Stable representation of a naturalistic movie emerges from episodic activity with gain variability

Ji Xia, Tyler D. Marks, Michael J. Goard & Ralf Wessel

Visual cortical responses are known to be highly variable across trials within an experimental session. However, the long-term stability of visual cortical responses is poorly understood. Here using chronic imaging of V1 in mice we show that neural responses to repeated natural movie clips are unstable across weeks. Individual neuronal responses consist of sparse episodic activity which are stable in time but unstable in gain across weeks. Further, we find that the individual episode, instead of neuron, serves as the basic unit of the week-to-week fluctuation. To investigate how population activity encodes the stimulus, we extract a stable one-dimensional representation of the time in the natural movie, using an unsupervised method. Most week-to-week fluctuation is perpendicular to the stimulus encoding direction, thus leaving the stimulus representation largely unaffected. We propose that precise episodic activity with coordinated gain changes are keys to maintain a stable stimulus representation in V1.
stimulus-driven activity is highly variable across repeated trials within a recording session. Furthermore, in chronic recordings covering multiple stimulus sessions, session-to-session fluctuation tends to be qualitatively different from trial-to-trial variability within sessions. Even without learning, the same neuron population responds unstably under the same environmental and behavioral conditions across days. However, not all the brain areas share the same instability. For example, neural activity from posterior parietal cortex, hippocampus, and primary olfactory cortex exhibit large changes across days, while HVC (proper name) neural activity remains stable in long-term recordings.

How does stimulus-driven activity in V1 change across days under a nominally constant condition? Recently, several studies shed light on how V1 stimulus-driven activity changes in the long term in responses to drifting gratings. Even though day-to-day variations were larger than trial-to-trial variations, stable tuning over weeks was found in most tuned neurons. Yet few reported on the long-term stability of neural responses to natural movies. Natural movie responses are sparser and more precise than neural responses to artificial stimuli such as drifting gratings. Moreover, responses to natural stimuli cannot be predicted from responses to drifting gratings. Thus, the long-term stability of neural responses to natural movies is not necessarily the same as that to drifting gratings. Indeed, data from our group showed that single neural responses to natural movies were significantly more unstable than drifting grating responses.

This session-to-session fluctuation raises an important question: Is there a stable representation of natural stimuli hidden in the unstable neural activity in V1? Stable stimulus representation is possible when neural fluctuations reside in a space orthogonal to the stimulus encoding dimensions. Intuitively, if one neuron’s session-to-session fluctuation affected the encoding of stimulus, then the other neurons’ fluctuation could compensate for its influence. Moreover, the stimulus could be encoded in a low-dimensional subspace of the high-dimensional population activity. In that case, the random fluctuation in the high-dimensional neural space would likely be perpendicular to the low-dimensional subspace of stimulus encoding, often referred to as the stimulus encoding dimension. Clarification of these possibilities requires long-term recordings in response to repeated stimulation, identification of the stimulus encoding dimensions, and quantification of neural fluctuation within the high-dimensional population activity.

To address the question of stable stimulus representation in unstable neural activity, we analyzed a dataset from longitudinal two-photon calcium imaging of excitatory neurons in the primary visual cortex of awake, head-fixed mice during visual stimulation with repeated natural movies across weeks. We found that single neural responses consisted of episodic activity that was precise in time during the natural movie across weeks. However, firing rates during those spiking episodes were unstable across weeks. Moreover, within the same neuron, firing rates of different spiking episodes varied in distinct temporal patterns across weeks. By fitting a linear model, we found that episodic activity was the basic unit of the week-to-week fluctuation. Importantly, despite the unstable episodic activity, we extracted a low-dimensional stable representation of time in the natural movie from neuronal population activity across weeks. We propose that precise episodic activity with coordinated gain changes are keys to maintain a stable stimulus representation in V1.

Single neuron responses to natural movies are unstable across weeks. To investigate the long-term variability of cortical responses, we used a dataset that consisted of chronic GCaMP6s imaging of excitatory neurons in V1 L2/3 of awake, head-fixed mice (9 mice; 10 imaging fields) during visual stimulation with repeated natural movies (30 trials per session; one session per 7 ± 1 days; over 5–7 weeks) (Fig. 1a). Single neuron responses varied in a largely stochastic manner across trials within a recording session (week) as described before, and, importantly, varied in a qualitatively different manner across weeks (Fig. 1b). We quantified this response variation across weeks in terms of the “similarity”, defined as the correlation coefficient between trial-averaged neural responses (within a week) for a given neuron between pairs of weeks and averaged across all neurons. Similarity largely decreased over time using the first week of recording as the reference (Fig. 1c). Specifically, the similarities of the fifth week were significantly lower than the similarities of the second week (one-sided Wilcoxon signed-rank test, p = 0.0035, ten imaging fields). In a complementary analysis, to compare how single neuronal activity varied across weeks, we computed the difference of trial-averaged activity across weeks (Supplementary Fig. 1a). The change of trial-averaged ∆F/F across weeks was significantly higher than baseline variability within a week (Supplementary Fig. 1b). Thus, the long-term stability of neural responses to natural movies is not necessarily the same as that to drifting gratings. Indeed, data from our group showed that single neural responses to natural movies were significantly more unstable than drifting grating responses.

Single neuron responses consist of episodic activity with distinct episode-specific rate variations across weeks. The episodic nature of cortical neuron responses to naturalistic visual stimuli provides the unique opportunity to study neural variability with respect to episodic spiking. Neurons in the visual cortex are known to respond to naturalistic movies sparsely with temporally precise, but stochastic, spiking within a few well-timed “spiking episodes”. The change of single neuron spiking across weeks (Fig. 1c and Supplementary Fig. 1) dominated by changes in spike timing or by changes in spike counts? To address this question, we inferred spiking activity and defined spiking episodes (Fig. 2a; see “Methods”) based on peaks in the smoothed peristimulus time histogram (PSTH). Note that the inferred spiking activity might correspond to bursts of spikes instead of a single spike due to limitations of calcium imaging. A neuron usually possessed multiple spiking episodes and episodes from different neurons overlapped (Fig. 2b). To quantify the precision of spiking episodes across weeks, we computed the durations of spiking episodes (Fig. 2c). The right-skewed distribution of durations showed that most of the spiking episodes had short durations (median duration: 0.66 ± 0.17 s, ten imaging fields). Furthermore, compared with spiking episodes defined based on PSTH within weeks, the median of durations of spiking episodes defined based on PSTH across weeks only increased by at most 2 time steps (0.2 s) for each imaging field (Fig. 2c, median duration of spiking episodes based on PSTH within weeks: 0.59 ± 0.07 s, ten imaging fields). The short durations of spiking episodes and a small increase compared with data within weeks indicated that episodic activity had rather precise and stable timing across weeks. In contrast, the inferred spike rates during those spiking episodes changed more from trial-to-trial across weeks than that within each week (Fig. 2d). Importantly, inferred spike rates during each episode for a given neuron varied in different patterns from week to week (Fig. 2e). The diverse inferred spike rate variation for different spiking episodes raised the question whether inferred spike rates during spiking episodes within the same neuron change independently across weeks. We quantified the similarity between inferred spike rate variability...
population activity. Specifically, we organized neuronal responses into a three-dimensional tensor (neuron × time × trials) and decomposed this tensor into R components, each consisting of a neuron factor, a temporal factor, and a trial factor (Fig. 3a). Thus, TCA achieves a simultaneous, interlocked dimensionality reduction across neurons, time, and trials. For each component, (i) the neuron factor indicates how the component is shared across neurons, (ii) the temporal factor reflects the component’s temporal profile on every trial, and (iii) the trial factor enumerates how the component’s gain changes across trials. Within this framework, a neuronal response can be approximated by the reconstructed response, which is a linear combination of these TCA components (Fig. 3b). As TCA components mainly capture correlated activity across neurons or trials, the reconstructed responses from TCA components can be viewed as denoised responses, i.e., the responses from which independent noise has been removed.

