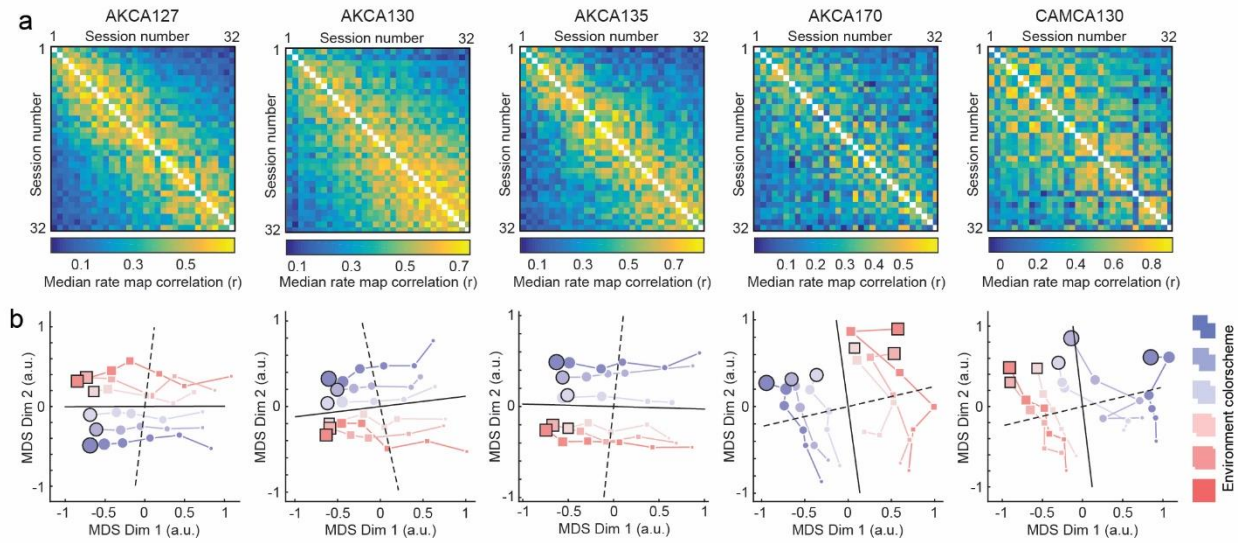

Figure 2. Dimensionality reduction reveals distinct temporal and contextual representational components in the CA1 spatial code. *a) Population-level representational similarity matrices (RSMs) across all recording sessions for all five mice. Each RSM is computed by taking the median pairwise similarity across all tracked cells. b) Session similarity structure when embedded in a two-dimensional space extracted via nonmetric multidimensional scaling. Dot size indicates session number, with earliest sessions indicated by the smallest dots and latest sessions indicated by the largest dots. Color indicates environmental geometry. Final sessions outlined in black. Note that estimated time (solid black line) and spatial context (dashed black line) dimensions are nearly orthogonal in this subspace (see Methods).*

The observation of distinct population-level representational components could be the product of a distributed code across neurons that exhibit mixed-selectivity. Conversely, it could also be a product of separate neural ensembles coding for individual representational components. To adjudicate between these possibilities, for each cell with at least 28 comparisons we created a representational similarity matrix (*RSM*) of the pairwise rate map correlations between all tracked sessions (Fig. 3a)[17]. (Twenty-eight was chosen as the number of pairwise comparisons within a single bookend morph sequence.) Next, we constructed predicted RSMs on the basis of similarity in time, spatial geometry, and contextual group as defined by the attractor dynamics (Fig. 3a; see *Methods*). Using these matrices, we computed the amount of variance in each cell RSM explained by a model containing the three predicted RSMs as well as all possible interactions (*total* r^2). We then determined the amount of total explained variance attributable to each factor by quantifying the reduction in explained variance when selectively excluding that factor (and its interactions).

The results of this analysis revealed remarkable selectivity in representational content and long-term dynamics across simultaneously-recorded cells (Fig. S3). Across all cells with a full model r^2 of at least 0.1915 (90th percentile of the shuffled distribution, n = 1316 of 2745, 47.9% of cells with sufficient comparisons, binomial test: p = ~0.0), explained variance was typically attributable to a single factor (Fig. 3b). Moreover, reorganization of RSMs for time-, attractor-, and geometry-loading cells revealed clear distinctions in their temporal and contextual dynamics (Fig. 3c; Fig S4). To quantify these observations, we computed null distributions by randomly shuffling RSM values within each comparison across cells. The resulting null distributions thus preserve the population-level statistics while destroying any within-cell cross-session effects. Relative to these distributions, the first-ranked factor was attributed slightly but significantly more variance, while the second- and third-ranked factors were attributed significantly less variance than would be expected given the variability within the population alone (Fig. 3d) – a pattern which held across a range of inclusion criteria (Fig. S5). These results provide evidence that simultaneously-recorded neural ensembles are selectively tuned to distinct representational components, governed by differing long-term dynamics.

