Supplemental Information

Population-Level Neural Codes Are Robust
to Single-Neuron Variability from a
Multidimensional Coding Perspective

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Supplemental figures

Figure S1, related to figure 1. Neuronal responses to drifting gratings are often stable across several weeks. **a**, Four tuning curves of example neurons showing raw data (blue) and von Mises fit (red). **b**, Data from example animal acquired over a period of 29 days, showing estimated preferred orientation per neuron (y-axis) per recording session (x-axis), which suggests that most neurons exhibit a fairly stable preference for the orientation of drifting gratings. The top panels show stably tuned neurons included in further analyses (significant non-uniform distribution of preferred orientation across days quantified using a Rayleigh test (uncorrected p<0.05)), while the bottom panels show variable, non-responsive, or non-orientation-tuned neurons that were excluded from further analyses (Rayleigh test, uncorrected p>0.05). Left-hand panels show preferred orientation, while right-hand panels show orientation selectivity index (OSI). **c**, Distribution of mean pairwise angular distance in preferred orientation across recording sessions for all neurons (both included and excluded) shows a heterogeneous distribution with a peak for neurons with relatively stable tuning (up to ~30 degrees) and a smaller group of neurons with relatively unstable tuning (~35 degrees upwards). **d**, On average neurons with more stable tuning had higher orientation selectivity indices (linear regression, p<0.001).
Figure S2, related to figure 1. Separately calculating the dF/F0 for soma and neuropil, and post-hoc subtracting neuropil dF/F0 from somatic dF/F0 leads to higher decoding accuracy and less neuropil-contaminated dF/F0 traces than subtracting raw neuropil fluorescence from raw somatic traces. 

a, Arrows show two example neurons with traces shown in c-g. 

b, Overlay of same recording as in a, showing regions of interest in red (neuronal somata) and their surrounding neuropil annuli in yellow. 

c, Somatic dF/F0 traces without neuropil subtraction. Note the repetitive small activity bumps due to neuropil contamination in the blue neuron’s trace that closely follow the neuropil responses in (d) (e.g., red arrow). 

d, Neuropil dF/F0 shows small calcium transients that are unrelated to somatic activation whenever a stimulus is presented. 

e-f, Somatic dF/F0 traces with different forms of neuropil subtraction. 