Within this unsupervised TCA dimensionality reduction method (Fig. 3c), the pronounced peaks in the temporal factors (Fig. 3c, center) revealed shared episodic activity across neurons (Fig. 2a, b). Importantly, the distribution of the temporal factors across all 40 TCA components (Fig. 3c, center) revealed the scattering of the episodic activity across the duration of a trial (Fig. 2b). For a given TCA component (of the chosen R = 40 components), the neurons with a high neuron factor value (Fig. 3c, left) had episodic activity timed near the peak in the temporal factor (Fig. 3c, center). Any given neuron tended to display high neuron factor values in multiple components (Fig. 3c, left), thus reflecting the occurrence of multiple activity episodes for any one neuron (Fig. 2a). The co-activation of neurons within a given component (i.e., multiple neurons with a high neuron factor; Fig. 3c, left) revealed the temporal overlap between episodic activity from different neurons (Fig. 2b). Further, the

Latent factors resembling episodic activity with gain changes capture the across-week fluctuations. To identify the basic unit of the week-to-week fluctuation in an unbiased fashion, we switched from single-neuron analysis (Figs. 1, 2) to population analysis (Fig. 3 and Supplementary Figs. 2, 3), thus including the potential impact of coordinated activity. We decomposed population activity into latent factors that can have independent gain changes across trials. For this purpose, we chose the recently introduced tensor component analysis (TCA), which provides an unsupervised way to identify latent factors of the recorded population activity. Specifically, we organized neuronal responses during different spiking episodes as the mean correlation coefficient between mean inferred spike rate across weeks (Fig. 2e). For most neurons, the similarity of inferred spike rate changing patterns across spiking episodes was low, although significantly higher than the chance level (Fig. 2f, one-sided Mann–Whitney U test, $p = 2.45 \times 10^{-42}$, 1404 neurons with more than 1 spiking episodes). This means that different spiking episodes within the same neuron have different, but not completely independent, inferred spike rate variations across weeks. Moreover, the similarity between inferred spike rate changing patterns was significantly lower than that expected from i.i.d. Poisson statistics (Fig. 2f, one-sided Mann–Whitney U test, $p = 1.03 \times 10^{-143}$, 1404 neurons with more than 1 spiking episodes). Consequently, assuming spike trains of all the trials were independent Poisson spike trains, the inferred spike rates of distinct spiking episodes within the same neuron followed significantly different variations across weeks. The difference in inferred spike rate changing patterns of spiking episodes within the same neuron suggests that the basic unit of the week-to-week fluctuation is the spiking episode instead of neuron.

Fig. 1 Single neuron responses to natural movies are unstable across weeks. a Experimental setup. We performed chronic calcium imaging of excitatory neurons in the primary visual cortex of awake, head-fixed mice during visual stimulation with repeated natural movies. The visual cortex (contralateral to visual stimulus delivery) is retinotopically mapped in Emx1-Cre::TITL-GCaMP6s mice. V1 fields are chosen from the region selective for the center of the presentation screen. Widefield scale bar = 1 mm; two-photon scale bar = 100 μm. The average activity of four example well-tracked neurons across weeks are shown in the bottom panel. b ΔF/F responses of one example neuron during the same natural movie clip for 30 trials per experimental session for 6 weeks (movie starts at 5 s and lasts for 30 s duration). We recorded one experimental session per week. c Similarity (correlation coefficient between trial-averaged ΔF/F) averaged over neurons during week 1 and that during other weeks are plotted for all the recorded imaging fields. Different imaging fields are denoted by different colors. The black curve with the error bar denotes the mean and standard deviation of similarity over imaging fields. Ten imaging fields have recordings for weeks 1–5. Only a subset of imaging fields has recordings on week 6 (seven fields) and week 7 (five fields). Specifically, the similarities of the fifth week were significantly lower than the similarities of the second week (one-sided Wilcoxon signed-rank test, $p = 0.0035$, ten imaging fields).
diverse variation of the trial factor values (Fig. 3c, right) reflected the diverse gain variability of episodic activity (Fig. 2d), even for any given neuron (Fig. 2e, f).

In summary, the TCA dimensionality reduction confirmed in an unsupervised manner the episodic activity of single neurons (Fig. 2a), the temporal overlap of episodic activity from different neurons (Fig. 2b), and the diversity of week-to-week fluctuations of episodic activity within a given neuron (Fig. 2d–f). In conclusion, the results from the TCA analysis (Fig. 3c) support the hypothesis that cortical coordination resides at the level of episodic activity, rather than at the level of neurons, as is commonly assumed36.

Visual inspection of the trial factors across weeks indicated vastly diverse dynamics across weeks for different components. To illustrate this diversity of dynamics, we sorted the components by their trial factors using K-means clustering, choosing 5 or 6 clusters (Fig. 3c and Supplementary Fig. 3). Within each thus determined cluster, we further ordered the components by the time to peak in their temporal factors. This reorganization of the TCA analysis display revealed two important insights. First, trial
attempt to preserve the manifold topology of neuronal population recorded neurons, the denoised instantaneous population (see "the unsupervised algorithmic approach, Isomap, for the mapping the stability of the activity on this manifold (Fig. 4). For N activity onto a low-dimensional space (manifold) and investigated tion responses (reconstructed responses; Fig. 3b) of episodic neural manifold using dimensionality reduction. summarized by TCA components in the previous section (Fig.3). As expected from the interconnected nature of cortical circuits, we observed population-wide correlated neural fl.

Stable manifolds exist in unstable population activity. As factors changed in a distinctly different manner across weeks for different clusters of components. For instance, while the trial factors for the first cluster of components were largely homogeneous across weeks, the trial factors for the second cluster largely faded away after the second week. Of functional significance, with such vanishing trial factors, the second cluster of components would contribute little to a stimulus representa- tion in week 4 and beyond. We observed such diverse dynamics of trial factors across weeks for all imaging fields studied (Fig. 3d, e and Supplementary Fig. 3). Second, within each cluster of components, the pronounced peaks in the temporal factors were largely evenly distributed across the duration of the trial. Assuming that the peaks in the temporal factors (or equivalently the spiking episodes; see Fig. 2) contribute to cortical stimulus representation, the even distribution of these peaks suggests that every moment in the movie was evenly represented, however by different groups of neurons at different weeks. In conclusion, the diverse dynamics of trial factors across weeks for different components indicates a fluid long-term stimulus representation in visual cortex. Importantly, the fluid stimulus representation was structured at the level of episodic activity rather than the neuron.