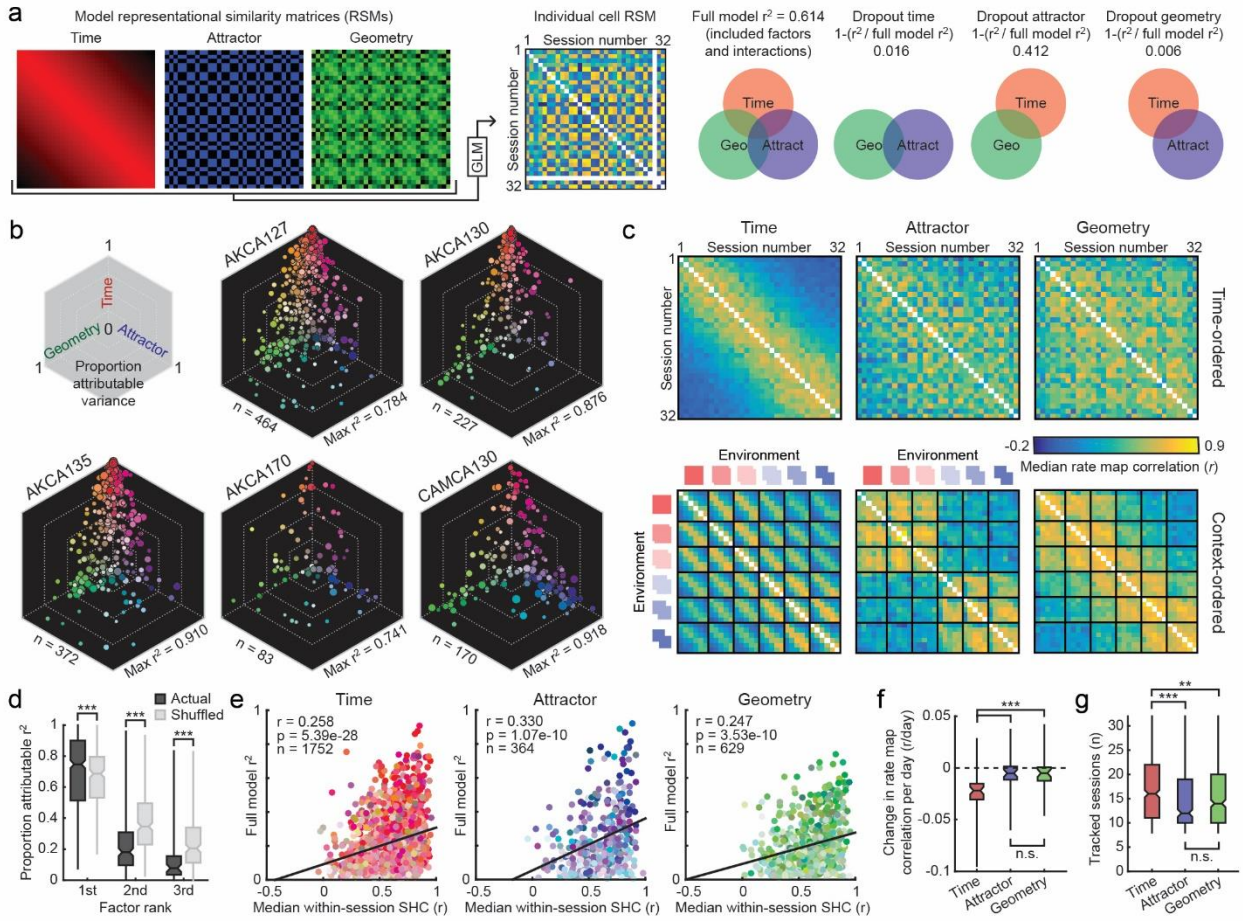

Figure 3. Characterizing individual cell RSM variance reveals representational selectivity at the single-cell level. All tests reported in this caption are Wilcoxon rank sum tests. *a) Each cell RSM was fit with a general linear model consisting of time, attractor, and geometry components including interactions. Then the relative reduction in r^2 was computed when excluding each factor. Cell RSMs were Fisher-transformed before fitting. Note that model attractor and geometry RSMs tend to be correlated, limiting the amount of variance that can be attributed to one or the other. b) Proportion attributable variance for cells meeting inclusion*

criteria. Note that cells tend to lie on the axes, indicating that most of their attributable variance is driven by a single factor. Dot size scaled to full model r^2. c) RSMs of time, attractor, and geometry-loading cells meeting criteria, ordered by time (top) and spatial context (bottom). Mouse CAMCA130 was excluded from this panel because of large changes in the attractor transition point across sequences. d) Proportion of attributable variance when factors are ranked. The primary factor was attributed more variance (Z = 4.27, p = 1.99e-05), while the secondary (Z = -16.15, p = 1.19e-58) and ternary (Z = -17.48, p = 1.89e-68) factors were attributed less variance than would be expected by chance given population statistics. e) Full model r^2 and median within-session split-half rate map correlation (SHC) were similarly correlated across all cells regardless of the primary factor. f) Change in rate map correlations per day for cells meeting criteria (time vs attractor, Z = -15.40, p = 1.62e-53; time vs geometry, Z = -18.65, p = 1.16e-77; attractor vs geometry, Z = 0.14, p = 8.91e-01)*. g) Number of sessions in which a cell was tracked for cells meeting criteria* (time vs attractor, Z = 4.21, p = 2.51e-05; time vs geometry, Z = 2.85, p = 4.33e-03; attractor vs geometry, Z = -1.71, p = 8.65e-02)*. **p < 0.01, ***p < 0.001*

The observation of a subset of persistent representational components raises the question of whether differently-loading cells are distinguished by their representational quality. To address this possibility, we characterized a number of properties as follows. Within-session split-half rate map correlations were similarly high in all groups (Fig. S6), indicating comparable spatial reliability on a timescale of minutes. A similar positive relationship between split-half correlations and full model r^2 was observed regardless of primary factor loading (Fig. 3e), indicating that full model r^2 was associated with more reliable spatial maps in the short-term regardless of long-term dynamics. Cells meeting the full model r^2 criterion tended to have higher mean firing rates than cells not meeting this criterion, and attractor-loading cells exhibited higher mean firing rates than time- and geometry-loading cells, possibly reflecting excitability-dependent recruitment dynamics[9] (Fig. S6).To characterize the decay in map similarity across sessions separated by increasing amounts of time, we computed the least squares linear fit of rate map correlations as a function of temporal lag between sessions. Time-loading cells exhibited a modest but reliable decrease in rate map correlations for each day separating compared sessions, which was significantly greater than that observed in attractor-loading or geometry-loading cells (Fig. 3f). These results indicate that time-loading cells were characterized by gradual changes in their spatial firing properties, while corroborating the differing long-term representational dynamics between groups. Time-loading cells were tracked across more sessions than attractor-loading or geometry-loading cells (Fig. 3g), consistent with a lack of context-selectivity in these cells and indicating that variance attributed to time was not the result of cell dropout. The proportion of geometry- and attractor-loading cells within a population was positively related to the decorrelation between familiar environment representations, while the proportion of time-loading cells was negatively related to this decorrelation, also consistent with differences in context-selectivity between groups (Fig. S1). Signal-to-noise differences between groups were minor (Fig. S6). No clear anatomical organization according to primary factor was observed (Fig. S7). Together, these results suggest that time-, attractor-, and geometry-loading cells are distinguished primarily by their content and long-term dynamics, rather than the quality of their representations.

These findings have a number of important implications. First, by assaying representational content across extended experience, these results go beyond prior work[8,18] to demonstrate that differing long-term dynamics governing the CA1 spatial code are mapped to distinct representational components, including time, spatial geometry, and experience-dependent attractor dynamics. Components representing the spatial context, such as those coding spatial geometry and prior experience in similar environments, are persistently reinstated

across the timescale of weeks. This mix of persistent and time-varying representational dynamics is consistent with many theories of episodic memory and hippocampal function, such as indexing theory[19] and temporal context models[20]. In such theories, a time-varying component allows for the encoding and memory of the temporal relationship between different events, while the persistent context-dependent component allows for linking events occurring within the same spatial context across time. Our finding that these distinct components coexist within the population code further adds to a growing literature that challenges the traditional view of a representationally homogenous CA1 spatial code[21–23], and in turn also challenges models which assume a single state space[24] or basis set[25] as the source of the CA1 spatial code. Additionally, these results suggest that long-term changes in the CA1 spatial code do not reflect homogenous representational drift, which could result from uniform plasticity at the level of the population[9]. Rather, these changes likely reflect either the selective influence of plasticity on a distinct subpopulation within CA1 and/or the influence of time-varying input(s) mapped onto this subpopulation, such as those found in the lateral entorhinal cortex[26,27] and hippocampal subregion CA2[28]. Such time-varying inputs may include surrounding soundscapes, scentscapes, motivations, and intervening experience that cannot be completely controlled in this paradigm, as well as a representation of time per se. Finally, these results provide strong evidence of the heterogenous and malleable nature of hippocampal remapping[29,30], one that is linked to a dynamic recruitment and subsequent reinstatement of distinct neural ensembles[9].