e, Subtraction method used in this study (eq. S1), showing almost complete elimination of neuropil signal
when dF/F0 is first calculated for both soma and neuropil, and post-hoc subtracted. f, Subtraction of raw neuropil fluorescence from the raw somatic fluorescence with fixed correlation values (r) as used by Chen et al. (2013) and Akerboom et al. (2012) can lead to undersubtraction and inflated dF/F0 values (note difference in scale with e). g, Neuropil-somata correlation-dependent neuropil subtraction as used by Greenberg et al. (2008) leads to less strongly inflated dF/F0 values, but an often stronger undersubtraction of the neuropil signal. h, Quantification of the neuropil-subtraction performance with a simple naive Bayes orientation decoding of drifting gratings (same as Independent decoder in fig. S6e) shows that somatic signals provide more information on stimulus identity when treated with our post-hoc neuropil subtraction procedure (as in e) compared to the other two example methods (paired t-tests, post-subtraction vs. pre-subtraction with fixed strength (r=0.7); p<0.005, post-subtraction vs. pre-subtraction with variable strength (r=corr); p<0.001, pre-subtraction with fixed vs. variable strength; p<0.001). Asterisks indicate statistical significance; ** p<0.005, *** p<0.001.
Figure S3, related to figure 1. Eye-tracking and pupil location analysis shows that epochs of strong eye movement are similar to epochs of large pupil size (compare with fig. 1e), albeit statistically less significant. **a**, Eye-tracking example, showing normalized size (top), x-position (middle) and y-position (bottom) of the mouse’s pupil. **b**, Example frames with detected pupil areas during time points t=1 and t=2 as marked in panel a. **c**, Splitting trials by strongest and weakest 50% of eye movement, as for pupil size in figure 1e, shows similar effects: mean neuronal activity is not different (left panel, paired t-test across animals, N=8, p=0.469), variability is increased (middle panel, p<0.005), and pairwise noise correlations show a non-significant trend towards higher values (right panel, p=0.083).
Figure S4 related to figure 1. Single neuron examples illustrate the response variability on different time scales for grating (a-b) and natural movie (c-d) stimuli. a. The trial-by-trial response to the preferred orientation of four example neurons (from left to right) for oriented drifting gratings. Black lines show single trial responses to presentation of the same orientation across one imaging session. The red line shows the mean over repetitions. The grey area indicates stimulus presentation. The mean, standard deviation (S.D.) and coefficient of variation (C.V.) as shown in the upper right for each panel were calculated by first averaging over the three second stimulus period, and then taking the mean, standard deviation, and S.D. / mean.
across repetitions respectively. b, The average response for the same neurons as in a for ten imaging sessions performed on different days. The mean, standard deviation and coefficient of variation now show the response variability over recording sessions. c,d, as a,b, but now for neuronal responses to natural movies. Note the large variability of responses within single recording sessions in c. The C.V. shown in c,d, is calculated for the movie frame where the mean response was highest.
Figure S5, related to figure 2. Long-term instability of population codes is not due to neuropil contamination of somatic signals. a-c, Long-term stability of signal correlations. a, Soma-to-neuropil intensity of fluorescence (SNIF) ratio-weighted inter-recording correlations are very similar to the original, unweighted correlations (compare with figure 2b). b, Analyzing population code stability on neuropil annulus (NPA) signals show very different values, and a much larger spread. c, Comparison of the similarity of original and SNIF-weighted half-lives (left), original and neuropil-based half-lives (middle), and SNIF-weighted and neuropil-based half-lives (right) shows that half-lives calculated on neuropil signals are very different from somatic signal derived half-lives (p<0.001). d-f, as a-c for noise correlations. Also noise correlations are more similar between original and SNIF-ratio weighted values than for original-NPA (p<0.001) and SNIF-NPA (p<0.001). Asterisks indicate statistical significance; *** p<0.001. a,b,d,e: Grey curves in right-hand panels show fits on single animal data; red curves show fits on aggregate data (pooling all points from all animals).
Figure S6, related to figure 4. Pairwise neuronal responses are interdependent and their distribution resembles heavy-tailed two-dimensional Gaussians. This analysis validates the assumption that multivariate Gaussians are suitable to decode orientation from neuronal response distributions. a, dF/F0 activity of example neuronal pair during repeated trials of the same stimulus shows a long-tailed distribution. Contour lines indicate the distance in number of standard deviations to the mean of a multivariate Gaussian fit on the data. b, Binning and blurring the data in panel (a) allows a decoder to use the pairwise neuronal response structure without assuming a particular distribution a priori, which is used by the CONDOR (Correlation Organization Naive with Discretized Observed Responses) decoder (see Supplemental Experimental Procedures and c, black line). c, Assuming a multivariate Gaussian distribution of the pairwise response can be computationally efficient for decoding stimuli, but removes
heavy tails from the neuronal activity. This method is used by the GECKO (Gaussian-Estimated Correlation Kernel Operation) decoder (see Supplemental Experimental Procedures and e, red line). d, Pairwise responses distributions resemble bivariate Gaussians (panel a,b), but have long tails towards occurrences where both neurons are highly active. White-shaded area shows for an example animal the distribution of angles between the center of mass of the data and the center of mass of the residuals (data minus Gaussian fit) across all neuronal pairs. Black arrows pertain to all mice (n=9) and indicate the mean angle per animal. The curved line outside the polar plot shows the 95% confidence interval (CI) of the mean across animals. e, Assuming non-interdependent pairwise responses as with an independent Naive Bayes decoder (“Independent”) yields lower cross-validated (CV) decoding accuracy of grating orientation than when pairwise response structures are taken into account (paired t-test at sample size 14; CONDOR vs. Independent, p<0.005; GECKO vs. Independent, p<0.001). Using a distribution-naive pairwise approach (CONDOR) rather than assuming multivariate Gaussian responses (GECKO) makes little difference (p=0.723, n.s.). This shows that pairwise neuronal response distributions are approximated well with multivariate Gaussians, even though noise correlations are significantly reduced by the removal of non-Gaussian outliers (panel h). f, Schematic illustrating the calculation of the Vector of Association (VoA; see Supplemental Experimental Procedures) used to quantify correlations in binned pairwise responses, as in panel h; points along the positive diagonal increase the correlation value (red pluses), while points along the negative diagonal decrease the correlation value (blue minuses) (see Supplemental Experimental Procedures; CM, center of mass). g, There is strong correspondence between the correlation calculated using the Vector of Association and using Pearson’s r. The mean explained variance of Pearson correlation vs. VoA across trials over animals (n=9) is R²=0.948. h, Assuming a bivariate Gaussian distribution decreases pairwise neuronal noise correlations when compared to the non-fitted data (paired t-test, p<0.001). i, Orientation decoding accuracy
of greedy decoder using a Naive Bayes algorithm as in e (see also Supplemental Experimental Procedures), plotted as a function of the number of most informative neurons used in the decoding process. This analysis shows that when correlations are ignored during the decoding procedure, the first 7-9 most informative neurons are sufficient to provide over 90% of the information on stimulus orientation that is present in the whole population. j. Decoding accuracy using a greedy classifier approach as in i, but now using our Mahalanobis-space based decoder, as described in the main manuscript. k, Orientation decoding accuracy of the algorithm that takes into account multidimensional correlations (j) is higher than the accuracy of the independent Naive Bayes algorithm that does not (i), even in the case when the selection of neurons included in the decoding process is optimized for the algorithm. Blue line and shaded area show the mean ± SEM across animals. A t-test was performed by first taking the mean change in accuracy over dimensionalities, and then testing these mean values vs. 0 across animals; p<0.05, N=9 mice. l, The effect of correlations on decoding accuracy is relatively robust to the size of the analysis window used (compare with fig. 4d): using high-dimensional correlations leads to higher decoding improvement over shuffled than using only pairwise correlations for all window sizes, except when using a single acquisition frame (paired t-test of decoding improvement over shuffled, maximum dimensionality (max. dim.) vs. pairwise for different window sizes: 79 ms, p = 0.143, n.s.; 236 ms, 394 ms, 551 ms, p < 0.05; 709 ms, 866 ms, 1024 ms, 1181 ms, p < 0.005.
Figure S7, related to figure 4. The effect of multidimensional correlations in dF/F0 space is to confine correlated variability to directions parallel, but not orthogonal to decision boundaries.