The manifold mediates a stable representation of the time within the movie clip. To extract the stimulus representation potentially encoded in the stable neural manifold, we applied spline parameterization for unsupervised decoding (SPUD) to population activity embedded in the first few Isomap dimensions. Here we only showed results for the first two Isomap dimensions for visualization, but the following results also hold for up to the first five dimensions (Supplementary Fig. 5a). The decoding process consisted of the following steps (Fig. 5a). First, we randomly split the two-dimensional neural manifold into a training set (80%) and a test set (20%). Second, we fit a one-dimensional spline to the training set, and then assigned coordinates to the fitted spline. Third, we assigned each dot in the test set a value according to the coordinate of its nearest point on the spline. Last, we circularly shifted or flipped the coordinates on the spline such that we achieved the best decoding performance (circular least...
mean squared error) for time in the movie of the test set. We did this because when we assigned coordinates to the spline, the origin and direction of the coordinates were arbitrarily determined. To match the assigned coordinates with the actual time, we need to determine the origin and direction of coordinates using the test set. The decoded time $\alpha$ closely traced the actual time $t$ in the movie for population activity across weeks (Fig. 5b). We summarized the decoding error (circular absolute difference between $t$ and $\alpha$) from all the recorded imaging fields (Fig. 5c). As a comparison, the decoding error of SPUD was significantly lower than the decoding error from that of linear decoders (Supplementary Fig. 5b). In general, the decoding performance improved with an increasing number of recorded neurons in the imaging field (Fig. 5c). To further investigate the stability of neural representation of time in the trial, we also trained SPUD on neural data from odd trials in week 1 and tested its performance on neural data from even trials in week 1 and trials from other weeks. The decoding errors pooled from later weeks were not significantly different from the decoding errors for week 1 across imaging fields ($p = 0.11$, two-sided Mann–Whitney U test; Supplementary Fig. 5c, d). This additional analysis showed that week-to-week variability does not affect the coding of time in the movie.
of each component, and trial factors describe the modulation of the component across trials. We ordered components according to the K-means clustering on their trial factors. Within each cluster, we further ordered the components by the time to peak in their temporal factors. We ordered neurons in the neuron factors by their dominant components. Correlation coefficient (CC) between trial factors shown in (c). CC between neuron factors averaged across trial pairs within week plotted against CC between trial factors averaged across trial pairs across weeks. Different colors denote different imaging fields. The week-to-week variability of trial factors was significantly larger than the corresponding trial-to-trial variability within each week (for avg CC, one-sided Wilcoxon signed-rank test, p = 0.0025, ten imaging fields).

One of the key reasons for the high decoding performance of population activity as analyzed by SPUD resides in the isometric representation\(^3\). Time in the movie was evenly represented along the fitted spline direction. In other words, equal amounts of population activity variations along the spline direction contributed to equal amounts of change across time in the trial. This isometric representation was related to the evenly distributed episodic activity across time in the trial, as shown by TCA components (Fig. 3c). If we removed episodic activity during a certain time window in the trial from the population activity, then the corresponding section in the manifold ring would collapse into the hyperplane perpendicular to the spline direction (Supplementary Fig. 6).

Due to the high trial-to-trial variability in population activity, the ring-shaped neural manifold had many outlier dots. The outlier dots in the center of the ring corresponded to low amplitude of population activity, while outlier dots on the outside of the ring corresponded to high amplitude of population activity (Supplementary Fig. 4b). The decoder failed at a few outlier dots. However, most of the neural variability seemed to be perpendicular to the direction of the fitted spline, thus, harmless to decoding. This observation gave us a hint about the mechanism that maintains stable neural correlates in the face of dynamical population activity.

Both week-to-week fluctuation and trial-to-trial variation within the week is restricted to non-coding directions. In order to quantify to what extent neural variability influences the stimulus coding, we calculated the variance of instantaneous population activity on the manifold along the direction parallel or perpendicular to the fitted spline. Specifically, we computed the parallel and perpendicular component of the instantaneous population activity variance employing the following steps. First, we reconstructed \(\Delta F/F\) population activity based on 40 TCA components (Fig. 6a). Second, we used Isomap to project the population activity of all trials into a two-dimensional space. Third, as illustrated in Fig. 5, we separated the projected instantaneous population activity into a training set and a test set. Fourth, we calculated the fitted spline to the training set. Fifth, we computed the coordinates on the spline, based on the test set data (Fig. 6b, left panel). Sixth, for each time point in the movie, we calculated the variance of instantaneous population activity in the test set along the direction parallel or perpendicular to the spline (Fig. 6b, right panel). Finally, we summarized the variance for all the time points in the movie (Fig. 6c). The variance of population activity along the spline direction was significantly smaller than that perpendicular to the spline direction. This observation held for eight out of ten imaging fields (Fig. 6d). In this computational framework, the spline direction signifies the stimulus coding direction. In conclusion, the comparatively small contribution of neural variability to stimulus encoding direction directly explains why the high neural variability we observed in spiking episodes (Fig. 2) did not harm the decoding performance of SPUD (Fig. 5).
direction? To answer this question, we quantified week-to-week variability and trial-to-trial variability within each week separately. For week-to-week fluctuations, first, we calculated the trial-averaged projected population activity in the two-dimensional space for each week (Fig. 6e). Second, we calculated the variance of those trial-averaged instantaneous population activity across weeks along the direction parallel or perpendicular to the spline. Finally, we summarized the variance for all the time points in the movie (Fig. 6f, left panel). The significantly larger week-to-week variance along the direction perpendicular to the spline compared with that of parallel direction suggested that the week-to-week fluctuation was also constrained to the non-coding direction. For trial-to-trial variability within each week, first, we calculated the variance of single-trial population activity for each week separately. Second, we summarized the variance for all the weeks and all the time points in the movie (Fig. 6f, right panel). The trial-to-trial variability within each week was larger along the direction perpendicular to the spline compared with that of parallel direction. Furthermore, the same observation held for most imaging fields (Fig. 6g). In conclusion, both week-to-week fluctuations and trial-to-trial variability within each week were restricted to the non-coding direction.

The precisely timed episodic activity constrains neural variability to non-coding directions. How is neural variability largely constrained to the direction perpendicular to stimulus coding direction? Is it caused by the reproducible timing of episodic activity, by the coordination between different episodes, or by the combination thereof? To answer these questions, we applied the previous analyses to shuffled reconstructed $\Delta F/F$ population activity.

First, we checked whether the neural manifold was an artifact of the method by applying Isomap to shuffled data. To remove both the reproducible timing of episodic activity and the coordination of episodic activity across neurons in the shuffled data, we circularly time-shifted reconstructed $\Delta F/F$ responses by a random amount for every trial of each neuron independently (Fig. 7a). In other words, only the temporal statistics of $\Delta F/F$ responses were kept. As expected, neural trajectories from different trials were not aligned (Fig. 7b). However, trajectories were continuous instead of being a noisy point cloud. Such continuous trajectories arise from the smooth nature of shuffled reconstructed $\Delta F/F$ responses. This sanity check showed that the ring structure of the neural manifold (Fig. 6b) arose from the timing and coordination of the population activity and was not an artifact of the method.