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Methods

Subjects

Naive male mice (C57Bl/6, Charles River) were housed in pairs on a 12-hour light/dark cycle at 22°C and 40% humidity with food and water ad libitum. All experiments were carried out during the light portion of the light/dark cycle, and in accordance with McGill University and Douglas Hospital Research Centre Animal Use and Care Committee (protocol #20157725) and with Canadian Institutes of Health Research guidelines.

Surgeries

During all surgeries, mice were anesthetized via inhalation of a combination of oxygen and 5% Isoflurane before being transferred to the stereotaxic frame (David Kopf Instruments), where anesthesia was maintained via inhalation of oxygen and 0.5-2.5% Isoflurane for the duration of the surgery. Body temperature was maintained with a heating pad and eyes were hydrated with gel (Optixcare). Carprofen (10 ml kg^{-1}) and saline (0.5 ml) were administered subcutaneously at the beginning of each surgery. Preparation for recordings involved three surgeries per mouse.

First, at the age of six to ten weeks, each mouse was transfected with a 350 nl injection of the calcium reporter GCaMP6f via the viral construct AAV5.CaMKII.GCaMP6f.WPRE.SV40 (CAMCA130) or AAV9.syn.GCaMP6f.WPRE.SV40 (all other mice). The original titre of the AAV9.syn.GCaMP6f.WPRE.SV40 construct, sourced from University of Pennsylvania Vector Core, was 3.26e14 GC-ml and was diluted in sterile PBS (1:1 AKCA170; 1:5 AKCA127; 1:10 AKCA130; 1:15 AKCA135) before surgical microinjection. The original titre of the AAV5.CaMKII.GCaMP6f.WPRE.SV40 construct, sourced from Addgene, was 2.3e13 GC-ml and was diluted in sterile PBS (1:3) before surgical microinjection.

One to three weeks post-injection, either a 1.8mm (AKCA127, AKCA130, AKCA135) or 0.5mm (AKCA170, CAMCA130) diameter gradient refractive index (GRIN) lens (Go!Foton) was implanted above dorsal CA1 (Referenced to bregma: ML = 2.0 mm, AP = -2.1 mm; Referenced to brain surface: DV = -1.35 mm). Implantation of the 1.8mm diameter GRIN lens required aspiration of intervening cortical tissue, while no aspiration was required for implantation of the 0.5mm diameter GRIN lens. Results observed using 1.8- or 0.5-mm diameter GRIN lenses were similar. In addition to the GRIN lens, two stainless steel screws were threaded into the skull above the contralateral hippocampus and prefrontal cortex to stabilize the implant. Dental cement (C&B Metabond) was applied to secure the GRIN lens and anchor screws to the skull. A silicone adhesive (Kwik-Sil, World Precision Instruments) was applied to protect the top surface of the GRIN lens until the next surgery.

One to three weeks after lens implantation, an aluminum baseplate was affixed via dental cement (C&B Metabond) to the skull of the mouse, which would later secure the miniaturized fluorescent endoscope (miniscope) in place during recording. The miniscope/baseplate was mounted to a stereotaxic arm for lowering above the implanted GRIN lens until the field of view contained visible cell segments and dental cement was applied to affix the baseplate to the skull. A polyoxymethylene cap with a metal nut weighing ~3 g was affixed to the baseplate when the mice were not being recorded, to protect the baseplate and lens, as well as to simulate the weight of the miniscope.

After surgery, animals were continuously monitored until they recovered. For the initial three days after surgery mice were provided with a soft diet supplemented with Carprofen for pain management (MediGel CPF, ~5mg kg^{-1} each day). Familiarization to both environments (while recording in the room A-associated environment to monitor imaging quality and habituate the mouse to recording) began 3 to 7 days following the baseplate surgery.

Data acquisition

In vivo calcium videos were recorded with a UCLA miniscope (v3; miniscope.org) containing a monochrome CMOS imaging sensor (MT9V032C12STM, ON Semiconductor) connected to a custom data acquisition (DAQ) box (miniscope.org) with a lightweight, flexible coaxial cable. The DAQ was connected to a PC with a USB 3.0 SuperSpeed cable and controlled with Miniscope custom acquisition software (miniscope.org). The outgoing excitation LED was set to between 2-8% (~0.05-0.2 mW), depending on the mouse to maximize signal quality with the minimum possible excitation light to mitigate the risk of photobleaching. Gain was adjusted to match the dynamic range of the recorded video to the fluctuations of the calcium signal for each recording to avoid saturation. Behavioral video data were recorded by a webcam mounted above the environment. Behavioral video recording parameters were adjusted such that only the red LED on the CMOS of the miniscope was visible. The DAQ simultaneously acquired behavioral and cellular imaging streams at 30 Hz as uncompressed avi files and all recorded frames were timestamped for post-hoc alignment.

All recording environments were constructed of a grey Lego base and black Lego bricks (Lego, Inc) according to the dimensions specified in the main text and supplemental figures. All external walls had a height of 22 cm. During recording, the environment was dimly lit by a nearby computer screen, which could serve as directional cue. During familiarization and all unrecorded experience the environments were well-lit by overhead room lighting. All sessions were 20 min, and only one session was recorded per day to avoid photobleaching. The mouse was always placed in the same corner at the start of the session and was allowed to explore the environment for 15 to 30 s prior to the start of data acquisition. Following each recording the environment was cleaned with disinfectant (Prevail).

Data preprocessing

Calcium imaging data were preprocessed prior to analyses via a pipeline of open source MATLAB (MathWorks; version R2015a) functions to correct for motion artifacts[12], segment cells and extract transients[13,14], and infer the likelihood of spiking events via deconvolution of the transient trace through a second-order autoregressive model[15]. The motion-corrected calcium imaging data were manually inspected to ensure that motion correction was effective and did not introduce additional artifacts. Following this preprocessing pipeline, the spatial footprints of all cells were manually verified to remove lens artifacts. Position data were inferred from the onboard miniscope red LED offline following recording using a custom written MATLAB (MathWorks) script and were manually corrected if needed. Cells were tracked across sessions on the basis of their spatial footprints and/or centroids[16].

Data analysis

All analyses were conducted using the vector of inferred likelihood of spiking events, treating this vector as if it were the firing rate of the cell (henceforth *firing rate*) [15]. Only periods of

movement for which the mouse speed exceeded 2 cm s^{-1} were included in the analysis, though no qualitative differences were observed if this criterion was relaxed.