**a.** Raw variability orthogonal and parallel to decision boundaries shows no clear difference between orthogonal and parallel variability. However, when normalizing this variability relative to the variability when correlations are destroyed by shuffling (b), it becomes clear the effect of multidimensional correlations is to confine variability mostly to directions parallel to decision boundaries, so as not to impair the population code of drifting grating orientation (c).

**d.** Correlation-induced variability in directions parallel to decision boundaries is higher than orthogonal (one-sample t-test of values at maximum dimensionality for each mouse, \( p<0.005, n=9 \)).

**e-h,** Analysis similar as for a-d, but now for natural movie scenes. The effect of multidimensional correlations on the encoding of natural movies is also to confine variability to directions parallel to decision boundaries (panel h, one-sample t-test, \( p<0.05 \)).
Supplemental Experimental Procedures

Animals and surgical procedures

All experimental procedures were in accordance with the Dutch national guidelines on the conduct of animal experiments and approved by the ethics committee of the University of Amsterdam. Fourteen C57BL/6 wild type mice were used in this study, of which the age on the last day of the experiment ranged from 98 to 254 days (repeated imaging across days, n=9) and 74-223 days (within same day, n=5) on the last day of the experiment. Animals were group housed on a reversed day/night cycle so that recordings were performed in their active phase.

Mice were implanted with a chronic head-bar for head fixation during experiments. Analgesic compound (Temgesic, 0.05 mg/kg bodyweight) was injected subcutaneously 30 minutes prior to anesthesia induction (3% isoflurane in 100% O2). Isoflurane concentration was lowered to 1-2% during surgery. A custom-built titanium head bar was cemented to the skull centered above the visual cortex (~4 mm caudal and ~2.5 mm lateral from bregma) using C&B Superbond (Sun Medical, Japan). A layer of cyanoacrylate glue (Loctite 401, Henkel, Germany) was applied to the skull to avoid infections. After several days of recovery (n=6 animals), or immediately after implantation of the head bar (n=8 animals), we performed viral injections with GCaMP6 using similar analgesia and anaesthesia as for the head post implantation procedure. The skull was thoroughly cleaned, a small hole was drilled in the skull (~0.1mm) and 200-300 nl of AAV1-Syn-GCaMP6m-WPRE was injected (Penn Vector Core, PA, USA) (Chen et al., 2013) at 1 mm anterior of the target imaging site (i.e., V1). For injection we used a mineral-oil backfilled glass capillary pipet operated by a Nanoject II Auto-Nanoliter injector (Drummond Scientific, PA, USA) at a depth of 600-700 µm below the dura. Following the viral injection a circular craniotomy was made (Ø 3 mm) and a double layered coverglass (top layer, Ø 5 mm, thickness ~150 µm; bottom layer, Ø 3 mm, thickness ~300 µm) was attached to the skull using cyanoacrylate glue to prevent skull regrowth (Goldey et al., 2014) (fig. 1a).
Apparatus and stimulus presentations

