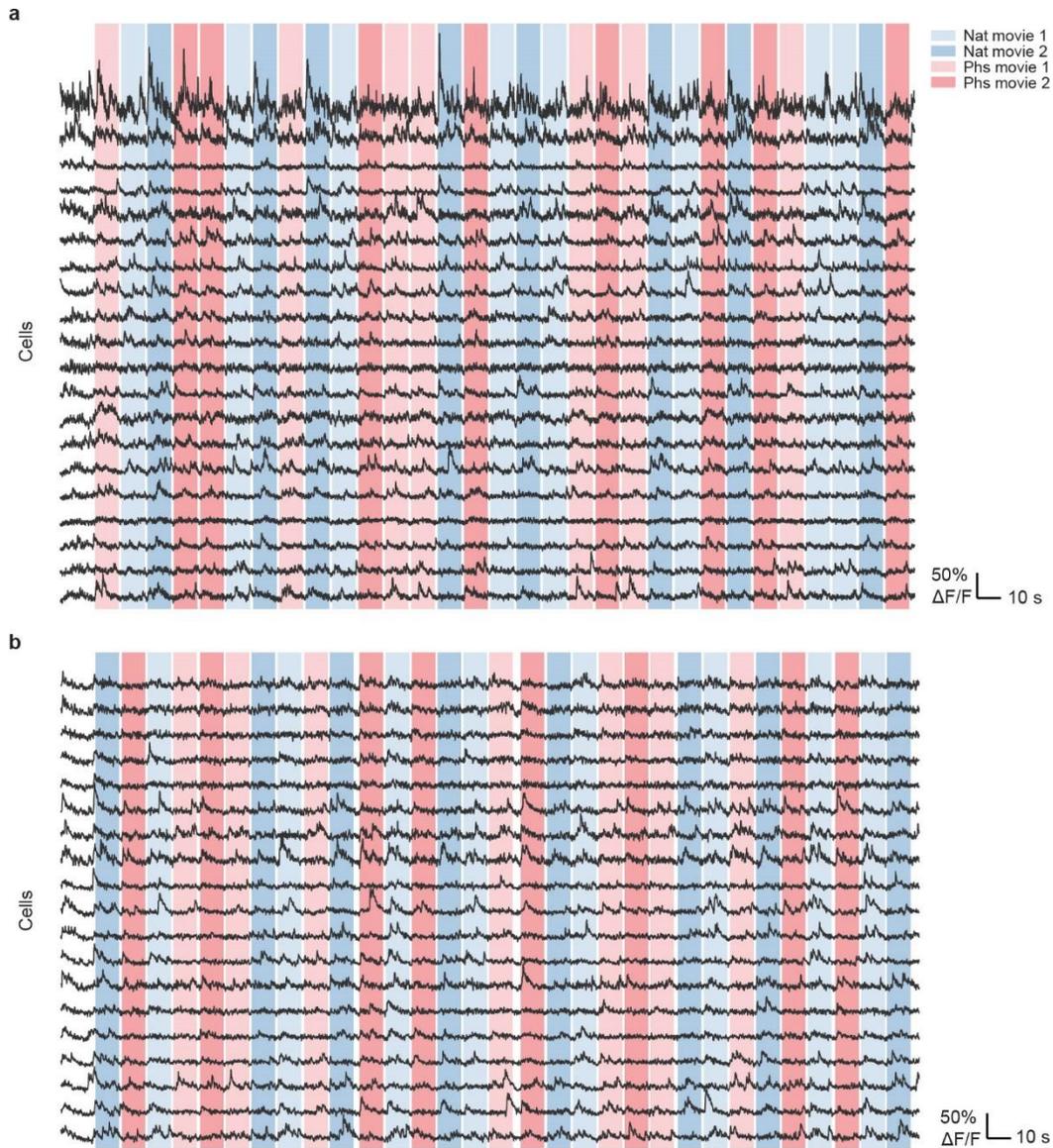


## Supplementary information

# Population code in mouse V1 facilitates read-out of natural scenes through increased sparseness

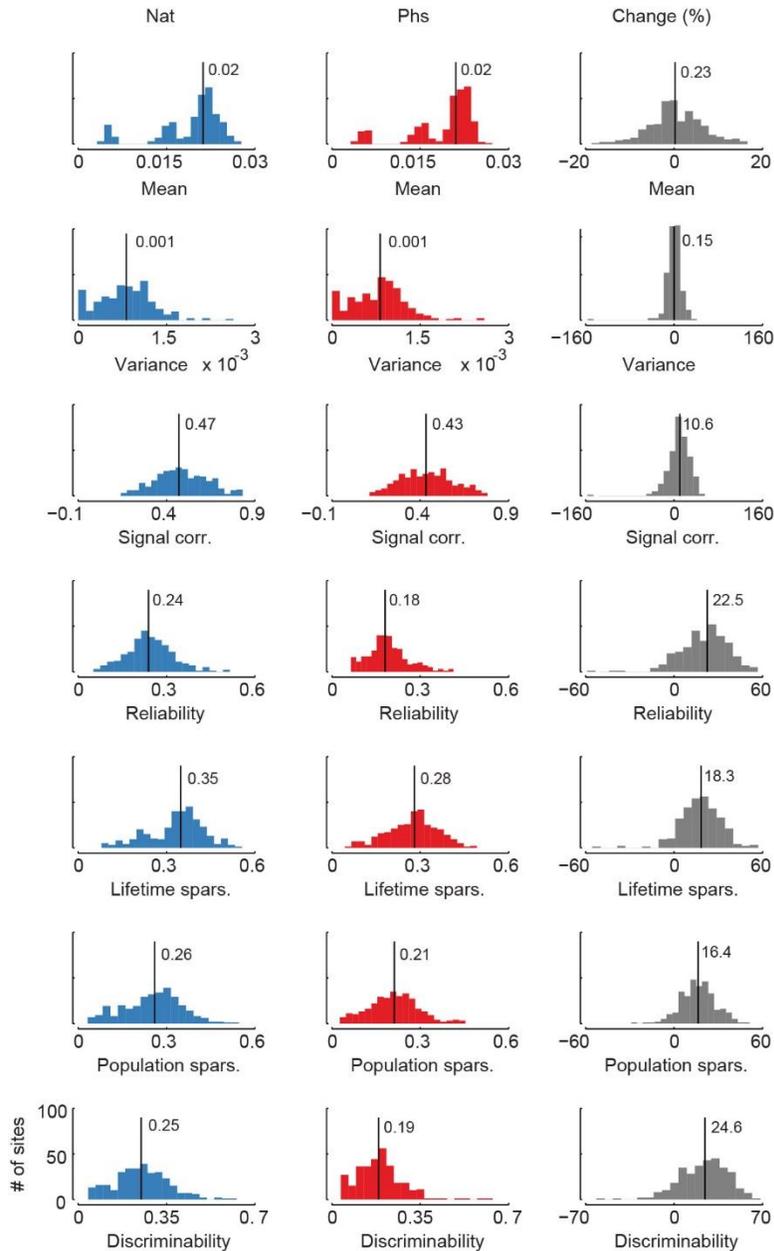
Emmanouil Froudarakis, Philipp Berens, Alexander S. Ecker, R. James Cotton, Fabian H. Sinz, Dimitri Yatsenko, Peter Saggau, Matthias Bethge, Andreas S. Tolias

Supplementary Figure 1: Examples of raw data for 3D and 2D scanning .....	2
Supplementary Figure 2: Histograms of all measures of population activity .....	3
Supplementary Figure 3: Dependence of sparseness on population and bin size .....	4
Supplementary Figure 4: Correlation of lifetime and population sparseness .....	5
Supplementary Figure 5: Control analysis for the effect of eye movements .....	6
Supplementary Figure 6: Measures of population activity for the V1 models .....	7
Supplementary Figure 7: Example behavioral traces during the awake experiments. ....	8
Supplementary Table 1: Overview over experimental data .....	9
Supplementary Table 2: Processing parameters and regression analysis .....	10