Rate maps were constructed by first binning the position data into pixels corresponding to a 2.5 cm x 2.5 cm grid of locations. Then the mean firing rate was computed for each pixel and then smoothed with a 4 cm standard deviation isometric Gaussian kernel. For all comparisons between rate maps, similarity was measured as the Pearson's correlation between corresponding pixels. Individual cell representational similarity matrices (RSMs) were thus computed as the Pearson's correlation between pairwise rate map comparisons for tracked sessions. Population-level RSMs were computed by taking the median across all cells for each pairwise comparison.

Within-sequence analyses summarized morph sequence dynamics with a transition plot, which captured the similarity of all 6 morph environment maps to the 2 familiar environment maps. To this end, rate map correlations between each environment and familiar rate maps at the beginning and end of the morph sequence were computed. Only comparisons between cells whose within-session split-half rate map correlation (SHC) exceed the 99th percentile of a shuffled control for at least one of the compared sessions were included. The shuffled control was computed for each cell by randomly circularly shifting its firing rate vector relative to the position data by at least 30 sec and recomputing the SHC 1000 times to create the null distribution. The median across all pairwise cell comparisons was taken as the measure of central tendency for transition plots. To characterize these plots, the Fisher-transformed median values comparing the morph environments to each of the two familiar environments were both fit to minimize mean squared error with a 4-parameter sigmoid of the form

$$f(x) = p_3 + \left(1 + \frac{1}{p_2 e^{(-x+p_1)}}\right) / p_4$$

where x is the position of the current environment in the shapespace (arbitrarily chosen to range from 1 to 6), and parameters p determine the shape of the sigmoid. The intersection of these two sigmoids was taken as the measure of the transition point between familiar maps. The maximum absolute difference between these two sigmoids within the sampled shapespace was taken as the measure of maximum map decorrelation. Sigmoidality was computed as the mean absolute difference between each sigmoid and a linear fit from endpoint to endpoint, with endpoints normalized to range from zero to one.

Two-dimensional embeddings of the population representation were computed and quantified as follows. First, the population RSM was computed by taking the median across cells for each comparison. Next, this RSM was computed to a distance matrix as 1-(RSM) with the diagonal set to a distance of 0. Finally, this distance matrix was reduced to a two-dimensional embedding via Kruskal's nonmetric multidimensional scaling, implemented by the MATLAB function mdscale with the default parameterizations. To estimate the context dimension within this embedding, the average difference separating neighboring-in-time familiar environment recordings was computed. To estimate the time dimension within this embedding, the average difference between the first and last recordings of each familiar environment was computed.

Characterization of individual cell RSM variance was computed as follows. Only cells tracked across at least 28 pairwise comparisons (8 sessions) were included. Model RSMs were computed on the basis of temporal, geometric, and attractor group similarity, as schematized in

Figure 3a. The time RSM was computed as the difference in session number between pairwise comparisons. The geometry RSM was the difference in environment shape between pairwise comparisons, assuming that consecutive environments mapped linear steps through the shapespace separating the two familiar environments. The attractor RSM was computed such that if a pairwise comparison was between two sessions on the same side of the within-sequence transition point then those environments were assigned a similarity of 1; otherwise, that comparison was assigned a similarity of 0. Models consisting of all factors and their interactions (full model) as well as models with each factor and their interactions removed were then fit to the Fisher-transform individual cell RSMs as described in the main text. Notably, geometry and attractor factors were often correlated (though the extent of their correlation differed depending on transition point dynamics and the particular subset of tracked sessions for each cell), often limiting the amount of explained variance that could be attributed to one or the other. The time RSMs tended to be uncorrelated with either the geometric or attractor RSMs.

Signal-to-noise (SNR) for each dF/F trace was computed as a measure of data quality prior to spike estimation via deconvolution as described in previously[31]. Briefly, because calcium transients around the baseline can only be positive, for each trace the variance of a normal noise distribution was estimated by scaling the standard deviation of trace values below baseline via a half-normal distribution, such that:

$$\mathrm{NOISE} = \frac{\mathrm{std}(\mathbf{t}(\mathbf{t} < 0))}{\sqrt{1 - \frac{2}{\pi}}}$$

where t is the detrended filtered calcium trace and $\mathbf{t}(\mathbf{t} < 0)$ are all filtered trace values below baseline. Next, we z-score the trace $\mathbf{t}$ such that $z(\mathbf{t}) = \mathbf{t}/\mathrm{NOISE}$. These z-scored values are next transformed into probabilities $p(\mathbf{t}) = \varphi(-z(\mathbf{t}))$, where φ denotes the cumulative distribution function of the standard normal distribution. Then we compute the lowest probability event over a time window of 0.4 s (N = 12 frames), the duration around which a GCamp6F transient is typically at its maximum, such that:

$$p_{\min} = \min_i \left(\prod_{j=0}^{N-1} \mathrm{p}(\mathbf{t}(i+j)) \right)^{1/N}$$

where $\min_i$ is the minimum value across all timepoints i from $i = 1$ to $i =$ the length of $\mathbf{t} - N + 1$. Finally, we define the average peak SNR as:

$$\mathrm{SNR} = -\varphi^{-1}(p_{\min})$$

where φ^{-1} is the quantile function of the standard normal distribution (logit function).

Histological validation of expression and recording targets

After experiments, animals were perfused to verify GRIN lens placement. Mice were deeply anesthetized and intracardially perfused with 4% paraformaldehyde in PBS. Brains were dissected and post-fixed with the same fixative. Coronal sections (50 μm) of the entire hippocampus were cut using a vibratome and sections were mounted directly on glass slides.

Sections were split and half of all sections were stained for DAPI and mounted with Fluoromount-G (Southern Biotechnology) to localize GRIN lens placement and to evaluate virus expression. Due to the large imageable surface but restricted miniscope field of view (~0.5 mm x ~0.8 mm), we were unable to determine more specific localization of populations within the hippocampus for mice recorded with 1.8 mm lenses.

Statistics and reproducibility

All statistical tests are noted where the corresponding results are reported throughout the main text and supplement. All tests were uncorrected 2-tailed tests unless otherwise noted. Z-values for nonparametric Wilcoxon tests were not estimated or reported for comparisons with fewer than 15 datapoints. In all cases, the median served as the measure of central tendency unless otherwise noted. Box plots portray the minimum and maximum (whiskers), upper and lower quartiles (boxes), and median (cinch).

Code availability

All custom code written for reported analyses are publicly available at [insert Github link] or via request to the corresponding authors.

Data availability

The complete dataset for all experiments are publicly available at [insert DRYAD link] or via request to the corresponding authors.

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Author Contributions

ATK contributed to experimental design, surgeries, recordings, analysis of data, as well as drafting and revising the manuscript. CAM contributed to surgeries and histology. MPB contributed to experimental design, analysis of data, as well as drafting and revising the manuscript.