Recording procedures were similar to those described previously (Goltstein et al., 2013; Montijn et al., 2014). We performed single-channel two-photon imaging recordings (filtered at 500-550nm for GCaMP6 emission spectrum) with a 512 x 512 pixel frame size at a sampling frequency of 12.7Hz. We used an in vivo two-photon laser scanning microscopy setup (modified Leica SP5 confocal system) with a Spectra-Physics Mai-Tai HP laser set at a wavelength of 880-910 nm to simultaneously excite GCaMP6 molecules. Mice (n=14 in total, n=9 across multiple days) were awake and head-fixed during the two-photon calcium imaging recordings (5-8 recordings/animal). Mice were presented with visual stimuli consisting of either 10 or 12 repetitions of bidirectionally moving square-wave drifting gratings in 8 different orientations (n=80 or 96 trials/recording). Visual stimulation lasted 3 seconds, and the direction of the drifting grating switched 180 degrees after 1.5 seconds. Visual drifting gratings (diameter 60 retinal degrees, spatial frequency 0.05 cycles/degree, temporal frequency 1Hz) were presented within a circular cosine-ramped window to avoid edge effects at the border of the circular window. Stimulus presentations were alternated with a 5 second blank inter-trial interval during which an isoluminant grey screen was presented. A subset of these animals (n=4 out of 9 animals, 5-8 recordings/animal) were also presented with natural movies (frame rate 25Hz) which consisted of four scenes (durations: scene one and three, 3.6 seconds (90 frames); scene two and four, 6.4 seconds (160 frames)) taken from the BBC’s Earthflight (Winged Planet) - Condor Flight School. Each presentation lasted 20 seconds and was repeated 31 times per recording session without an inter-trial interval between repetitions. Drifting gratings and natural scenes were displayed on a 15 inch TFT screen with a refresh rate of 60Hz positioned at 16cm from the mouse’s eye, which was controlled by MATLAB using the PsychToolbox extension (Brainard, 1997; Pelli, 1997). A field-programmable gate array (FPGA, OpalKelly
XEM3001) was connected to the microscope setup and interfaced with the stimulus computer to synchronize the timing of the visual stimulation with the microscope frame acquisition.

**Data preprocessing**

Data processing was performed similarly as described in (Montijn et al., 2014). After a recording was completed small x-y drifts within the recording were corrected (Guizar-Sicairos et al., 2008) and stability was checked along the z-axis. All recordings appeared stable and none were rejected due to excess z movement. Regions of interest (i.e., neuronal somata) were determined semi-automatically using custom-made MATLAB software and subsequently $dF/F_0$ values for all neurons were calculated. For each image frame $x$ a single $dF/F_0$ value was obtained for each neuron as well as its surrounding neuropil (annulus between two and five microns from soma) by calculating the baseline fluorescence ($F_0$), taken as the mean of the lowest 50% of fluorescence values during a 30-second window surrounding image frame $x$. $dF$ is defined as the difference between the fluorescence in the given frame and the sliding baseline fluorescence ($dF_x = F_x - F_0$). After calculating the somatic and neuropil $dF/F_0$ values, we subtracted the neuropil $dF/F_0$ from the somatic $dF/F_0$ to avoid neuropil contamination of our data (see (Chen et al., 2013) and fig. S2,3):

$$
\text{dF/F}_{0,\text{corr}} = \text{dF/F}_{0,\text{soma}} - \text{dF/F}_{0,\text{neuropil}} \quad \text{(eq. S1)}
$$

The mean number of simultaneously recorded neurons present in all recordings was 113.2 (range: 43 – 181). Eye-tracking was performed during the acquisition of neural data in eight of the animals used in the current study. Pupil detection was performed using custom-made MATLAB software (fig. 1f, fig. S3).
**Neuronal responses to drifting gratings and selection of neurons**

A neuron’s response $R$ to a 3.0 second stimulus was defined as the mean $dF/F_0$ value of all 38 frames recorded during that single trial’s grating presentation. To obtain a measure of a neuron’s orientation selectivity, we calculated each neuron’s preferred direction by fitting a von Mises distribution to the neuron’s responses across 8 different orientations:

$$f(x|\theta, \kappa, \mu_0) = \frac{e^{\kappa \cos(x-\theta)}}{2\pi I_0(\kappa)} + \mu_0$$  \hspace{1cm} (eq. S2)

Here, $I_0(\kappa)$ is the modified Bessel function of order 0 and $x$ represents the stimulus angle. As can be seen in the equation, we defined the free parameters as $\theta$ (preferred direction), $\kappa$ (concentration parameter at $\theta$) and $\mu_0$ (baseline response). We calculated each neuron’s preferred orientation separately for each recording session. A neuron was included for further analysis if it was visibly present in all recording sessions and its preferred orientation, calculated separately for each recording session, was distributed non-randomly across recordings, using a Rayleigh test ($p < 0.05$; fig S1b).

