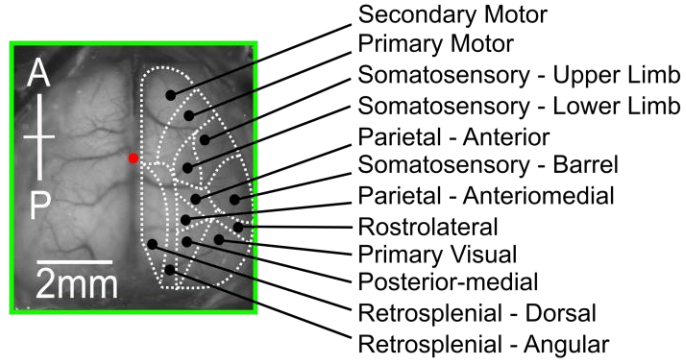
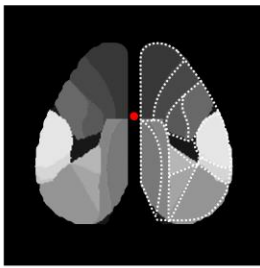
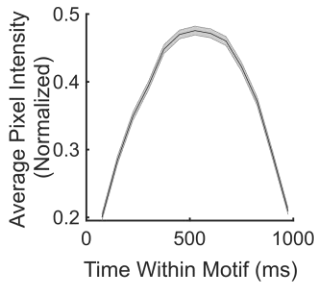


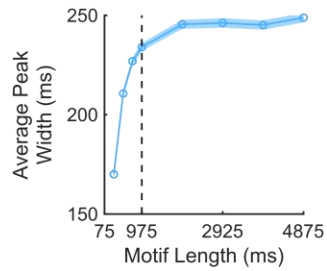
A 2D Allen Brain Atlas



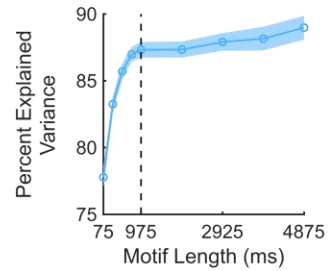
B Neural Activity Resolves Within ~1s Motif Duration



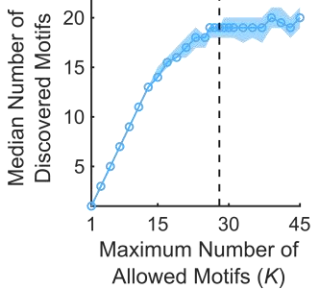
C Choice of ~1s Motif Duration (L) Did Not Restrict Motif Activity



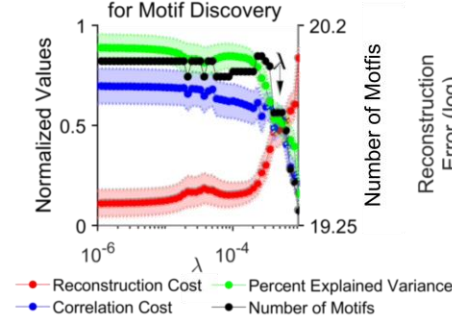
D Choice of ~1s Motif Duration (L) Did Not Limit Quality of Motif Fit



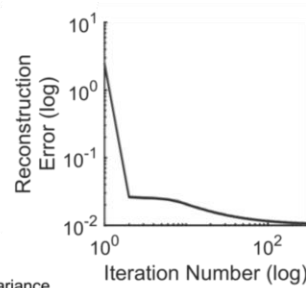
E Choice of Maximum Number of Motifs (K) Did Not Restrict Discovered Motifs



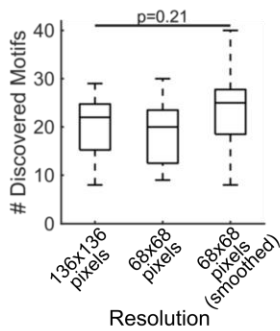
F Fitting CNMF Regularization Parameter λ for Motif Discovery



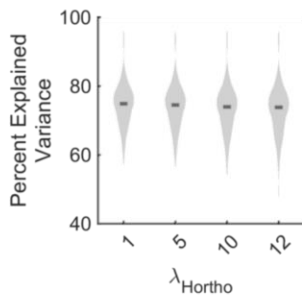
G Reconstruction Error During Fit



H Number of Discovered Motifs is Robust to Spatial Resolution



I Explained Variance is Robust to CNMF Temporal Sparsity Parameter λ_{Hortho} When Refitting To Withheld Data