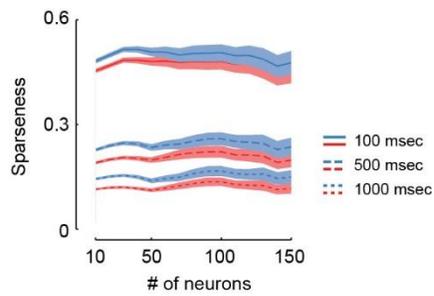
### Supplementary Figure 1: Examples of raw data for 3D and 2D scanning

(a) 3D scanning:  $\Delta F/F$  calcium signals from the 20 cells shown in **Fig. 1d** out of the 139 total recorded in this site. The 4 different colors indicate presentations of multiple repetitions of the 4 different movies. (b) 2D scanning: Raw  $\Delta F/F$  calcium signals from the 19 cells recorded in this site.



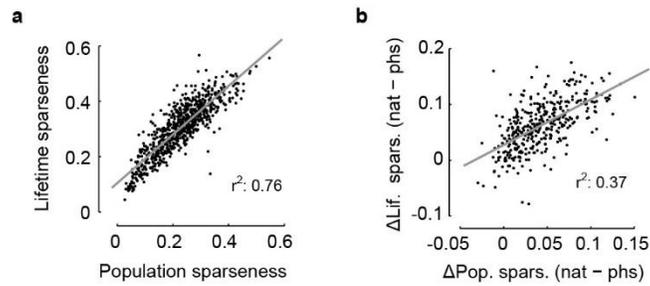
### Supplementary Figure 2: Histograms of all measures of population activity

Histograms of all the measures of population activity shown in **Fig. 3** for main dataset (L2/3; anesthetized animals;  $n = 315$  sites). Columns from left to right: histograms for natural movies, phase scrambled movies and the percentage difference between the two conditions relative to the natural condition. Black line and number indicate the median of each distribution.



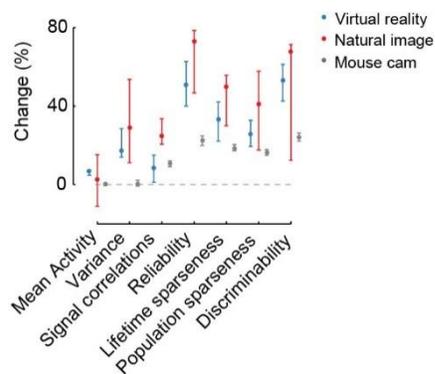
### Supplementary Figure 3: Dependence of sparseness on population and bin size

Population sparseness for 100 ms, 500 ms and 1000 ms bins (indicated by the line style) and natural (blue) and phase scrambled (red) movies. Population sparseness does not depend on population size but larger bin sizes reduce the apparent sparseness. Shaded area around the mean represents  $\pm 1$ SEM.



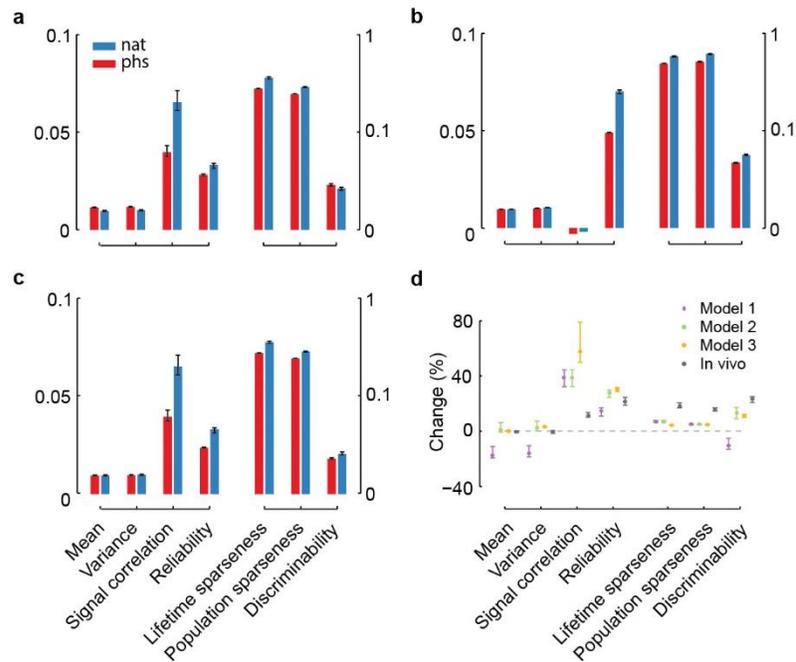
**Supplementary Figure 4: Correlation of lifetime and population sparseness**