Note that consistent changes in neuronal responses across time, such as in fig. 2, are unlikely to arise from consistent drift in focus depth across recordings, because on each recording day the same set of neurons was manually located and brought into focus, with the first recording day as reference. Such random variation would decrease the absolute correlation values in fig. 2, but cannot explain the exponential decay we observed as a function of inter-recording period.

**Signal correlations**

To quantify the temporal stability of orientation tuning in V1 neurons to drifting gratings, we calculated signal correlations between all neuronal pairs in each recording. We defined each
orientation as a separate stimulus class and calculated a neuron’s mean response vector $\bar{R}$, where the elements of $\bar{R}$ are the neuron’s mean responses to each orientation $\theta$ ($\bar{R}_\theta$):

$$\bar{R} = [\bar{R}_0, \bar{R}_{22.5} \ldots \bar{R}_{157.5}]$$  \hspace{1cm} (eq. S3)

We then filled a correlation matrix $\rho_{signal}$ with all pairwise signal correlation values, using for each element the Pearson correlation between the response vectors of two neurons $(i,j)$:

$$\rho_{i,j}^{signal} = \text{corr}(\bar{R}_i, \bar{R}_j)$$  \hspace{1cm} (eq. S4)

The correlation between two of these matrices, comprising all neuronal pairs, recorded at different points in time measures the similarity of the entire population’s response to different stimulus orientations.

**Noise correlations**

Complementary to signal correlations, noise correlations give an indication of the similarity in trial-by-trial response variability between neurons. We first calculated a response vector for each stimulus orientation $\theta$, where each element in the vector is the neuron’s response to a single presentation $t$ of that stimulus orientation:

$$R_\theta = [R_{\theta_t} \ldots R_{\theta_n}]$$  \hspace{1cm} (eq. S5)

Here, $n$ is the number of repetitions per orientation. Because we aim to compute a single noise correlation value per neuronal pair, we took the mean noise correlation over all eight stimulus orientations:
Similarly to signal correlations, we used the inter-recording similarity of these matrices to investigate the temporal stability of non-stimulus-related inter-trial variability.

**Exponential decay times**

To quantify the population response stability over time, we calculated the decay of population correlation structures over days. For each pair of recordings performed at different days on the same population, we calculated the correlation between the recordings’ signal and noise correlation matrices, which yields a value indicative of the similarity in stimulus-related responses (signal correlations) and fluctuations in those responses (noise correlations). An exponential decay function was fitted over all inter-recording similarity values to obtain a half-life for signal and noise correlations per animal:

\[ N(t) = N_0 e^{-\lambda t} \]  

(\text{eq. S7})

Here, \( N_0 \) is the intercept at time \( t = 0 \) and \( N(t) \) is the correlation at time \( t \). The parameter \( \lambda \) controls the decay time and can be transformed to the half-life by \( t_{1/2} = \ln(2)/\lambda \).

**Half-logistic growth functions**

To estimate an upper limit to the dimensionality-based increase in decoding performance we fitted the data with a modified logistic function where the steepest point of the curve is centered at \([0;0]\) rather than the \([0;0.5]\) of regular logistic functions:
\[ f(x) = 2L \left( \frac{1}{1 + e^{-kx}} - 0.5 \right) + y0 \]  

(eq. S8)

We defined the free parameters as \( L \) (the asymptote), \( k \) (the slope), and \( y0 \) (the offset along the y-axis).

**Variability orthogonal vs. parallel to decision boundaries in normalized space**

We hypothesize that the optimal coding strategy for neuronal populations is to minimize neuronal response variability orthogonal to decision boundaries. We therefore computed the neuronal variability orthogonal and parallel to decision boundaries between adjacent orientations \((\theta_1, \theta_2)\) as follows. For each orientation, the mean population response can be represented as a multidimensional vector \( \mu = [m_1 \ldots m_N] \), where \( m \) is the mean neuronal activity in dF/F0 for that stimulus and \( N \) is the number of neurons. We set the origin of responses at the mean response to stimulus class \( \theta_1 \), and calculated for each trial \( t \) the population response \( r_t \) as a projection along the line crossing both the origin (mean response of \( \theta_1 \)) and the multidimensional mean response of \( \theta_2 \)’s projection onto \( \theta_1 \). The distances orthogonal (\( d_{\text{orth}} \)) and parallel (\( d_{\text{parallel}} \)) to the decision boundary are then given by:

\[
d_{\text{orth}} = \left\| \left( \frac{n \cdot \mu_{\theta_2}^T}{\mu_{\theta_2} \cdot \mu_{\theta_2}} \odot \mu_{\theta_2} \right) - \mu_{\theta_2} \right\| \tag{eq. S9}
\]

\[
d_{\text{parallel}} = \left\| \left( \frac{n \cdot \mu_{\theta_2}^T}{\mu_{\theta_2} \cdot \mu_{\theta_2}} \odot \mu_{\theta_2} \right) - r_t \right\| \tag{eq. S10}
\]