Second, we checked whether the reproducible timing of episodic activity was sufficient to constrain the neural variability by applying Isomap to shuffled data with preserved trial structure. To merely remove the coordination between different episodes, but to maintain the amplitude of the covariance of neural activity, we chose to shuffle TCA factors instead of shuffling reconstructed population activity. In contrast, shuffling reconstructed population activity would decrease the covariance between neural activity.
Fig. 6 Both week-to-week fluctuation and trial-to-trial variation within the week is restricted to non-coding directions. a TCA components of one imaging field (n = 166). We ordered components by the time to peak in their temporal factors. We ordered neurons in the neuron factors by their dominant components. Colormap maximum values are set to 2 for all the factors. b Left: two-dimensional neural manifold extracted from reconstructed (denoised) ΔF/F population activity (n = 166) across weeks using Isomap. Each dot represents instantaneous population activity in the test set. The color of the dot (same colormap as Fig. 4a) indicates the corresponding time in the trial. The black line is the fitted spline to the training set. Right: zoom-in view on the neural manifold. Instantaneous population activity corresponding to 28 s in the trial was highlighted with black shade. The blue arrow denotes the direction perpendicular to the spline, and the red arrow denotes the direction parallel to the spline. c Histogram of the variance of population activity parallel or perpendicular to the spline for the imaging field shown in (a, b). d Median variance of population activity parallel to the spline plotted against median variance of population activity perpendicular to the spline for all the imaging fields. The variance of population activity parallel to the spline was significantly smaller than the variance perpendicular to the spline for most imaging fields (one-sided Wilcoxon signed-rank test, 350 timepoints, p-values for different imaging fields are 3.93 × 10−31 (n = 63), 2.61 × 10−55 (n = 84), 1.78 × 10−40 (n = 140), 2.61 × 10−58 (n = 150), 2.91 × 10−59 (n = 166), 7.37 × 10−57 (n = 185), 2.06 × 10−59 (n = 293), 3.03 × 10−31 (n = 353), except for two imaging fields (p = 1.0 (gray, n = 49) and p = 0.997 (brown, n = 88)). The two outliers are from two imaging fields whose neural manifold didn’t have a clear ring shape (Supplementary Fig. 5a). e The same zoom-in view on the neural manifold as shown in (b) (right). Each triangle represents instantaneous population activity within a week. The color of the triangle denotes different weeks. Each cross represents the trial-averaged instantaneous population activity within a week. The color of the cross also denotes different weeks. f Left: histogram of the variance of trial-averaged population activity within a week parallel or perpendicular to the spline for the imaging field. Right: histogram of the variance of single-trial population activity within a week parallel or perpendicular to the spline for the imaging field. g Median variance of trial-averaged population activity or single-trial population activity within a week parallel to the spline plotted against median variance of population activity perpendicular to the spline for all the imaging fields. Y-axis is clipped at 9 for visualization. The variance of population activity parallel to the spline was significantly smaller than the variance perpendicular to the spline for both across weeks and within a week cases for most imaging fields (across weeks: one-sided Wilcoxon signed-rank test, 350 timepoints, p-values for different imaging fields are 1.45 × 10−27 (n = 63), 1.41 × 10−39 (n = 84), 1.55 × 10−33 (n = 140), 5.13 × 10−57 (n = 150), 8.50 × 10−58 (n = 166), 1.29 × 10−54 (n = 185), 1.26 × 10−57 (n = 293), 1.25 × 10−29 (n = 353), two outliers p = 1.0 (gray, n = 49), p = 0.99 (brown, n = 88); within a week: one-sided Wilcoxon signed-rank test, number of samples = 350 timepoints × number of weeks, p-values for different imaging fields are 7.36 × 10−97 (n = 63), 1.04 × 10−219 (n = 84), 1.53 × 10−177 (n = 140), 6.13 × 10−171 (n = 150), 6.23 × 10−273 (n = 166), 0.0 (n = 185), 0.0 (n = 293), 2.99 × 10−120 (n = 353), two outliers p = 1.0 (gray, n = 49), p = 1.0 (brown, n = 88)).

Activity across neurons, in addition to removing the coordination between episodic activity. For each TCA component, we randomly shuffled the neuron order in the neuron factor, and we circularly shifted the temporal factor and the trial factor by a random amount (Fig. 7c). Thus, by shuffling the factors for each component independently, we removed all the significant coordination between episodic activity. As expected, the removal of coordination between episodic activity resulted in a new manifold and a new spline (Fig. 7d). However, the variability of reconstructed population activity (based on shuffled TCA factors)
continued to be largely constrained to the direction perpendicular to the spline (Fig. 7d). The smaller variability of population activity parallel to the spline is visible in the separation of dots of different colors, where color indicates the time in the trial of the instantaneous population activity (Fig. 7d). Indeed, the quantification of variance showed that the amplitude of neural variability along the spline was significantly smaller than that perpendicular to the spline (Fig. 7e). Moreover, the significant difference between variance along the direction parallel and perpendicular to the spline held for shuffled data with preserved trial structure from all the imaging fields (Fig. 7f). In conclusion, the fact that episodic activity is precise in time across trials (Fig. 2c) alone is sufficient for constraining neural variability to the direction perpendicular to the stimulus encoding direction. In contrast, the coordination among episodic activity plays no role in this constraint.

However, coordination between episodic activity is essential for uniquely representing time points in the trial. The neural manifold of shuffled data with preserved trial structure had a collapsed ring structure (Fig. 7d and Supplementary Fig. 7a) in contrast to the clear ring structure from original data (Figs. 5a, 6b). The collapsed ring structure would lead to ambiguous decoding due to the overlap between instantaneous population activity from different time points in the trial (Supplementary Fig. 7b).
We quantified the shape of the neuronal manifold for original and shuffled data with preserved trial structure by calculating the distance from each dot representing instantaneous population activity to the center of the manifold (see "Methods"). For nine out of ten imaging fields, the neuronal manifold of shuffled data with preserved trial structure had a more collapsed ring structure than the manifold of original data, as shown by a significantly smaller radius (Fig. 7g). The collapsed ring structure of shuffled data showed that stable representation of the time in the natural movie requires not only that some neurons display reliable responses over sessions (shuffled data also have neurons with reliable responses), but also coordination between episodic activity.

In summary, both the nature of the precise episodic activity and the coordination between different activity episodes contributes to encode time in the natural movie. However, episodic activity reproducible in time alone is sufficient for restricting neural variability to non-coding directions.

Discussion

We showed that single neuronal responses to the natural movie in V1 consisted of episodic activity with variability in gain across weeks. Importantly, we found a stable low-dimensional subspace inside the highly variable high-dimensional neural space. Time in the movie was represented on a one-dimensional ring manifold isometrically, where equivalent changes on the ring indicated equivalent changes in time. Moreover, we found that the limited influence of neural variability and week-to-week fluctuations on the stable representation of the natural movie was mediated by the fact that most of the neural variability was constrained in the non-coding direction, augmenting the previous literature on population coding and neural variability.40–42 Furthermore, we found that stable episodic activity was sufficient for restricting neural variability to non-coding directions independent of coordination between episodic activity.

To study the neural representation in V1, it is common practice in the field to measure tuning curves (trial-averaged single-neuron activity) with respect to external variables or decode external variables from neuronal activity with supervised methods, such as linear decoders.11,46,47 In contrast, recent work introduced unsupervised methods in revealing the internal representation using neural data alone without reference to external variables.39,48 Here, we identified an internal representation of time in the natural movie by parameterizing the neural manifold, without using any external information or prior assumptions.

There are several advantages in the dissociation of internal and external variables. First, such dissociation avoids the biases introduced by the chosen external variables. One caveat of interpreting the neural activity through the lens of the chosen external variable is that the encoded variable might be different but correlated with the chosen external variable. Thus, non-trivial tuning curves or supervised decoding results do not necessarily reveal the actual neural representation. Second, dissociation of internal and external variables permits discovering representation of cognitive variables. It is possible that the internal variable represents the animal’s inference about an external variable. For example, as hypothesized by the sampling-based neural variability theory,49,50 neural variability in V1 might represent the perceptual uncertainty of certain visual features. In the future, it will be interesting to investigate whether the thickness of the ring manifold (Fig. 6) reflects the animal's perceptual uncertainty of certain scenes in the movie.

Even though population activity may never visit the same state in the high dimensional space, there exists a stable readout direction as indicated by the fitted spline (Fig. 5). The liquid state machine (LSM), a computational paradigm for recurrent neural networks, describes a similar situation. Instead of viewing neural networks as “feature detectors”, LSM views the network as liquid, continuously receiving external perturbations. Although the liquid neural trajectory keeps changing across time, we can get a stable readout by training a linear readout unit. Note that our work is different from LSM in the readout method, as we obtained stable readout in an unsupervised manner. LSM suggests that trial-to-trial variability reflects an accumulation of information instead of noise, as recurrent network activity implicitly contains the previous external perturbations. This recurrent network perspective can be instructive for our future work. In our work, we found that trial-to-trial variability is mostly constrained in the direction perpendicular to the spline direction (Fig. 6). However, we did not interpret the latent variables encoded in other directions except for the spline direction. Moreover, recent
works suggest that V1 encodes various behavior and state variables besides visual-related variables. A new experimental design with behavior or state recordings might provide a more complete picture of internal representation in V1.