J Dynamic Motifs Capture Majority of Spatial Variance in Neural Activity

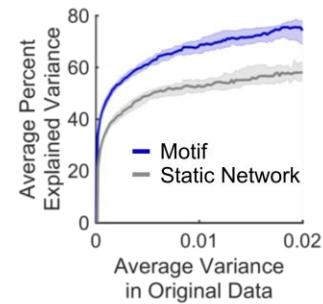


Figure S1. Anatomical parcellation, and impact of CNMF parameters on motif discovery. Related to Figures 1, 2, and 3. (A) Left: 2D projection of Allen Brain Atlas anatomical parcellation. **Right:** Allen Brain Atlas anatomical region labels overlaid on example mouse brain. Dotted white lines indicate manually drawn region outlines overlaid on motifs in main figure text. **(B)** Average pixel intensity during the motif timecourse across $N=2622$ discovered motifs. Prior to averaging, motifs were aligned to a random template motif using their maximum temporal cross correlation. Line and shaded regions show mean and SEM, respectively. Motif activity follows a parabolic trajectory, starting and returning to baseline within the ~ 1 second motif duration. **(C)** Duration of activity within motifs as a function of maximum motif length (parameter L ; see STAR Methods). Motifs were discovered using motif durations up to 5 seconds. Half-peak width (MATLAB; *findpeaks*) was used to estimate the relative duration of motif activity. Line and shaded regions show mean and SEM, respectively. Dotted line shows L value (975ms) used for main experiments. **(D)** Variance in neural activity captured by motifs as function of L across $N=144$ epochs. Display follows **C**. The variance in neural activity explained by the motifs plateaued at $L=975$ ms, suggesting that our choice of L did not constrain the explanatory power of the discovered motifs. **(E)** The median number of discovered motifs across $N=144$ epochs as a function of the maximum number of allowed motifs (parameter K ; see STAR Methods). Line and shaded regions show median and 95% CI, respectively. Dotted line shows K value (28) used for main experiments. The median number of discovered motifs plateaued before $K=28$, suggesting that our choice of K did not constrain the number of discovered motifs. **(F)** The effect of changing the spatio-temporal regulation parameter (λ) in the CNMF algorithm on reconstruction cost (red), correlation cost (blue), explained variance (green) and number of identified motifs (black). Each data point indicates the mean value from 20 fit epochs (randomly selected; no replacement); shaded regions indicate SEM. Y-axis units are arbitrary; values were normalized between 0 and 1 across λ values for each of the 20 fits. Chosen λ is indicated by arrow. **(G)** Reconstruction error as a function of iteration number of CNMF algorithm. All motif discovery factorizations were run for 300 iterations, at which point there was minimal improvement in reconstruction error. **(H)** Spatial resolution did not change number of discovered motifs. Spatial resolution indicated along x-axis. When smoothed, a 2D gaussian filter ($\sigma = [1,1]$ pixels; see STAR Methods) was convolved across each frame. Line, box, and whiskers denote median, 25th-75th percentile, and range respectively. Significance estimated with one-way ANOVA. **(I)** Percent of variance in neural activity explained by motif reconstructions as a function of temporal sparsity parameter λ_{Hortho} . Full distribution shown. Dark lines indicate median. **(J)** Percent of variance in neural activity explained by motif reconstructions (purple) and static networks (gray) of withheld epochs per timepoint. The explained variance was separately calculated for each timepoint of each epoch (1560 timepoints per epoch, 144 epochs; see STAR Methods). Timepoints were then binned according to the variance across the image in the original data (100 equal bins). The explained variance captured by the reconstruction was averaged per bin per epoch. Dark lines indicate median of 144 epochs. Shading indicates 95% confidence interval. Analyses performed on withheld data within the same animals (as in Figure 3D; purple).