In these equations * indicates vector product, \( \odot \) element-wise multiplication, \( ^T \) vector transpose and \( \| \cdot \| \) vector norm. The variability for this pair of stimuli orthogonal and parallel to the decision boundary can be retrieved by taking the standard deviation of the distances. We
compared this variability relative to baseline variability where we reiterated the procedure, but shuffled all trial indices independently for each neuron, thereby destroying noise correlation structures. The values shown in fig. 4h,5f are relative to this baseline variability. To study the dependency on dimensionality, we took the mean of 100 neuronal groups of different size, as described above for the Mahalanobis-space based decoder.

In other words, for every pair of stimuli, the multidimensional population responses to one of these stimuli are projected onto the mean population response of the other. Because the mean response to stimulus 1 (s1) is used as origin, the vector from this new origin to the mean response to stimulus 2 (s2) will lie on the axis that crosses both s1 and s2, and is orthogonal to the decision boundary between them. Because this line can cross diagonally to dimensions defined by the neurons, the expected mean orthogonal variability will grow as a function of dimensionality. The parallel axis for each instance of population activity can now be defined as the shortest vector from each point (i.e., a single instance of a population response to s2 in multidimensional neural space) to the orthogonal axis. By this definition, the parallel and orthogonal axes are perpendicular to each other. Similar to the orthogonal variability, the expected mean parallel distance will grow as a function of dimensionality, at the same rate as the orthogonal variability. Note that therefore the bias towards larger variability as a function of dimensionality is identical for orthogonal and parallel directions. This effect can be observed in fig. S7a,b,e,f. As the rate of growth is dependent on many factors, such as the heterogeneity, mean level and sparseness of population activity, only the variability normalized to shuffled responses, as shown in fig. 4h, will provide an answer to the question whether correlations restrict the variability to particular directions in multidimensional neural space.
Natural movie analysis

Natural movies were analyzed with the Mahalanobis-distance based decoder as described in the main text for drifting grating orientations. Instead of orientation, we defined the separate stimulus classes as either an entire scene (fig. 5), yielding n=4 different stimuli; or as the duration of a single calcium imaging acquisition frame (fig. 6,7), yielding n=253 different stimuli per movie repetition. Note that although the natural scenes were presented at 25Hz, the limiting factor of temporal accuracy was the acquisition speed of the microscope setup. The duration of a single calcium imaging frame was 79ms (12.7 Hz).

Prediction of instantaneous single-neuron noise

To investigate if we could predict instantaneous neuronal noise (i.e., the fluctuation of a neuron’s activity relative to its mean activation across repetitions within a time bin of ~79 ms), we proceeded as follows. Given a population of N neurons, we predicted neuron i’s activity based on the activity of the rest of the population; neurons [1 … N-1]. At a single time point within a movie, the population response space of neurons [1 … N] can be visualized as an N-dimensional probability distribution. Each repetition of a single time point therefore represents a random sample from this multidimensional distribution. When predicting neuron i’s response for repetition t of its preferred movie frame (i.e., highest neuronal response; fig. 7b), we fitted a multivariate Gaussian on all other repetitions (leave-one-repetition-out cross-validation), and calculated the highest probability density of this multivariate Gaussian for dimension N given the population response vector:

\[ \mathbf{r}_{t,-i} = [r_{t,1} \ldots r_{t,N-1}] \]  

(eq. S11)
This amounts to “reading out” the most probable activation level of neuron $i$ from the multivariate Gaussian, given the activation of the rest of the population at that point in time. This procedure can be performed for any dimensionality (size of the group of neurons; $N$) to investigate the effect of higher-dimensional response interdependencies on the predictability of instantaneous noise.