The low-dimensional internal representation offered us a better reference point to understand neural dynamics than the high-dimensional population activity. As a promising future direction, it would be informative to study neural dynamics on or off the manifold with perturbations. One way ofperturbation is to modulate the visual stimulus and observe whether the neural trajectories first deviate from the ring manifold and then flow back. Another way of perturbation is to directly control neural activity with optogenetics. As suggested by the TCA analysis, episodic activity shared across neurons was the building block for the ring manifold (Fig. 3). It will be interesting to see how the optogenetically mediated changes ofspiking timing or amplitude of episodic activity impact population dynamics on or off the manifold. Previous studies suggest behavioral variables such as locomotion and arousal could lead to gain modulations in single neural activity. However, the reported modulations by behavioral variables were homogeneous across neurons, and whether changes in behavioral variables would contribute to the heterogeneous gain modulations across episodic activity within the same neuron is not clear. Furthermore, although we checked the limited impact of eye movement and pupil size on neuronal responses in an earlier work, how behavioral variables would affect the neural trajectories remains to be explored in the future.

At the neural circuit level, there are several possible mechanisms that could lead to the observed drift. First, the turnover of boutons and dendritic spines in V1 at the baseline condition would cause changes in the recurrent inputs to single neurons. Second, potential changes in the feedforward connectivity from LGN to V1 or drift in LGN responses would cause drift in the feedforward inputs to single neurons. Third, slowly varying top-down inputs related to visual information processing could contribute to the drift as well. Model investigations and simultaneous chronic recordings from LGN or high-order visual areas would be helpful for distinguishing contributions from these potential mechanisms in the future.

Methods

Animals. For imaging visual cortical responses, a Emx1-Cre (Jax Stock #005628) x ROSA-LNL-TTA (Jax Stock #011008) x TtTL-GCaMP6s (Jax Stock #24104) triple transgenic mouse line (n = 9) was bred to express GCaMP6s in cortical excitatory neurons. Mice ranging in age from 6 to 20 weeks of both sexes (four males and five females) were implanted with a head plate and cranial window and imaged starting >2 weeks after recovery from surgical procedures and up to 10 months after window implantation. The animals were housed on a 12 h light/dark cycle in cages of up to five animals before the implants, and individually after the implants. All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of California, Santa Barbara.

Surgical procedures. All surgeries were conducted under isoflurane anesthesia (3.5% induction, 1.5–2.5% maintenance). Prior to incision, the scalp was infiltrated with lidocaine (5 mg/kg subcutaneous) for analgesia and meloxicam (1 mg/kg subcutaneous) was administered prophylactically to reduce inflammation. The full surgical procedure and equipment is available in the Supplementary Information. The information hardware can be found on the Goard lab website (https://goard.mcdb.ucsb.edu/resources).

Note that we performed glass prism implant surgeries on two of the mice to record from L2–5 neurons in V1. In this work, we only performed analysis on L2/3 neurons.

Two-photon imaging. After >2 weeks’ recovery from surgery, GCaMP6s fluorescence was imaged using a Prairie Investigator two-photon microscopy system with a resonant galvo scanning module (Bruker). Prior to two-photon imaging, epifluorescence imaging was used to identify the visual area being imaged by aligning to areal markers measured with widefield imaging. For fluorescence excitation, we used a Ti:Sapphire laser (Mai-Tai eHP, Newport) with dispersion compensation (Deep Sea, Newport) tuned to λ = 920 nm. For collection, we used GaAsP photomultiplier tubes (Hamamatsu). We used a 16×/0.8 NA microscope objective (Nikon) at 1× or 2× magnification, obtaining a square field of view with a width ranging from 414 to 828 μm. Laser power ranged from 40 to 75 mW at the sample depending on GCaMP6s expression levels. Photobleaching was minimal (<1%/min) for all laser powers used. A custom stainless-steel light blocker (https://goard.mcdb.ucsb.edu/resources) was mounted to the head plate and interlocked with a tube around the objective to prevent light from the visual stimulus monitor from reaching the PMTs. During imaging experiments, the polypropylene tube supporting the mouse was suspended from the behavior platform with high tension springs to reduce movement artifacts.

For imaging across multiple weeks, imaging fields on a given recording session were manually aligned based on visual inspection of the average map from the reference session recordings generated by stable structural landmarks such as blood vessels and neurons with high baseline fluorescence. Physical controls were used to ensure precise placement of the head plate and the visual stimulus screen relative to the animal, and data acquisition settings were kept consistent across sessions. Recordings were taken once every 7 ± 1 days for 5–7 weeks. To acclimate to head fixation and visual stimulus presentation, mice were head-fixed and presented the full series of visual stimuli for 1 to 2 full sessions prior to the start of their experimental run.

Two-photon post-processing. Images were acquired using PrairieView acquisition software and converted into TIF files. All subsequent analyses were performed in MATLAB (Mathworks) using custom code (https://goard.mcdb.ucsb.edu/resources). For Y Z volumes, a frame-by-frame projection by registration to a reference image (the pixel-wise mean of all frames) using two-dimensional cross-correlation. Next, to align recordings to the reference session, we used a semi-automated method similar to prior work. First, anchor points were automatically generated from matching image features between average projections detected by the ‘Speeded-Up Robust Features’ (SURF) algorithm (Computer Vision Toolbox, Mathworks), and were manually corrected and added through visual inspection when necessary. These anchor points defined a predicted displacement vector field that would be used to map coordinates from one session to the other. For each coordinate, the predicted vector was defined by the average (weighted inversely by distance) of the vectors for all defined anchor points. This vector field was then applied to every frame of the recording to warp the coordinates of each image to the reference coordinate plane.

To identify responsive neural somata, a pixel-wise activity map was calculated using a modified kurtosis measure. Neuron cell bodies were identified using local adaptive threshold and iterative segmentation. Automatically defined ROIs were then manually checked for proper segmentation in a graphical user interface (allowing comparison to raw fluorescence and activity map images). To ensure that the response of individual neurons was not due to local neuropil contamination of somatic signals, a corrected fluorescence measure was estimated according to:

\[ F_{\text{corrected}}(t) = F_{\text{norm}}(t) - \alpha \cdot (F_{\text{norm}}(t) - F_{\text{unnorm}}) \]

where \( F_{\text{unnorm}} \) was defined as the fluorescence in the region <30 μm from the ROI border (excluding other ROIs) for frame n (see Supplementary Fig. 8 for example neuropil signal traces). \( F_{\text{norm}} \) is \( F_{\text{unnorm}} \) averaged over frames. \( \alpha \) was chosen from [0, 1] to minimize the Pearson’s correlation coefficient between \( F_{\text{corrected}} \) and \( F_{\text{unnorm}} \). Empirically, \( \alpha \) is typically close to 1 and does not change significantly across weeks. The ΔF/ΔF for each neuron was then calculated as:

\[ \Delta F/\Delta F = (F_n - F_0)/F_0 \]

Where \( F_0 \) is the corrected fluorescence \( F_{\text{corrected}} \) for frame n and \( F_0 \) is defined as the mean of the corrected fluorescence density distribution across the entire time series.

To minimize potential artifacts introduced by misalignments of the imaging field across sessions, we manually inspected the average projection and pixel-wise activity maps underlying every defined ROI across all sessions. We assigned each ROI a quality rating as follows: ROIs rated a quality of 4 or 5 were cells that were clearly present across sessions, and the cell...
structure could be clearly resolved in both the average projection and activity map. ROIs rated a quality of 3 were also cells unambiguously tracked across sessions but had average maps that were often noisier than rated 4 or 5 (for example, they may be identifiable solely by their appearance on the activity map). ROIs rated a quality of 2 were either cells that were not well-tracked or were not unequivocally neuronal somata. ROIs rated a quality of 1 were cells that were not present on the reference session. Each ROI was also marked as either present or not present on each session. For analysis, we only included ROIs which were presented on all the sessions and with a quality larger than 3.