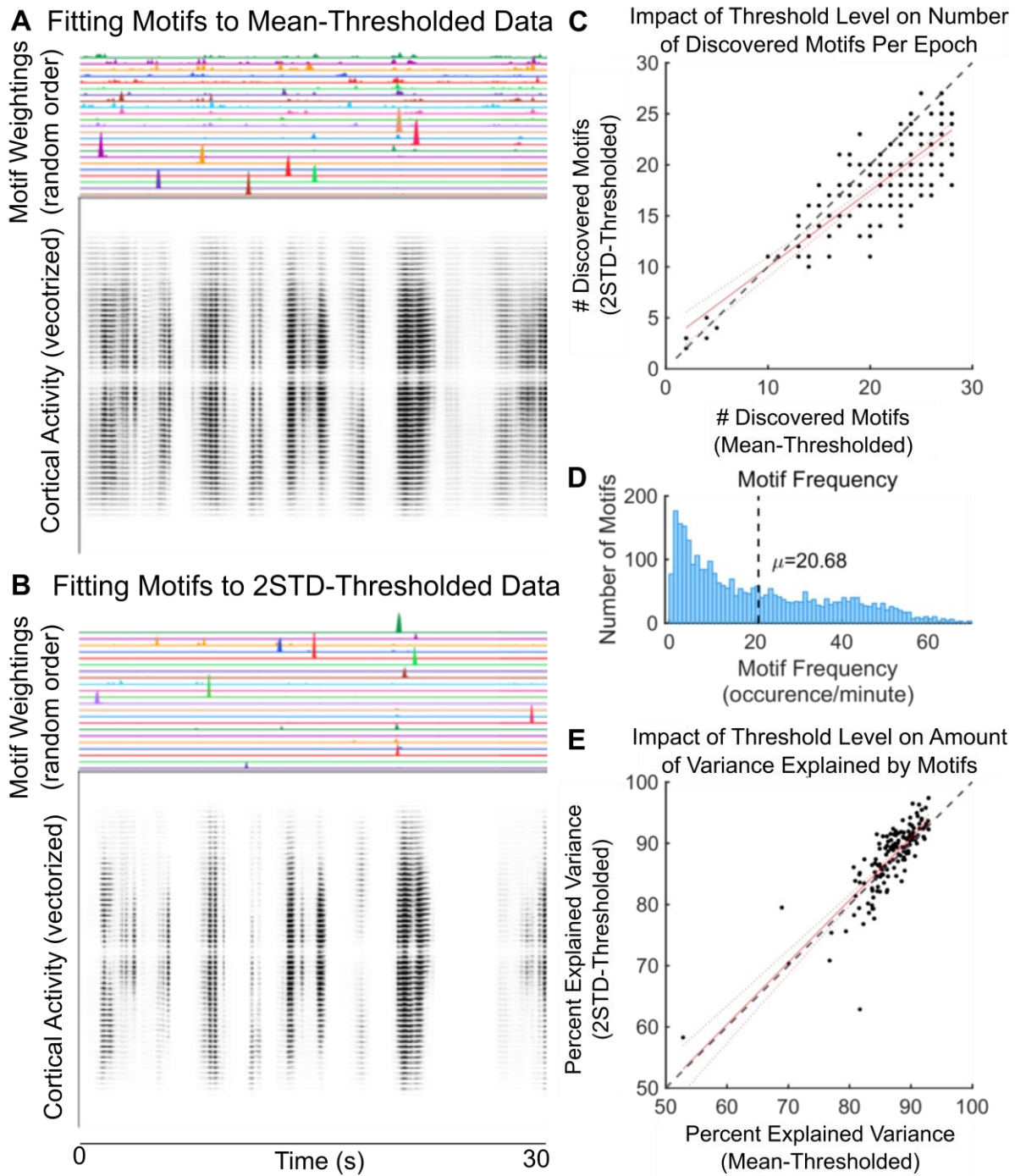


Figure S2. Impact of threshold level on discovered motifs (A-B). Related to Figures 1 and 2. 30-second epoch showing temporal weightings (top) of motifs fitted to widefield imaging data (bottom) in (A) data thresholded at the mean and (B) data thresholded at 2 standard deviations (2STD) above the mean. As individual motifs are unique to a given fit (due to randomness in CNMF fitting procedure), their temporal weightings are displayed in random order in each panel. As desired, applying the threshold removed low amplitude background signals, which sparsified

the observed activity patterns but maintained the global structure of cortical dynamics. **(C)** Similar numbers of motifs were observed in data 2STD-thresholded and mean-thresholded data. Black points show pairwise relationship between number of motifs discovered per (N=144) epochs in 2STD-thresholded and mean-thresholded data. Red line denotes linear least square fit to the data. Red dotted lines show 95% confidence bounds of fit. **(D)** Frequency of motif occurrence when fit to mean-thresholded data. Display follows Figure 2C. **(E)** Percent explained variance captured by motifs when fit to mean-thresholded and 2STD-thresholded data. Display follows **C**. Motifs fit at both threshold levels explained similar amounts of variance in neural activity (mean-thresholded: 87.25% CI: 86.70-87.98%; 2STD-thresholded: 89.05% CI: 87.78-89.68%).