(a) Scatter plot of population and lifetime sparseness for the main dataset (N=315). The two measures are highly correlated (linear correlation,  $r = 0.87$ ;  $p < 0.001$ ). Gray line indicates best fitting linear regression. (b) Scatter plot of difference in population and lifetime sparseness between the responses to natural and phase scrambled stimuli. The two measures are strongly correlated (linear correlation,  $r = 0.61$ ,  $p < 0.001$ ).



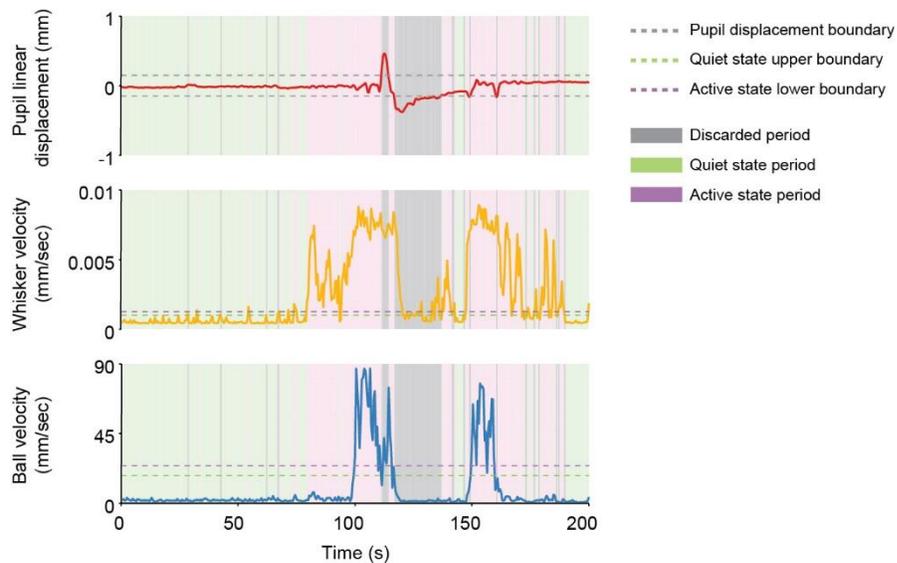
### Supplementary Figure 5: Control analysis for the effect of eye movements

Median difference in measures of population activity between unprocessed and phase scrambled version of the virtual reality environment movies generated by Wallace et al.<sup>20</sup> (n = 13 sites; blue), a natural image sequence with motion vectors extracted from the virtual reality movie and their phase scrambled version (n = 13 sites; red), and between stimulation with natural and phase scrambled movies from the mouse cam lacking eye movements (blue). Positive values indicate that the measure is higher under stimulation with natural stimuli. Error bars encompass the 95%-confidence intervals of the median.



### Supplementary Figure 6: Measures of population activity for the V1 models

(a) Median of the measures of population activity for the population responses of model 1 (LNP). Error bars encompass the 95%-confidence intervals of the median. (b) As in (a) but for model 2 (adaptive non-linearity). (c) As in (a) but for model 3 (divisive normalization). (d) Median difference in measures of population activity for the population responses between natural and phase scrambled sets separately for Model 1, Model 2, Model 3 and in vivo data (purple, green, yellow and gray, respectively). Positive values indicate that the measure is higher under stimulation with natural movies. Error bars encompass the 95%-confidence intervals of the median.



**Supplementary Figure 7: Example behavioral traces during the awake experiments.**

Linear pupil displacement from the median position across the experiment in millimeters, the whisker velocity and the ball velocity in mm/s are shown from top to bottom (red, yellow and blue, respectively). Periods with large pupil displacements, or running and whisking activity between the upper and lower boundaries (dashed lines), were discarded (gray background). Periods during which the animal was not whisking or not running were considered as quiet (green background, below green dashed line; see Methods). Periods during which the animal was either whisking, running or both were considered as active (purple trace, above purple dashed line; see Methods).