Visual stimuli. All visual stimuli were generated with a Windows PC using MATLAB and the Psychophysics toolbox. Stimuli used for two-photon imaging were presented on an LCD monitor (17.5 × 13 cm, 800 x 600 pixels, 60 Hz refresh rate) positioned 5 cm from the eye at a horizontal tilt of 30° to the right of the midline and vertical tilt of 18° downward, spanning 120° (azimuth) by 100° (elevation) of visual space in the right eye.

For natural movie visual stimulation, we displayed a grayscale 30 c/deg from Touch of Evil (Orson Welles, Universal Pictures, 1958) containing a continuous visual scene with no cuts (https://observatory.brain-map.org/visualcoding/stimulus/natural_movies). The clip was contrast-normalized and presented at 30 frames per second. We presented 30 repeats of the natural movie stimulus; each repeat started with 5 s of gray screen, followed by the 30 s of movie.

Spiking episodes. We first calculated deconvolved traces from ΔF/F using Suite-2p toolbox. For every neuron, we binarized the deconvolved trace by thresholding at 3 standard deviation above 0 to get inferred spikes. To calculate peristimulus time histogram (PSTH) for a given neuron, we first summed the inferred spikes across trials and smoothed them using Bayesian adaptive regression splines. The spiking episode in each neuron was defined in the following steps. First, we found peaks with a prominence larger than 3 in the smoothed PSTH. Second, the full width at half maximum (FWHM) of the peaks defined the duration of spiking episodes in most cases. When the FWHM of neighboring peaks overlapped, the duration was defined as the difference between the start of the first peak and the end of the last peak.

Nonnegative tensor decomposition with missing data. We organized our data into a three-way tensor \( \mathcal{X}(N \times T \times K) \) and let \( x_{ntk} \) represent the activity of neuron \( n \) at time \( t \) and trial \( k \). Nonnegative tensor decomposition decomposes \( \mathcal{X} \) into a sum of \( R \) rank-one tensors, where each rank-one tensor can be written as an outer product of three nonnegative vectors:

\[
    x_{ntk} \approx \sum_{r=1}^{R} \sum_{n=1}^{N} \sum_{t=1}^{T} \sum_{k=1}^{K} x_{ntr} v_{ntr}^T \chi_{ntr} \chi_{ntr}^T \chi_{ntr}^T
\]

Nonnegative TCA with missing values was fit to minimize the squared Frobenius norm of a tensor:

\[
    ||\mathcal{X} \otimes (\chi - \tilde{\chi})||_F^2 \quad \text{while} \quad W \geq 0, B \geq 0, A \geq 0
\]

Here, \( \chi \) denotes the reconstructed data, \( || \cdot ||_F \) denotes the squared Frobenius norm of a tensor:

\[
    ||\mathcal{Y}||_F^2 = \sum_{n=1}^{N} \sum_{t=1}^{T} \sum_{k=1}^{K} \chi_{ntr}^T \mathcal{Y}_{ntr} \mathcal{Y}_{ntr}^T \chi_{ntr}^T \chi_{ntr}^T
\]

\[
    ||\mathcal{Y}||_F^2 = \left\| \sum_{n=1}^{N} \sum_{t=1}^{T} \sum_{k=1}^{K} x_{ntr} v_{ntr}^T \chi_{ntr} \chi_{ntr}^T \chi_{ntr}^T \right\|_F^2
\]

\[
    \chi_{ntr} = \arg \min \left\{ ||\mathcal{X} \otimes (\chi - \tilde{\chi})||_F^2 \quad \text{subject to} \quad x_{ntk} \geq 0, \chi_{ntr} \geq 0 \right\}
\]

Nonnegative TCA model would overfit the training set. As previously reported, TCA is fit to optimize. To monitor this possibility, we calculated similarity between different TCA-reconstructed \( \chi_{ntr} \)'s. We chose 100 nearest neighbors for Isomap for shuffling results on the same dataset as described in. We found that the top K eigenvectors of the geodesic distance matrix represent the coordinates (Isomap dimensions) in the new k-dimensional Euclidean space.

With all 63,000 data points successfully mapped into a state-space of 63 dimensions, we recalled the assignment of each point to a given trial and to the time within the trial. This temporal sequence of data points formed the trajectory of population activity for a given trial in this low-dimensional space.

Shuffled data with circularly shifted responses across trials have much higher intrinsically lower dimensions than original data. To due to the curse of dimensionality and the smoothness of shuffled responses, we need to define a larger neighborhood size for Isomap to reveal a robust topology of the neural manifold in this case. Thus, we chose 100 nearest neighbors for Isomap for shuffled data.

Spline parameterization for unsupervised decoding (SPUD). We used the SPUD algorithm described in. We fitted the manifolds with piecewise linear curves. We chose to fit a curve \( \mathcal{L}(y) \) with ten knots to the data points \( x_{ntk} \) embedded in the twodimensional spaces by Isomap. Initially, the positions of knots were determined by \( K \)-means clustering centroids of the data points. Each knot was connected to the other knot with the highest data point density in between to form the initial curve. Then, positions of the knots were iteratively optimized to minimize \( \sum_{i} \left( \mathcal{L}(y_{i}) - x_{i} \right)^2 \). The length of the Euclidean distance between the data point and the nearest point on the curve, and \( ||\mathcal{L}(y)||_F \) is the length of the curve. We picked a random origin on the curve and assigned coordinates from 0 to 1 to the point on the curve. The coordinate of each data point \( x_{ntk} \) was decoded as the coordinate of its nearest point on the curve. We shifted or flipped the coordinates of the data points to minimize the mean squared error between the decoded coordinates and the rescaled actual time in the movie (rescaled to (0,1)). The decoded time for a given data point was set to the resulting coordinate scaled up to (0, 35) seconds.

Preprocessing of \( \Delta F/F \) data. \( \Delta F/F \) data were normalized such that the averaged squared sum of \( \Delta F/F \) traces over time equals 1 to 1 for every neuron:

\[
    \sqrt{\sum_{t=1}^{T} \sum_{k=1}^{K} x_{ntk}^2} / TK = 1
\]

This normalization step is crucial for ensuring TCA fitting is not biased by high firing rate neurons, since TCA is optimized to minimize the squared reconstruction error.

Choice of the number of components in TCA. We picked the number of TCA components such that they captured a significant amount of neural responses without over-fitting, checked with cross-validation as previously reported. To perform cross-validation, we randomly masked out 50% of tensor entries in \( \chi \). The remaining data was a training set and the masked-out data was a test set. We trained nonnegative TCA with missing values to fit the training set. And then we used the trained TCA model to fit the test set. As we increase the number of components in TCA, if the normal reconstruction error of the test set went up, the TCA model would overfit the training set. As previously reported, TCA is unlikely to overfit, even with up to 70 components. For this paper, we chose 40 components for TCA, given that 40 component TCA captured a significant amount of neural responses without over-fitting (Supplementary Fig. 2).

Isomap. The instantaneous (temporal frequency: 10 Hz) population response \( \Delta F/F \) of N recorded neurons is a point in an N-dimensional state space. Each axis in this state-space represents the activity of one neuron. A given trial of 35 s duration generates a discrete sequence (temporal frequency: 10 Hz) of 350 such points. The population activity from all trials (30 trials per recording session and six sessions) forms a cloud of 63,000 points in this N-dimensional state space. For the unsupervised transformation of the high-dimensional point cloud to a low-dimensional space, we ignored the association of a point to a given trial and to the time within the trial. We computed the Euclidean distance between all points, irrespective of the trial number and within-trial time. Based on the Euclidean distance we assigned 20 nearest neighbors to each point (choosing a higher number of nearest neighbors also works).