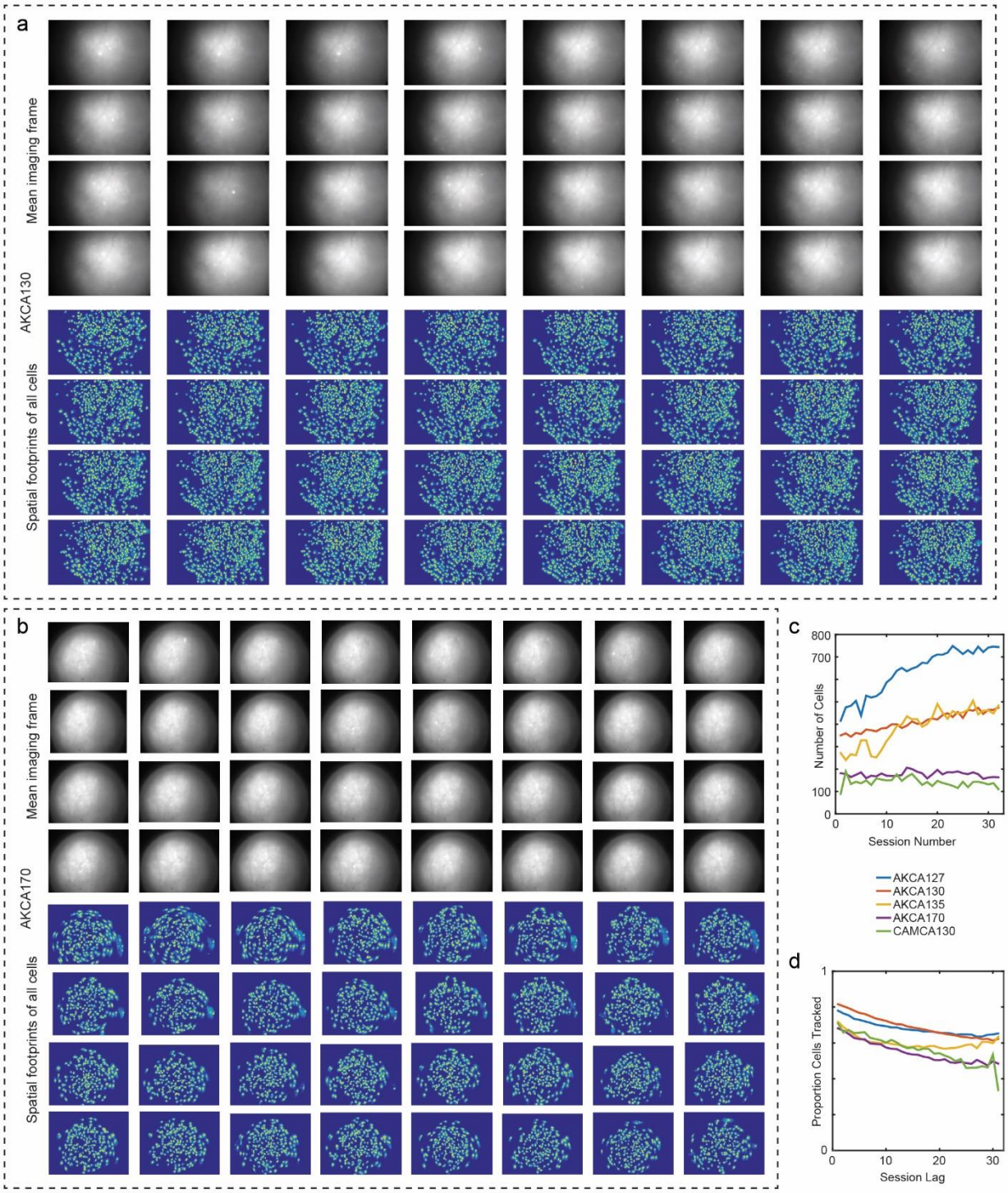
Competing interests.

The authors declare no competing interests.

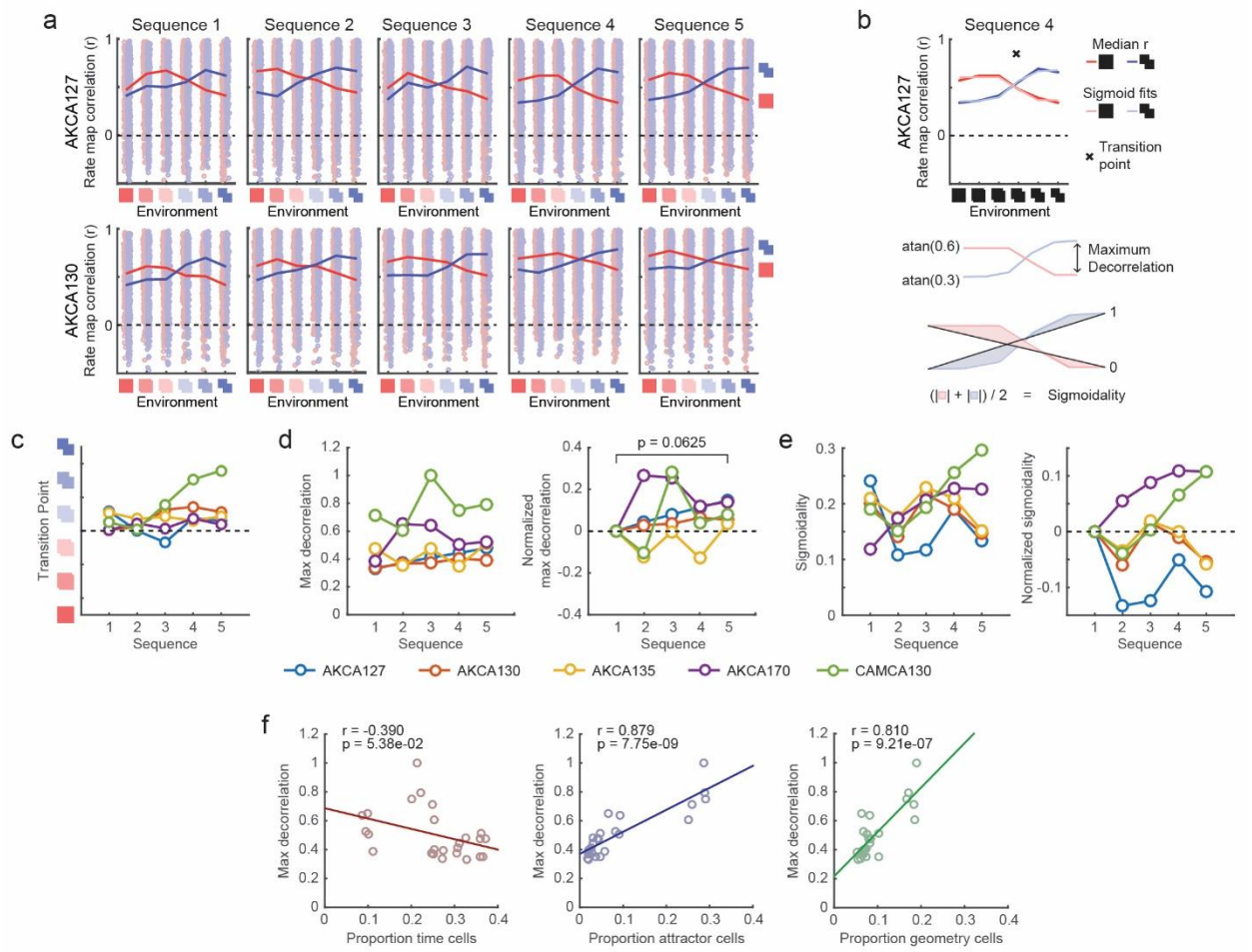
Supplementary information for:

Extended experience reveals distinct representational dynamics governing the CA1 spatial code

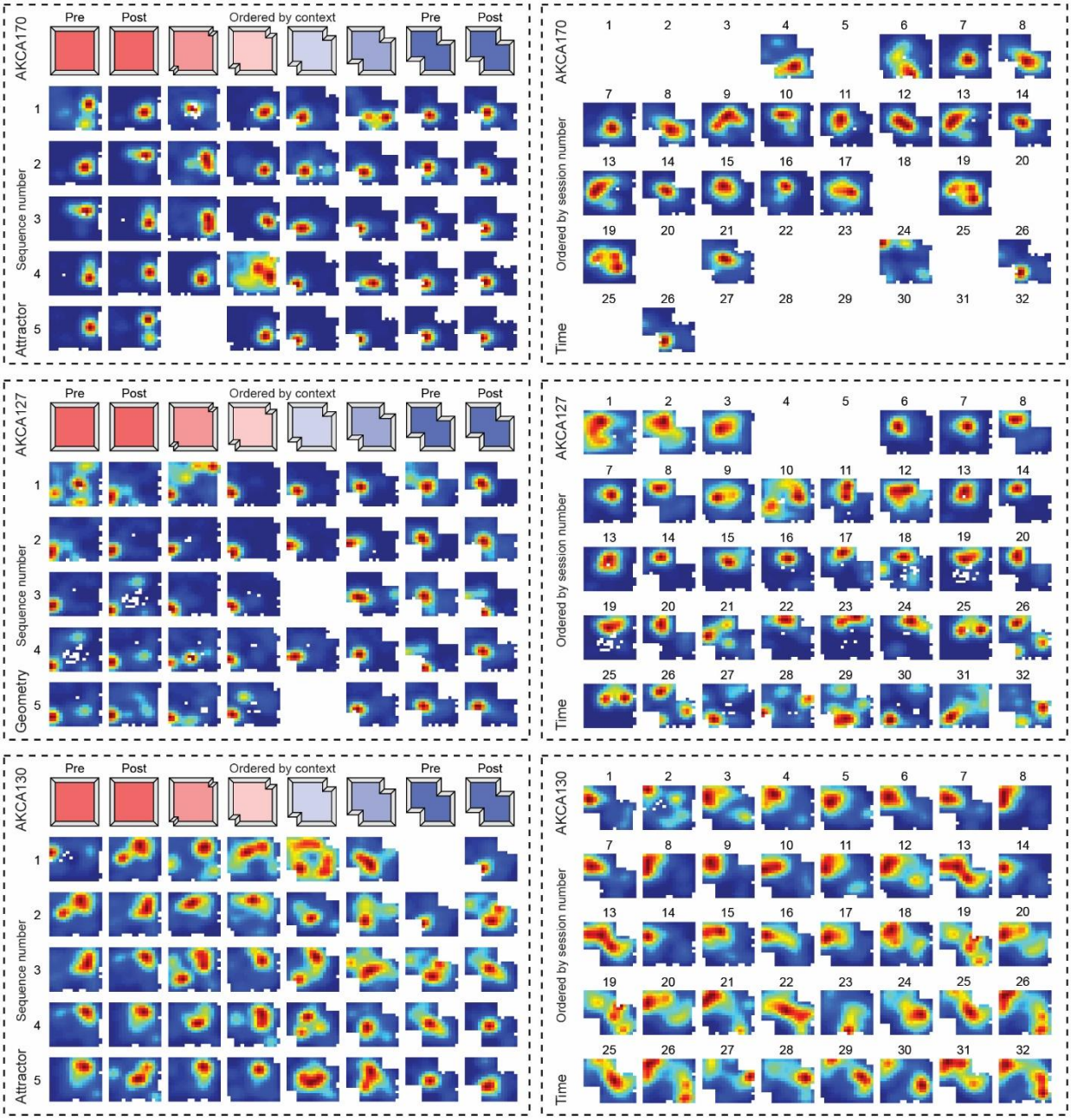
Keinath, et al.



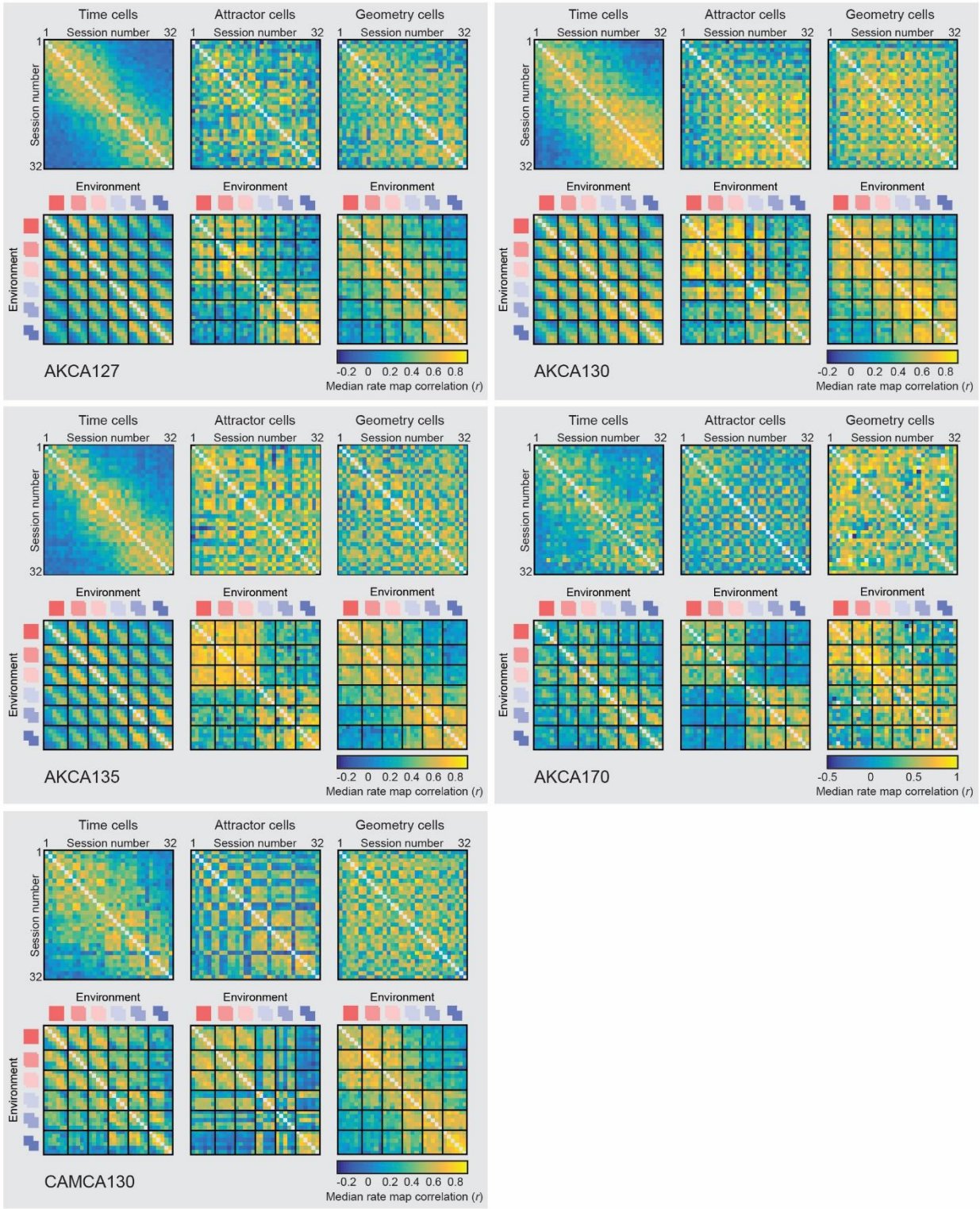
Supplementary Figure 1. Imaging across 32 days of recording. a,b) Arranged chronologically. Two example mice, AKCA130 (a) implanted with a 1.8 mm lens and AKCA170 (b) implanted with a 0.5 mm lens. Mean imaging frame normalized to maximum. Spatial footprints scaled and thresholded for ease of interpretation. c) Cell counts for each mouse across all 32 days. d) Proportion of the population registered as a function of lag between sessions for each mouse. Normalized to the smaller population for each pairwise comparison.