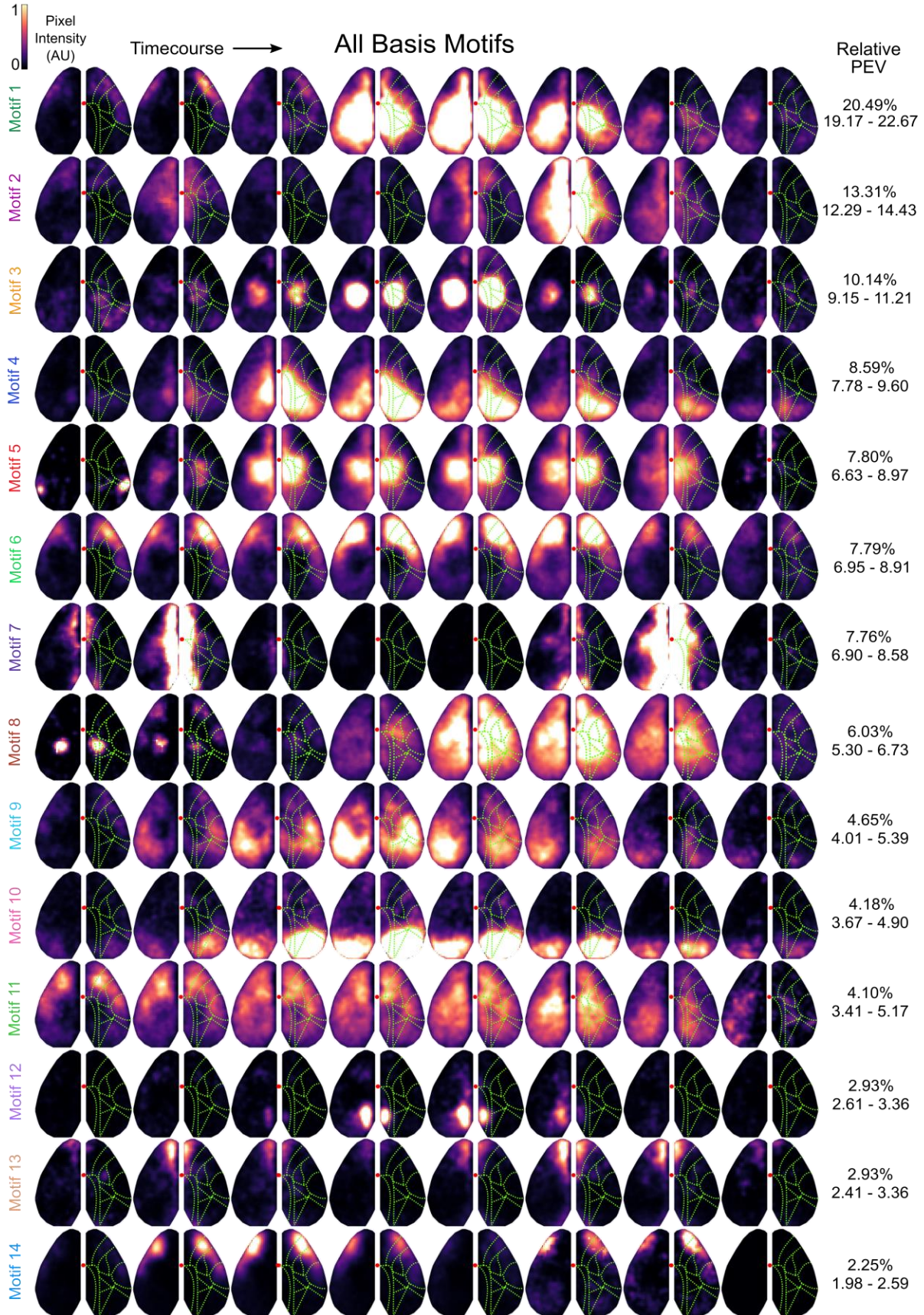
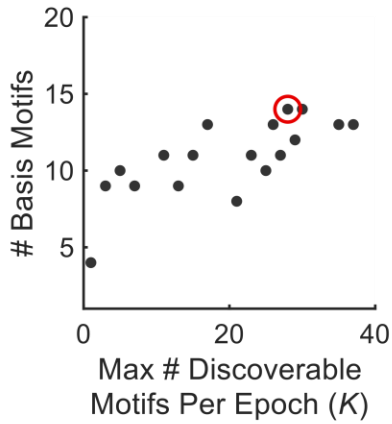
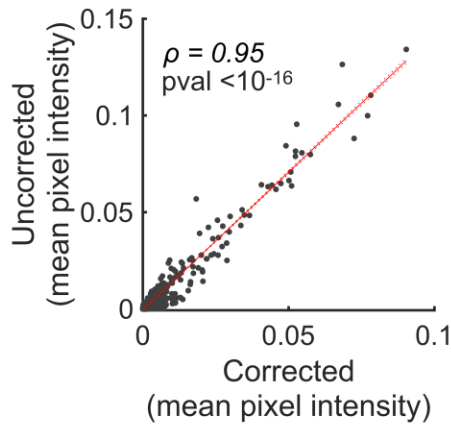


Figure S3. All Basis Motifs. Related to Figures 4-7. Display follows Figure 4C. Right column denotes the relative percent explained variance captured by each motif across N=144 recording epochs (mean and 95% CI). Motifs are ordered by decreasing explained variance. Complete timecourses are shown in Movie S2 and written descriptions are provided in Table S2.

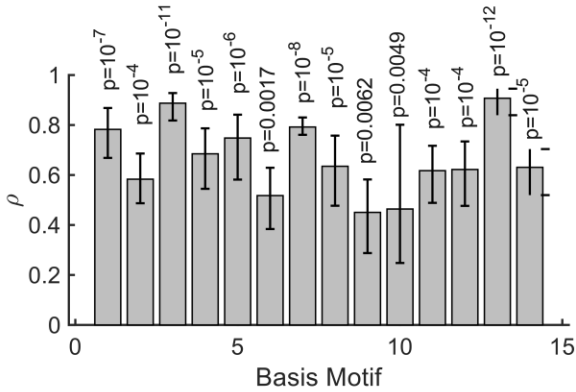
A Basis Motifs Are Robust to CNMF Parameters



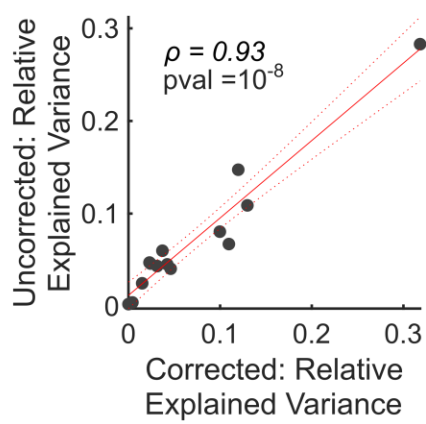
B Motif 1 Activity is Minimally Affected by Hemodynamics