This step of nearest neighbor assignment is sensitive with respect to the existence of independent fluctuations of \( \Delta F/F \) responses (i.e., independent noise). To discover meaningful structure in the population activity, we removed such independent noise. Rather than working with \( \Delta F/F \) directly, we conducted the nearest neighbor assignment based on the "TCA-reconstructed \( \Delta F/F \)," from which the independent noise was removed. By linking (edge) each point to its thus defined nearest neighbors, we translated the point cloud of population responses into a graph, i.e., a network of vertices (points) with edges (between a point and its nearest neighbor). The geodesic distance between two vertices in the graph is the distance of the shortest path connecting them. For our data set, the graph was described by the geodesic distance matrix of dimension 63,000 × 63,000.

Based on the pairwise geodesic distance between data points, we thus performed a transformation from the population responses in the N-dimensional space to a space of lower dimensions. This isometric mapping method ("Isomap") was chosen to incorporate the presumed (but a priori unknown) manifolds structure in the resulting transformation to a low-dimensional space. Isometric mapping preserves essential structure within the neuronal population responses. Note that the top K eigenvectors of the geodesic distance matrix represent the coordinates (Isomap dimensions) in the new k-dimensional Euclidean space.

With all 63,000 data points successfully mapped into a state-space of 63 dimensions, we recalled the assignment of each point to a given trial and to the time within the trial. This temporal sequence of data points formed the trajectory of population activity for a given trial in this low-dimensional space.

Shuffled data with circularly shifted responses across trials have much higher intrinsically lower dimensions than original data. Due to the curse of dimensionality and the smoothness of shuffled responses, we need to define a larger neighborhood size for Isomap to reveal a robust topology of the neural manifold in this case. Thus, we chose 100 nearest neighbors for Isomap for shuffled data.

The varience of population activity along/perpendicular to the coding direction. We calculated the variance of population activity along or perpendicular to the coding direction based on the coordinates of the projected instantaneous population activity onto the state-space. To be identified by SPUD. The variance of population activity reported in Figs. 6, 7 was calculated based on projected population activity in the first two Isomap dimensions.
Radius of points on the manifold. We quantified the shape of the neural manifold for original and shuffled data by calculating the distance from each dot representing instantaneous population activity to the center of the manifold. The Center of the manifold was calculated as averaged coordinates across all the points. Empirically, the center was close to the origin.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. Most of the hardware designs can be found on Michael Goard’s lab website (https://goard.mcdb.ucsb.edu/resources). Raw data analyzed in this study have been deposited in the Dryad https://doi.org/10.25394/D9M606.

Code availability. We used tools for fitting TCA in https://github.com/ahwillia/tensoertools. We used code available from https://betelab.mit.edu/code/ for SPUD. A sample dataset and a Jupyter notebook for reproducing some of the main figures are available from Supplementary Software. All the other code used for analysis is available upon request to the corresponding author.

Received: 11 December 2020; Accepted: 11 August 2021; Published online: 27 August 2021