**Supplementary Table 1: Overview over experimental data**

<b>State of animal</b>	<b>3D imaging</b>	<b>2D imaging</b>	<b>Electrophysiology</b>
Anesthetized	30 sessions 89 – 510 neurons/site 177 ± 90 neurons/site	462 sessions 16 – 144 neurons/site 53 ± 29 neurons/site	34 cells
Awake - Active		23 sessions 29 – 53 neurons/site 41 ± 7 neurons/site	
Awake - Quiet		100 sessions 25 – 70 neurons/site 43 ± 7 neurons/site	

Average reported as mean±SD

**Supplementary Table 2: Processing parameters and regression analysis**

	<b>Intercept</b>	<b>Mean</b>	<b>Variance</b>	<b>Signal Correlations</b>	<b>Reliability</b>	<b>Lifetime Sparseness</b>	<b>Population Sparseness</b>	<b>Spherical Variance</b>	<b>Explained Variance</b>
<b>As in text</b>	0.07	0.04*	-0.02*	0.01	0.46*	-0.29*	0.73*	-	0.73
<b>No Trial Averaging</b>	0.25*	0.04*	-0.02*	-0.19*	0.79*	0.03	0.09*	-	0.61
<b>100 ms Bin</b>	0.12*	0.11*	-0.07*	-0.02	0.11	0.19*	0.57*	-	0.77
<b>1000 ms Bin</b>	0.16*	0.03*	-0.02*	-0.03	0.61*	-0.37*	0.63*	-	0.66
<b>Support Vector Machine Decoder</b>	0.17*	0.04*	-0.02*	-0.03	0.34*	0.03	0.47*	-	0.69
<b>Lasso Regression</b>	0.09*	0.03*	-0.01	0	0.28*	0	0.61*	-	0.71
<b>Spherical Variance</b>	0.07	0.04*	-0.03*	-0.04	0.33*	-	-	0.62*	0.73
<b>Awake - Active</b>	0.14	-0.64*	-0.30*	-0.26	0.27	0.81	0.98*	-	0.72
<b>Awake - Quiet</b>	-0.09	1.17*	-0.42*	0.25*	-0.33*	0.62*	-0.20*	-	0.69
<b>LM/AL</b>	0.11	0.13*	-0.14*	0.02	0.57*	-0.78*	1.09*	-	0.78

\* indicates significantly different from zero (95%-CI does not include zero)

The difference in population sparseness is responsible for most of the change in classification performance between natural and phase-scrambled movies regardless of the time bin size we used, the decoding algorithm, the regression technique and the sparseness measure. We briefly explain the modified analysis indicated in the first column:

*No trial averaging.* If variability of the responses is very high, trial averaging can have a negative effect on sparseness. We computed lifetime and population sparseness on the single trial responses and then averaged across all trials.

*100 ms bin.* Since sparseness depends on the binning, we also used 100 ms bins.

*1000 ms bin.* Same as before but for 1000 ms.

*Support vector machine decoder.* In addition to the optimal linear classifier, we used a support vector machine classifier (MATLAB, The Mathworks) and leave-one-out cross-validation to estimate the decoding error between the neural representations of pairs of 500 ms scenes.

*Lasso Regression.* We used regularized least-squares elastic-net regression<sup>47</sup> with  $\alpha = 0.95$  to estimate the influence of the regressors difference in mean, variance, signal correlations, reliability, lifetime sparseness and population sparseness on the difference in classification performance across sites. The advantage of this regression technique is that it appropriately selects a minimal set of regressors if they are correlated. We used the 1-Standard-Error rule to select the optimal coefficient set.

*Spherical Variance.* Instead of using the two sparseness measures we also computed the spherical variance of the responses.

*Awake – Active.* Regression result when using only the periods where the animal was in an active state.

*Awake – Quiet.* Regression result when using only the periods where the animal was in a quiet state.

*LM/AL.* We performed the analysis also on the recordings from the secondary visual areas of the mouse LM and AL.