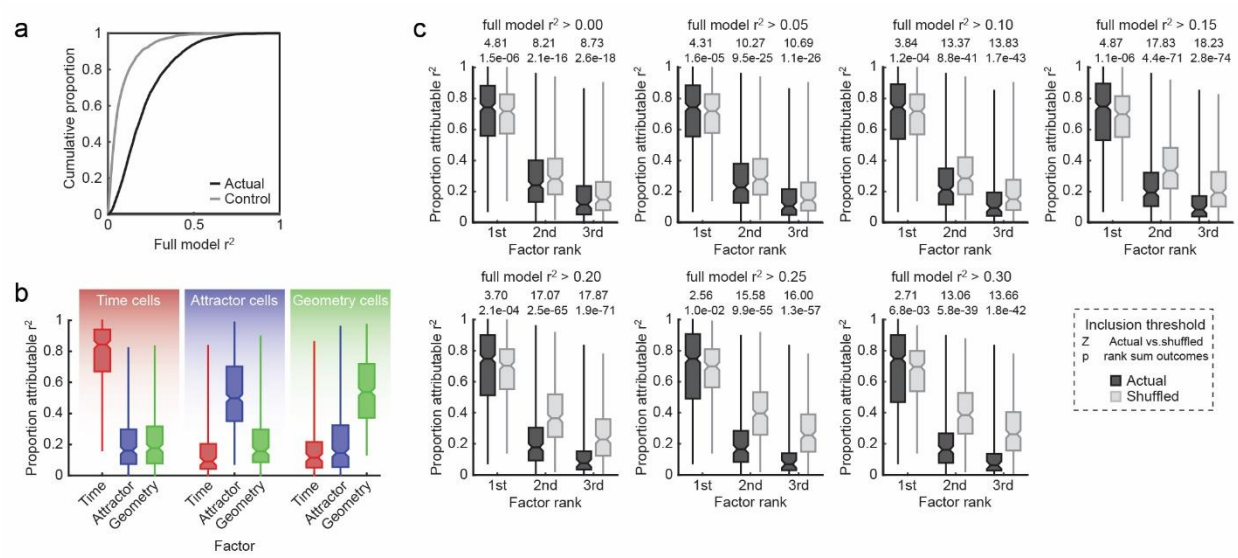
Supplementary Figure 2. Characterization of individual morph sequences. a) Rate map correlations between each environment and the familiar environments for each sequence as in Figure 1d for the two mice not shown there. b) To quantify transition characteristics, both median rate map correlations were fit with a sigmoid. Transition point was computed as the intersection of these sigmoids. Maximum decorrelation was computed as the maximum absolute difference between the Fisher transformation of these sigmoids. Sigmoidality was computed as the mean absolute difference between each sigmoid and a linear fit from endpoint to endpoint, with endpoints normalized to range from zero to one. c) Transition points across sequences for each mouse. Transition points tended to remain near the middle of the morph sequence except in the case of CAMCA130, which showed a progressive change in transition such that more morph environments resembled the square environment over time. Interestingly, the square environment was the room A-associated environment for this animal, where all test recordings took place. d) Max decorrelation by sequence and normalized by the first sequence. A trend toward increased decorrelation between the first and last sequences was observed (Wilcoxon rank sum test). e) Sigmoidality by sequence and normalized by the first sequence. No clear trend emerged. f) Relationship between maximum decorrelation and the mean proportion of cells meeting criteria for each primary factor within each sequence. A strong positive correlation was observed for attractor- and geometry-loading cells, and a marginal negative correlation for time-loading cells, consistent with a distinct contribution of each factor to representations of context and time, respectively.



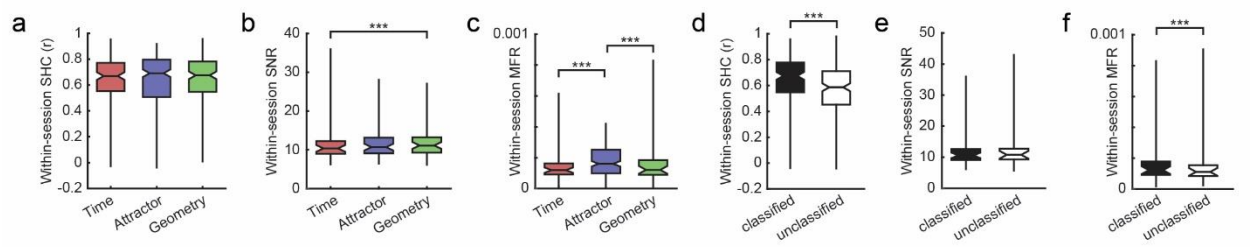
Supplementary Figure 3. Examples of individual cells across all sessions. Two simultaneously-recorded cells each from 3 mice. All cells met full model r^2 inclusion criteria, with their primary factors identified in the lower left of each panel. For ease of interpretation, context-loading cells are organized by context while time-loading cells are organized by session number. The attractor cell from AKCA170 is the example cell whose RSM is shown in Figure 3a.