References
1. Soltwy, W. R. & Koch, C. The highly irregular firing of cortical cells is inconsistent with temporal integration of random EPSPs. J. Neurosci. 13, 334–350 (1993).
2. Tomko, G. J. & Crapper, D. R. Neuronal variability: non-stationary responses to identical visual stimuli. Brain Res. 79, 405–418 (1974).
3. Xia, J., Marks, T. D., Goard, M. J. & Wessel, R. Diverse co-active neurons encode stimulus-driven and stimulus-independent variables. J. Neurophysiol. https://doi.org/10.1152/jn.00431.2020 (2020).
4. Wright, N. C., Hoseini, M. S. & Wessel, R. Adaptation modulates correlated subthreshold response variability in visual cortex. J. Neurophysiol. 118, 1257–1269 (2017).
5. Hoseini, M. S. et al. Dynamics and sources of response variability and its coordination in visual cortex. Vis. Neurosci. 36, E012 (2019).
6. Chambers, A. R. & Rumpel, S. A stable brain from unstable components: a stable brain from unstable components: an analysis of visual thalamocortical neurons. J. Neurophysiol. 98, 2657–2663 (2007).
7. Spacec, M. A. & Swindale, N. V. Cortical state and natural movie responses in cat visual cortex. Preprint at bioRxiv https://doi.org/10.1101/201401.970328 (2014).
8. Rule, M. E., O’Keefe, J., Latham, P. E. & Pouget, A. Neural correlations, population coding, and decorrelation in primary visual cortex during natural vision. Science 287, 1273–1276 (2000).
9. Baudot, P. et al. Animation of natural scene by virtual eye-movements evokes high precision and low noise in V1 neurons. Front. Neural Circuits 7, 206 (2013).
10. Oldhaufen, B. A. & Field, D. J. How close are we to understanding v1? Neural Comput. 17, 1665–1699 (2005).
11. David, S. V. Natural stimulus statistics alter the receptive field structure of V1 neurons. J. Neurosci. 24, 6991–7006 (2004).
12. Marks, T. & Goard, M. Stimulus-dependent representational drift in the primary visual cortex. Preprint at bioRxiv https://doi.org/10.1101/2020.12.09.420620 (2020).
13. Druckmann, S. & Chklovskii, D. B. Neuronal circuits underlying persistent representations despite time varying activity. Curr. Biol. 22, 2095–2103 (2012).
14. Cunningham, J. P. & Yu, B. M. Dimensionality reduction for large-scale neural recordings. Nat. Neurosci. 17, 1500–1509 (2014).
15. Gao, P. & Ganguli, S. On simplicity and complexity in the brave new world of large-scale neuroscience. Curr. Opin. Neurobiol. 32, 148–155 (2015).
16. Buracas, G. T., Zador, A. M., DeWeese, M. R. & Albright, T. D. Efficient discrimination of temporal patterns by motion-sensitive neurons in primate visual cortex. Neuron 20, 939–969 (1998).
17. Kumbhani, R. D., Nolt, M. J. & Palmer, L. A. Precision, reliability, and information-theoretic analysis of visual thalamocortical neurons. J. Neurophysiol. 82, 1099–1115.e8 (2008).
18. Spacec, M. A. & Swindale, N. V. Cortical state and natural movie responses in cat visual cortex. Preprint at bioRxiv https://doi.org/10.1101/201401.970328 (2014).
19. Herikstad, R., Baker, J., Lachaux, J.-P., Gray, C. M. & Yen, S.-C. Natural movies evoke spike trains with low spike time variability in cat primary visual cortex. J. Neurosci. 31, 15844–15860 (2011).
20. Pachitariu, M. et al. Suite2p beyond 10,000 neurons with standard two-photon microscopy. Preprint at bioRxiv https://doi.org/10.1101/201401.970328 (2014).
21. Ziv, Y. et al. Relationship between simultaneously recorded spiking activity and fluorescence signal in GCaMP transgenic mice. Elife 10, e51675 (2021).
22. Williams, A. H. & et al. Unsupervised discovery of demixed, low-dimensional neural dynamics across multiple timescales through tensor component analysis. Neuron 109, 1070–1115.e8 (2018).
23. Yuste, R. From the neuron doctrine to neural networks. Nat. Rev. Neurosci. 16, 487–497 (2015).
24. Lichtman, J. W., Pfister, H. & Shavit, N. The big data challenges of connectomics. Nat. Neurosci. 17, 1448–1544 (2014).
25. Tenenbaum, J. B. A global geometric framework for nonlinear dimensionality reduction. Science 290, 2319–2323 (2000).
26. Chauhduri, R., Gerck, B., Pandey, B., Peyrache, A. & Fiete, I. The intrinsic attractor manifold and population dynamics of a canonical cognitive circuit across waking and sleep. Proc. Natl Acad. Sci. USA 114, 1512–1520 (2019).
27. Averbeck, B. B., Latham, P. E. & Pouget, A. Neural correlations, population coding, and computation. Nat. Rev. Neurosci. 7, 358–366 (2006).
28. Zylberberg, J., Cafaro, J., Turner, M. H., Chase, S. M. & Kuhlman, S. J. Feature-selective circuits shape noise to ensure a precise population code. Neuron 89, 257–263 (2016).
29. Rumyantsev, O. I. et al. Fundamental bounds on the fidelity of sensory cortical coding. Nature 580, 100–105 (2020).
30. Hubel, D. H. & Wiesel, T. N. Receptive fields of single neurones in the cat’s striate cortex. J. Physiol. 148, 574–591 (1959).
31. O’Keefe, J. & Drostrovsky, J. The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. Brain Res. 34, 171–175 (1971).
32. Fyhn, M. Spatial representation in the entorhinal cortex. Science 305, 1258–1264 (2004).
33. Rule, M. E. et al. Stable task information from an unstable neural population. Neuron 89, e516210 (2021).
34. Stringer, C., Michaelos, M. & Pachitariu, M. High precision coding in visual cortex. Cell https://doi.org/10.1016/j.cell.2021.07.034 (2021).
35. Rubin, A. et al. Revealing neural correlates of behavior without behavioral measurements. Nat. Commun. 10, 4745 (2019).
36. Orbán, G., Berkes, P., Fiser, J. & Lengyel, M. Neural variability and sampling-based probabilistic representations in the visual cortex. Neuron 92, 530–543 (2016).
37. Berkes, P., Orbán, G., Lengyel, M. & Fiser, J. Spontaneous cortical activity reveals hallmarks of an optimal internal model of the environment. Science 331, 83–87 (2011).
38. Maas, W., Natschläger, T. & Markram, H. Real-time computing without stable states: a new framework for neural computation based on perturbations. Neural Comput. 24, 1447–1477 (2012).
39. Niell, C. M. & Stryker, M. P. Modulation of visual responses by behavioral state in mouse visual cortex. Neuron 65, 472–479 (2010).
40. Deichert, D., Rubin, A. & Ziv, Y. Representational drift in the mouse visual cortex. Preprint at bioRxiv https://doi.org/10.1101/2020.05.32.27049 (2020).
41. Vinje, W. E. & Gallant, J. L. Sparse coding and decorrelation in a multidimensional coding perspective. Curr. Opin. Neurobiol. 16, 2486–2498 (2016).
42. Montijn, J. S., Meijer, G. T., Lansink, C. S. & Pennartz, C. M. A. Population-level neural codes are robust to single-neuron variability from a multidimensional coding perspective. Curr. Opin. Neurobiol. 16, 2486–2498 (2016).
53. Stringer, C. et al. Spontaneous behaviors drive multidimensional, brainwide activity. *Science* **364**, 235 (2019).
54. Jazayeri, M. & Afraz, A. Navigating the neural space in search of the neural code. *Neuron* **93**, 1003–1014 (2017).
55. Saddler, P. T. et al. Neural constraints on learning. *Nature* **512**, 423–426 (2014).
56. Rikhye, R. V. & Sur, M. Spatial correlations in natural scenes modulate response reliability in mouse visual cortex. *J. Neurosci.* **35**, 14661–14680 (2015).
57. Ponce, C. R. et al. Evolving images for visual neurons using a deep generative network reveals coding principles and neuronal preferences. *Cell* **177**, 999–1009.e10 (2019).
58. Clawson, W. P., Wright, N. C., Wessel, R. & Shew, W. L. Adaptation towards scale-free dynamics improves cortical stimulus discrimination at the cost of reduced detection. *PLoS Comput. Biol.* **13**, e1005574 (2017).
59. Carrillo-Reid, L. & Yuste, R. Playing the piano with the cortex: role of neuronal ensembles and pattern completion in perception and behavior. *Curr. Opin. Neurobiol.* **64**, 89–95 (2020).
60. Carrillo-Reid, L., Han, S., Yang, W., Akrouh, A. & Yuste, R. Controlling visually guided behavior by holographic recalling of cortical ensembles. *Cell* **178**, 447–457.e5 (2019).
61. Marshel, J. H. et al. Cortical layer-specific critical dynamics triggering perception. *Science* **365**, eaaw5202 (2019).
62. Vinck, M., Batista-Brito, R., Knoblich, U. & Cardin, J. A. Arousal and locomotion make distinct contributions to cortical activity patterns and visual encoding. *Neuron* **86**, 740–754 (2015).
63. Lee, S., Park, J. & Smirnakis, S. M. Internal gain modulations, but not changes in stimulus contrast, preserve the neural code. *J. Neurosci.* **39**, 1671–1687 (2019).
64. Hofer, S. B., Mrsic-Flogel, T. D., Bonhoeffer, T. & Hubener, M. Experience leaves a lasting structural trace in cortical circuits. *Nature* **457**, 313–317 (2009).
65. Holtmaat, A. & Svoboda, K. Experience-dependent structural synaptic plasticity in the mammalian brain. *Nat. Rev. Neurosci.* **10**, 647–658 (2009).
66. Madisen, L. et al. Transgenic mice for interconnection targeting of neural sensors and effectors with high specificity and performance. *Neuron* **85**, 942–958 (2015).
67. Pho, G. N., Goard, M. J., Woodson, J., Crawford, B. & Sur, M. Task-dependent representations of stimulus and choice in mouse parietal cortex. *Nat. Commun.* **9**, 2596 (2018).
68. Huber, D. et al. Multiple dynamic representations in the motor cortex during sensorimotor learning. *Nature* **484**, 473–478 (2012).
69. Brainard, D. H. The psychophysics toolbox. *Spat. Vis.* **10**, 433–436 (1997).
70. Dimatteo, I., Genovese, C. R. & Kass, R. E. Bayesian curve-fitting with free-knot splines. *Biometrika* **88**, 1055–1071 (2001).
71. Kruskal, J. B. Three-way arrays: rank and uniqueness of trilinear decompositions, with application to arithmetic complexity and statistics. *Linear Algebra Appl.* **18**, 95–138 (1977).
72. Bellman, R. *Adaptive Control Processes: A Guided Tour* (Princeton University Press, 1961).

**Acknowledgements**

This work was supported by the following grants: Whitehall Foundation #20121221 (to R.W.), NSF CRCNS #1308159 (to R.W.), NIH R01 MH104259 (to M.J.G.), Whitehall Foundation #20181228 (to M.J.G.), NSF NeuroNex #1707287 (to M.J.G.), and R01 NS121919 (to M.J.G.).

**Author contributions**

J.X., T.D.M., M.J.G. and R.W. conceived and designed research; T.D.M. performed experiments; J.X. analyzed data; J.X., T.D.M., M.J.G. and R.W. interpreted results of experiments; J.X. prepared figures; J.X. drafted the paper; J.X., T.D.M., M.J.G. and R.W. edited and revised paper; J.X., T.D.M., M.J.G. and R.W. approved the final version of the paper.

**Competing interests**

The authors declare no competing interests.

**Additional information**

**Supplementary information** The online version contains supplementary material available at https://doi.org/10.1038/s41467-021-25437-2.

**Correspondence and requests for materials should be addressed to J.X.**

**Peer review information** *Nature Communications* thanks the anonymous reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

**Reprints and permission information** is available at http://www.nature.com/reprints

**Publisher’s note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

---

© The Author(s) 2021