C Basis Motif Activity in Corrected and Uncorrected Data is Highly Correlated



D Basis Motifs are Expressed Similarly in Corrected and Uncorrected Data



E Functional Parcellation of Mouse Cortex

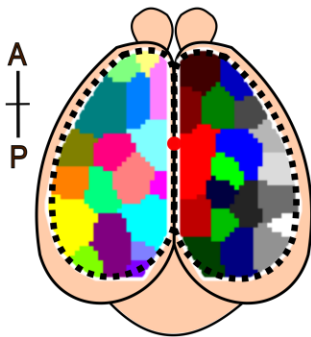


Figure S4. Additional characterizations and control experiments for basis motifs. Related to Figure 4. (A) Number of basis motifs discovered (y-axis) as a function of CNMF hyperparameter K, the maximum number of discoverable motifs allowed in a single epoch (x-axis). Motif discovery and clustering was repeated for each K value (see STAR Methods). Regardless of parameters, 10-14 basis motifs were identified. Red circle denotes K value (28) used for all experiments in the main text, conservatively chosen to maximize the number of basis motifs discovered. **(B)** Example correlation between average pixel intensity of motif 1 reconstruction before and after multiwavelength hemodynamic correction (see STAR Methods). Gray markers represent mean pixel intensity per timepoint. Solid and dotted red lines show linear least squares fit and 95% confidence bounds respectively. **(C)** The correlation in activity between corrected and uncorrected data was high for all motifs. Correlation is shown for N=30 2-min epochs across 2 animals. Mean and confidence intervals calculated on fisher z-transformed data before reconverting to Pearson's correlation coefficient. **(D)** Average relative variance explained by motifs in corrected and uncorrected epochs. Data points show mean of N=30 2-min epochs. Display follows **B**. **(E)** Estimating the 'working resolution' of widefield imaging approach. Parcellation of mouse cortex into functional clusters (N = 18 and 19 for left and right hemisphere, respectively). Functional clusters grouped pixels that were correlated over time (see STAR Methods). Each color denotes a separate functional cluster. Red dot indicates bregma.

Temporal Cross-Correlation Between Basis Motifs

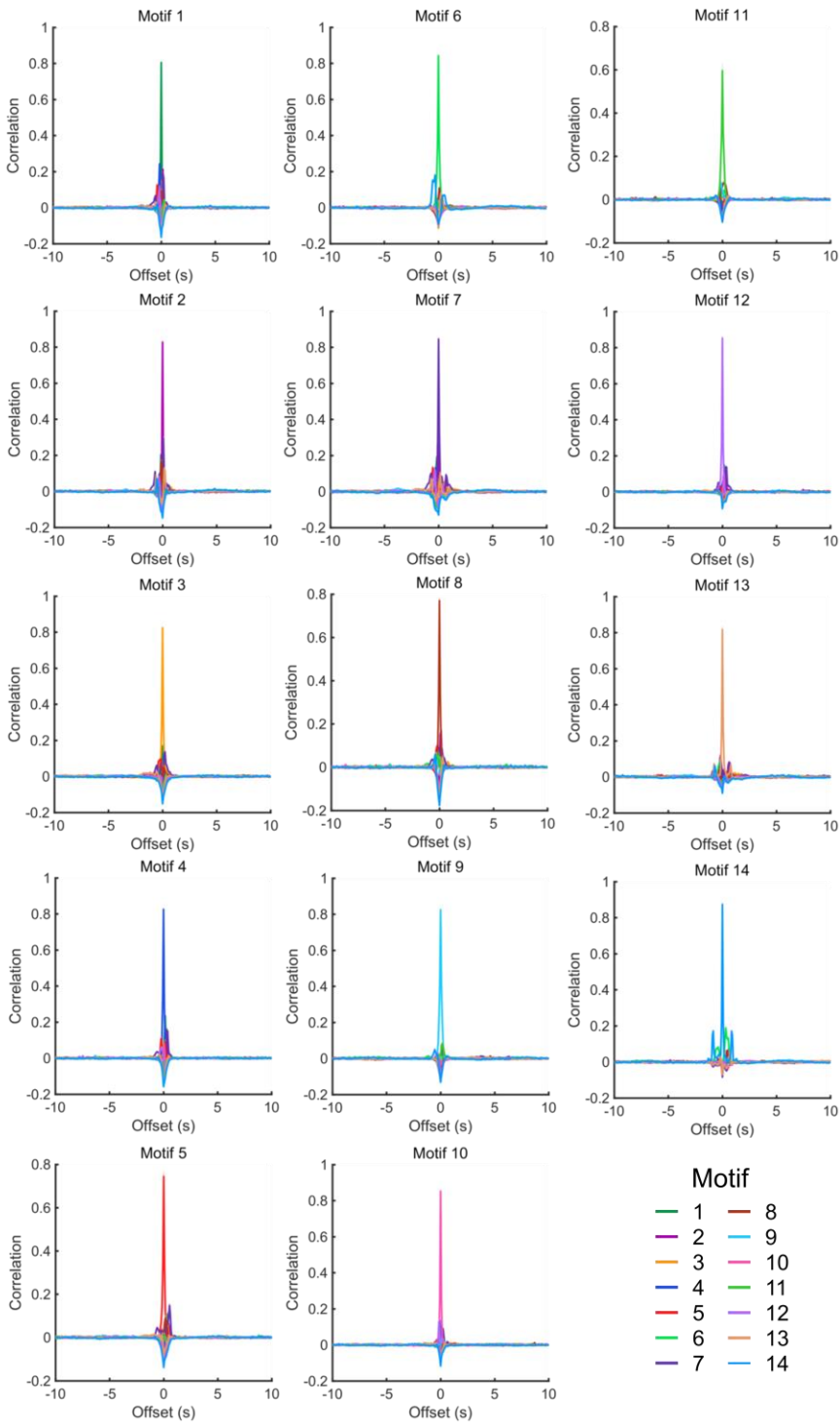
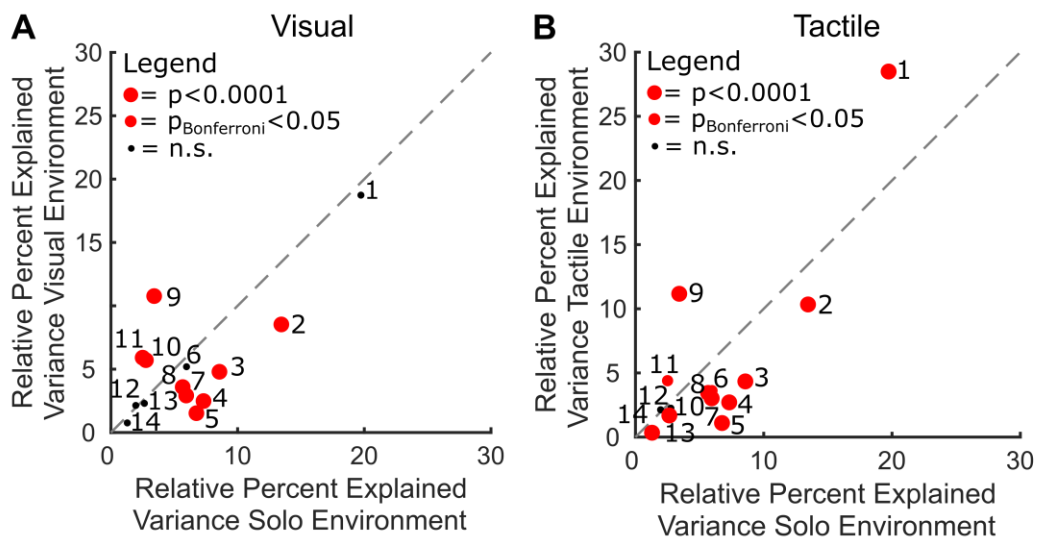