Soma-to-Neuropil Intensity of Fluorescence (SNIF) ratio analysis shows that the observed decay of noise and signal correlations is not due to changes in neuropil fluorescence

In calcium imaging with GCaMP6m, neuropil contamination can present a major problem. In an attempt to minimize the influence of contamination of the somatic signals by neuropil we analyzed only neuropil-subtracted data. In fig. S2 we presented example traces that show that our neuropil-subtraction method is very efficient, and even more effective than some traditional approaches (Greenberg, 2008; Akerboom, 2012; Chen, 2013). However, there is a slight chance that residual neuropil signals remained. To control for this, we performed additional analyses that quantified whether the amount of neuropil contamination influences the observed half-lives of the population code. We calculated for each neuron in each recording session a soma-to-neuropil intensity of fluorescence ratio (SNIF ratio): $\text{SNIF} = \frac{F_{\text{soma}}}{F_{\text{neuropil\_annulus}}}$. This ratio is a metric of the supposed severity of neuropil-contamination for each neuron that would be present without neuropil subtraction. We then used this SNIF ratio as a weighting factor in the correlation computation to check the effect of neuropil contamination on the long-term stability of population codes. We calculated the correlation between signal- and noise correlation matrices recorded on two separate days, weighting the Pearson correlation by the mean SNIF ratio of the pair of neurons averaged over the two days. If the long-term instability in population codes we report in the manuscript would be due to neuropil contamination of the
somatic signal (despite our use of neuropil-subtracted data), we should see a significant increase or decrease (depending on the stability of the neuropil signals) in the half-lives of the population code similarity values, as in this calculation more weight is given to less neuropil-contaminated pairs of neurons. As can be seen in fig. S5a,d, this is not the case. In fact, our SNIF-ratio weighted half-lives are almost identical to our non-weighted half-lives (fig. 2b,c), suggesting that our neuropil-subtraction method is highly efficacious. Still, it could be that half-lives calculated on neuropil signals are identical to those calculated using neuropil-subtracted or SNIF-ratio weighted data, and that therefore our SNIF-ratio weighting shows no difference. We therefore also computed the signal and noise correlation stabilities of the neuropil annulus signals, and found these yielded very different values, with a much larger spread, than the original and SNIF-ratio weighted data (fig. S5b,e). Some animals showed exceedingly large half-lives (> 10^6 days), and overall neuropil half-lives were markedly different from both the original non-weighted half-lives, as well as the SNIF-ratio weighted half-lives. An analysis of the relation of the half-lives obtained from the neuropil and neuropil-subtracted somatic signals showed that they were quite uncorrelated, and significantly more different than the original and SNIF-weighted somatic half-lives (fig. S5c,f, p<0.001 for both signal and noise correlations).

**Analysis of pairwise neuronal response distributions show they are mostly bivariate Gaussian**

A common (and sometimes implicit) assumption in the analysis of neuronal pairwise responses is that they show a bivariate Gaussian shape, and many theoretical predictions and experimental findings regarding noise correlations and population coding depend on this assumption (Averbeck et al., 2006; Cohen and Kohn, 2011; Dayan and Abbott, 2001; Ecker et al., 2011; Hansen et al., 2012; Simoncelli and Olshausen, 2001; Sompolinsky et al., 2001; Zohary et al., 1994). Because some of our analyses make the same assumption of Gaussian response
distributions, we investigated whether experimentally measured neuronal pairs show bivariate Gaussian responses. We found that our data show a generally good correspondence between experimentally measured pairwise neuronal responses and bivariate Gaussians (fig. S6). The only consistent deviation is that experimental data show heavier tails than Gaussian approximations: neuronal pairs show an excess of high activity trials. While removal of these tails slightly reduces correlation values, this did not noticeably alter the amount of information that can be extracted from the population response using a grating orientation decoding procedure (paired t-test across nine animals, performance of distribution-naive vs. Gaussian-approximation decoder, p=0.723, n.s.) (see below and fig. S6e).

Vector of Association

In order to be able to compute correlation values on gridded (discretized) probability density functions of pairwise neuronal responses, we developed a novel correlation metric we call the Vector of Association (Fig. S6). It is based on the resultant vector magnitude of the probability density function of the pairwise neuronal responses, and is conceptually similar to a Pearson correlation, but shows a slightly different scaling than Pearson’s r. First, we calculate the distribution’s center-of-mass:

\[ R = \frac{1}{M} \sum_{i=1}^{n} m_i r_i \]  

(eq. S12)

In this equation, \( R \) contains the coordinates of the center-of-mass in pairwise neuronal response space, \( m_i \) is the probability density at bin \( i \), \( r_i \) contains the coordinates of bin \( i \), and \( M \) is integral over the probability density function (sum of all \( m \)’s). Next, we calculate the offset of each bin in each dimension (i.e., x and y) to the center-of-mass:

\[ w_i = r_i - R \]  