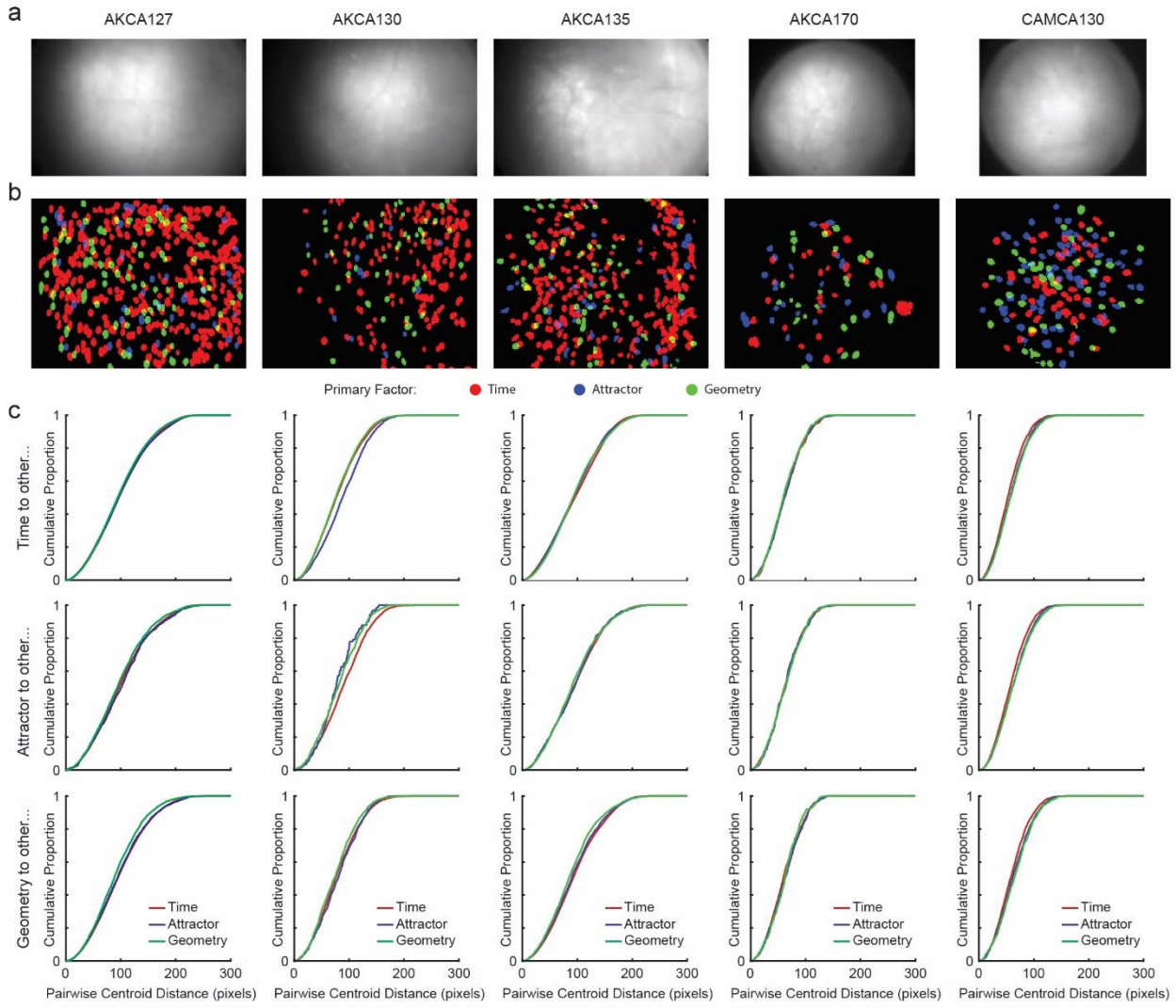
Supplementary Figure 4. Population RSMs for each cell group separated by mouse. RSMs of time, attractor, and geometry-loading cells meeting criteria, ordered by time (top) and context (bottom), separated by mouse.



Supplementary Figure 5. Reported ranked-factor results are robust to a variety of inclusion criteria. a) Cumulative distributions of full model r^2 for the actual and shuffled control populations (n = 2745). b) Proportion of attributable variance for each factor for cells exceeding a full model r^2 of 0.1915, grouped by their primary factor. c) Proportion of attributable variance when factors are ranked for a variety of minimum full model r^2 thresholds. In all cases, the primary factor was attributed more variance, while the secondary and ternary factors were attributed less variance than would be expected by chance given population statistics. All statistics denote the outcome of Wilcoxon rank sum tests between actual and control distributions.



Supplementary Figure 6. Within-session representational properties as a function of primary factor loading and as a function of meeting full model r^2 criterion. All statistical outcomes reported here are the results of Wilcoxon rank sum tests. a) Median within-session split-half rate map correlation (SHC) for cells meeting criteria, grouped by primary factor loading. No significant differences were observed (time versus attractor, Z = -0.82, p = 4.12e-01; time versus geometry, Z = -0.16, p = 8.74e-01; attractor versus geometry, Z = 0.56, p = 5.76e-01). b) Median within-session signal-to-noise ratio (SNR) for cells meeting criteria, grouped by primary factor loading. A small but significant difference in SNR was observed between time- and geometry-loading cells (time versus attractor, Z = -1.88, p = 6.01e-02; time versus geometry, Z = -3.60, p = 3.19e-04; attractor versus geometry, Z = -0.97, p = 3.31e-01). c) Median within-session mean firing rate (MFR) for cells meeting criteria, grouped by primary factor loading. MFRs of attractor-loading cells were significantly higher than those of other cells (time versus attractor, Z = -4.87, p = 1.12e-06; time versus geometry, Z = -0.55, p = 5.85e-01; attractor versus geometry, Z = 3.53, p = 4.11e-04). d) Median within-session SHC for cells meeting criteria (classified) versus cells not meeting criteria (unclassified; Z = 11.09, p = 1.36e-28). e) Median within-session SNR for cells meeting criteria (classified) versus cells not meeting criteria (unclassified; Z = -1.83, p = 6.72e-02). f) Median within-session MFR for cells meeting criteria (classified) versus cells not meeting criteria (unclassified; Z = 5.99, p = 2.13e-09).



Supplementary Figure 7. Anatomical organization of cells as a function of primary factors. a) Mean imaging frame for each mouse. b) Distribution of spatial footprints (SFPs) of all cells meeting full model r^2 inclusion criterion, color-coded by primary factor. c) Cumulative distribution of pairwise distances between SFP centroids for all combinations of cell groups separately for each animal. No evidence of reliably different anatomical distributions as a function of primary factor loading was observed.