Figure S5. Temporal cross correlation between motifs. Related to Figure 4. Temporal cross-correlations (and autocorrelations) performed on the temporal weightings of basis motifs refit to $N=144$ withheld epochs. Line and shading reflect mean and SEM respectively. No obvious hierarchical structure was observed in the activation of different motifs.

Basis Motifs Are Expressed Different In Sensory And Solo Environments



Some Basis Motifs are Time-Locked to Stimulus Onset

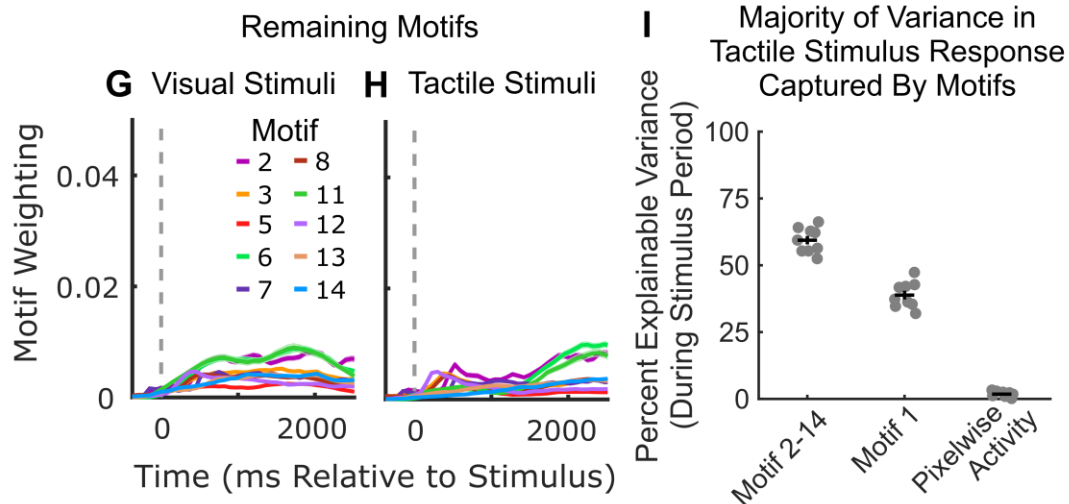
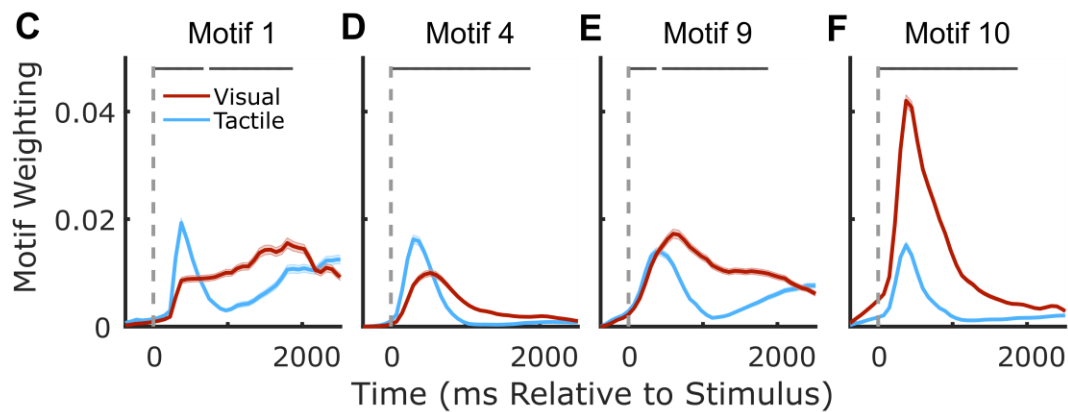