(eq. S13)
where \( w_i \) is the location of bin \( i \) relative to the center-of-mass and \( r_i \) contains the coordinates of bin \( i \). We can then perform an x/y-vector decomposition of \( w_i \), and for each bin take the product of these decomposition magnitudes and the square of the bin’s probability density:

\[
V_i = x_i y_i (m_i)^2, \quad \text{(eq. S14)}
\]

where \( V_i \) is the contribution of bin \( i \) and \( x \) and \( y \) are the decomposed values of \( w_i \) along the two cardinal dimensions. We can now take the sum over all points in \( V \) – the matrix of weighting values for all \( x \) and \( y \) –, divide by the sum of the absolute of all points to normalize the values in the interval \([-1,1]\), and take this value’s pseudo-square to normalize the magnitude for its dependence on two dimensions, but preserve its sign to calculate the vector of association \( r_v \):

\[
r_v = \frac{\sum V}{\sum |V|} \cdot \frac{\sum V}{\sum |V|} \quad \text{(eq. S15)}
\]

Note that this procedure can also be applied to non-binned observations; in this case \( w_i \) represents the location of observation \( i \) relative to the center-of-mass, and \( m_i \) is always one, because each point receives an identical weighting factor.

**Independent naive Bayes decoder**

To investigate the importance of pairwise neuronal response inter-relations we compared the performance of more sophisticated algorithms to an independent naive Bayes (Simple Neural Activity by Independent Likelihood; SNAIL) decoder (e.g., (Zhang et al., 1998)), which is identical to the Bayesian maximum-likelihood decoder described in (Montijn et al., 2014). In short, its population posterior probability assumes independent responses over all neurons (i.e., as if their stimulus responses were uncorrelated), using a product-rule to produce a population posterior probability per stimulus class for each trial:

\[
P(\theta | A_{\text{pop}}) \propto \prod_{i=1}^{n} P(\theta | A_i) \quad \text{(eq. S16)}
\]
Here, \( P(\theta|A_{\text{pop}}) \) is the posterior probability of stimulus \( \theta \) given the population activity \( A_{\text{pop}} \) for a certain trial, and \( P(\theta|A_i) \) is the posterior probability of neuron \( i \) for stimulus \( \theta \) given the cell’s activity \( A_i \), as defined by Bayes’ rule. The decoder uses a maximum-likelihood decision rule (its read-out is the stimulus whose posterior probability is highest).

**Correlation Organization Naive with Discretized Observed Responses (CONDOR) decoder**

Often multidimensional decoding algorithms make an a priori assumption about the shape of neuronal response functions. In most cases it is assumed pairs of neurons show a multivariate Gaussian probability density, but few studies have checked whether this assumption is valid (see main text). Therefore we created a decoding algorithm that makes no assumptions about the shape of pairwise correlations. To create pairwise likelihood distributions, but keep calculations computationally tractable, we binned the pairwise responses for each stimulus and each pair of neurons during all repetitions into a 21 x 21 grid. We applied a Gaussian filter (sd is 1 bin) to have a non-zero response probability in all bins of the grid (fig. S6a,b). Leave-one-repetition-out cross-validation was performed by recomputing for each to-be-decoded trial all pairwise likelihood grids without that repetition. This procedure yielded a likelihood value for all neuronal pairs and bins. The posterior population probability for each neuronal pair - trial combination was calculated by extracting the probability density from the bin corresponding to that pair’s dF/F0 response during that trial, and taking the product of the probabilities over all neuronal pairs (similar to eq. S16).

**Gaussian-Estimated Correlation Kernel Operation (GECKO) decoder**

To assess the influence of assuming multivariate Gaussian responses on the amount of information that can be extracted from pairwise neuronal responses, we made another decoding
algorithm similar to CONDOR. Here, we fitted the binned and blurred probability distribution of all neuronal pairs with a multivariate Gaussian instead of using CONDOR’s distribution-naive grid itself for constructing the likelihood (fig. S6c). Fitting multivariate Gaussians often captured >95% of the variance, but removed the heavy tails of the distribution (fig. S6b-d). This Gaussian-Estimated Correlation Kernel Operation (GECKO) decoder therefore used likelihood distributions with significantly lower correlation values than CONDOR (fig. S6e-h).

Greedy classifier analysis using independent naive Bayes decoder

An often used analysis in neurophysiological studies is a greedy-classifier approach; the decoding performance of the algorithm can be plotted as a function of number of neurons used in the decoding process, ordered from most to least informative. To allow comparison between our data and other studies presenting this metric, we also performed such an analysis. We used the independent naive Bayes (Simple Neural Activity by Independent Likelihood; SNAIL) decoder described above, where we decoded stimulus orientation for each animal. First we performed this decoding process using only a single neuron, consecutively for all neurons. We then took the most informative neuron N1 (i.e., the neuron that yielded the highest decoding performance), and added a second neuron N2, again iteratively repeating the procedure for all combinations with N1. We repeated this process until all neurons were included in the decoding process. The results from this analysis are presented in fig. S6i.

Supplemental references