Figure S6. Additional results of basis motif activity in response to tactile and visual stimuli. Related to Figure 5. (A-B) Basis motifs are expressed differently in sensory and solo environments. Scatter plots show the relative PEV for each basis motif in the solo (e.g. at rest) environment (x-axis; N=144) versus in response to **(A)** visual or **(B)** tactile stimulation (y-axis; N=1109 and N=1110 visual and tactile samples, respectively). Motif labels are indicated with numbers, red markers indicate significant differences in expression rate between solo (x-axis) and sensory (y-axis) environments. Identity line shown along diagonal. Significance computed with Mann-Whitney U-test. 9/14 motifs had different PEV in visual environment, 11/14 in tactile environment, all significantly different at $p_{\text{Bonferroni}} < 0.05$, Mann-Whitney U-test; N=1109 visual and N=1110 tactile presentations, N=144 original epochs. **(C-F)** Timecourses of activity of motifs 1, 4, 9, and 10, relative to stimulus onset (vertical dotted line). Display follows Figure 5C. Interestingly, both motif 1 and 4 show similar response trajectories to tactile stimulation and were correlated with the average response to tactile stimuli (Pearson's $r=0.90$, $p < 10^{-16}$; $r=0.76$, $p < 10^{-16}$, for Motif 1 and 4, respectively). This suggests the average response to tactile stimuli involved both motifs 1 and 4. However, this was not because motif 1 and 4 were co-active: their activity was anti-correlated across trials ($R = -0.13$ CI: -0.20 to -0.07 , $p = 10^{-4}$, permutation test, left-tailed vs no correlation; correlation used the peak amplitude in activity per trial). This suggests that the same tactile stimulus evoked either motif 1 or 4. **(G-H)** Timecourses of remaining motifs in response to **(G)** visual or **(H)** tactile stimuli. Display follows 5C except with individual motifs colored according to legend in **G**. **(I)** The majority of variance in neural activity could be explained by motif activity, not stimulus-specific activation. Plot shows the percent of explainable variance in the neural response to tactile stimuli that is captured by non-specific motifs (left column), the stimulus specific motif (motif 1; middle column) and stimulus-specific residuals (right column). Follows Figure 6C. Data points correspond to mice (N=9). Black horizontal bars indicate mean and vertical bars indicate SEM.

Basis Motifs Discovered in
Social Environment Generalize
to Solo Environment

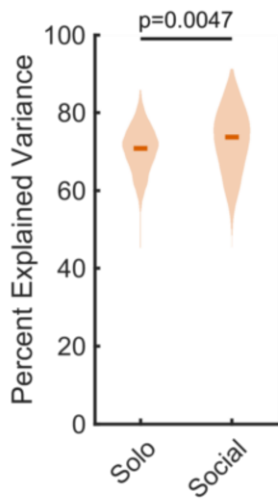


Figure S7. Basis motifs discovered on data from social environment recordings capture generalize to solo environments. Related to Figure 7. Display follows figure 7D. We sought to test whether basis motifs defined in the data from the social recording paradigm generalized to the solo recordings. Basis motifs were fit as in main text but were fit on 123 ‘discovery’ epochs from social, instead of solo, recordings. 11 basis motifs were identified. These basis motifs were then refit to withheld data as in Figure 7D (see STAR Methods). These basis motifs captured similar amount variance in both solo and social environments. solo=70.83% CI: 69.77-72.09%, social=73.72% CI: 71.54-75.05%; difference=2.89%, $p=0.0047$, Mann-Whitney U-test).

Mouse	9027	9029	9030	9031	9036	9040	9041	9042	9051
Median Number of Discovered Motifs	18.00	20.00	18.50	18.00	18.00	21.50	15.50	20.00	19.50
95% CI	16.00-19.00	17.00-24.00	17.00-20.00	14.00-20.00	15.00-19.00	19.00-23.50	13.00-18.00	18.50-23.00	13.50-22.00
Median Motif Frequency (per min)	2.23	2.28	2.06	3.28	2.63	2.47	3.08	2.19	1.92
95% CI	1.86-2.65	2.00-2.75	1.83-2.39	2.79-3.71	2.24-2.97	1.91-2.75	2.69-3.53	1.99-2.49	1.67-2.57
Median PEV Motif Discovery (%)	90.61	86.75	89.42	85.26	84.86	90.66	88.01	90.85	86.97
95% CI	89.33-91.35	82.63-94.18	87.60-90.43	82.09-88.32	81.38-89.11	86.87-92.47	84.15-91.12	89.07-92.02	79.19-90.67
Median PEV Motif Held out data (%)	76.40	74.59	74.49	74.64	75.02	74.05	78.09	75.88	73.59
95% CI	72.91-79.45	69.83-79.87	72.60-76.11	72.70-76.59	64.61-76.67	69.35-76.82	74.33-81.46	71.66-76.63	68.40-77.12
Median PEV Basis Motifs (%)	75.38	74.60	74.72	74.94	72.25	74.82	73.76	73.19	73.13
95% CI	71.67-77.93	69.47-78.15	72.24-78.06	71.90-76.10	68.37-79.24	71.19-76.37	71.87-78.84	69.99-75.39	72.48-74.23
Median Motif PEV During Sensory Trials (%)	62.06	57.02	55.47	61.54	58.9	61.2	62.35	66.08	55.4
95% CI	59.94-64.88	54.5-58.95	53.06-58.27	58.9-64.22	56.82-62.12	59.36-64.11	59.1-64.53	64.55-69.3	53.3-58.16

Table S1. Main text statistics computed for each mouse. Related to Figures 1-7. Statistics computed per epoch as described in main text and STAR Methods.

Basis Motif	Ordered List of Areas Activated	General Description and Duration
1	[anterolateral M1/M2] → [medial M1/M2, PC, anterior RSP] → [PC, V1]	Anterior-to-posterior wave of activity across cortex. 600ms
2	[anteromedial M1/M2] → [anteromedial M1/M2] → [RSP, medial PC]	Discrete anteromedial secondary motor cortex activity followed by anterior-to-posterior wave of activity from medial motor cortex to retrosplenial areas. 1125ms
3	[PC]	Localized burst in parietal regions. 750ms
4	RSP → [RSP, amPC] → [RSP, amPC, VC] → [posterior RSP, VC]	Posterolateral wave of activity from retrosplenial and visual areas. 825ms
5	RL → PC → [PC, medial M2]	Discrete rostrolateral activity followed by activity in parietal and medial regions. 1275ms
6	[anterolateral M1/M2, SS, BC] → [anteromedial M1/M2, SS]	Wave of activity from anterolateral somatosensory, motor, and barrel cortex to anteromedial somatosensory and motor areas. 975ms
7	[medial M2, RSP] → [medial M2, RSP]	Consecutive bursts in activity in medial regions. 975ms
8	PC → [PC, BC, anterolateral M1/M2] → all dorsal cortex	Discrete parietal and anterior-lateral somatosensory and motor area activity followed by generalized activity across entire cortex. 1275ms
9	[BC, RL] → [BC, RL, amPC, anterior VC] → VC	Posteromedial wave of activity from barrel cortex to visual areas. 975ms
10	[V1, PM, posterior RSP]	Localized burst in visual areas. 675ms
11	[anterolateral M1/M2, SS, BC] → [medial M1/M2/SS/BC] → PC → [RSP, PC, V1]	Anterior-to-posterior wave of activity across cortex. 1050ms
12	RSP	Burst in activity in retrosplenial cortex. 375ms
13	M2 → M2	Consecutive bursts in activity in secondary motor cortex. 1200ms
14	[anterolateral M1/M2, SS, BC] → [anterolateral M1/M2, SS] → [anterolateral M1/M2]	Posterior-to-anterior wave of activity within anterolateral somatosensory, motor, and barrel cortex, followed by burst of activity in anterolateral motor areas. 1125ms

[] denotes co-activated areas. → denotes sequential activation.
SM = secondary motor, PM = primary motor, SS = somatosensory, BC = Barrel Cortex, PC = parietal cortex (all), amPC = anterior-medial parietal cortex, aPC = anterior parietal cortex, RL = rostrolateral cortex, VC = primary visual cortex, RSP = retrosplenial cortex (all).
*Durations are approximate, as motifs are convolved with temporal weightings vectors to reconstruct neural activity

Table S2. Detailed description of each motif. Related to Figure 4. See also Figure S3 and Video S2.

	Discovery: Solo	Fit: Solo	Fit: Paired Social	Fit: Sensory	Discovery: Paired Social	Fit: Paired Social
K	28	14 (# basis motifs)	14 (# basis motifs)	14 (# basis motifs)	28	11 (# social basis motifs)
L	13	13	13	13	13	13
λ	0.0005	0	0	0	0.003	0
W_{init}	Random	Basis Motifs	Basis Motifs	Basis Motifs	Random	Complex Basis Motifs
H_{init}	Random	Random	Random	Random	Random	Random
λ_{orthoH}	1	1	1	0	1	1
λ_{orthoW}	0	0	0	0	0	0
Iterations	300	100	100	100	300	100
Tolerance	0	0	0	0	0	0
Shift	0	0	0	0	0	0
$W_{\lambda L1}$	0	0	0	0	0	0
$H_{\lambda L1}$	1	0	0	0	1	0
W_{fixed}	0	1	1	1	0	1
SortFactors	0	0	0	0	0	0
W_{update}	1	0	0	0	1	0

Table S3. CNMF parameters used in each experiment. Related to Figures 1-7. See STAR Methods for descriptions of parameter choices and fitting procedures.