
STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

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ABSTRACT

Information is processed by networks of neurons in the brain. On the timescale of sensory processing, those neuronal networks have relatively fixed anatomical connectivity, while functional connectivity, which defines the interactions between neurons, can vary depending on the ongoing activity of the neurons within the network. We thus hypothesized that different types of stimuli, which drive different neuronal activities in the network, could lead those networks to display stimulus-dependent functional connectivity patterns. To test this hypothesis, we analyzed electrophysiological data from the Allen Brain Observatory, which utilized Neuropixels probes to simultaneously record stimulus-evoked activity from hundreds of neurons across 6 different regions of mouse visual cortex. The recordings had single-cell resolution and high temporal fidelity, enabling us to determine fine-scale functional connectivity. Comparing the functional connectivity patterns observed when different stimuli were presented to the mice, we made several nontrivial observations. First, while the frequencies of different connectivity motifs (i.e., the patterns of connectivity between triplets of neurons) were preserved across stimuli, the identities of the neurons within those motifs changed. This means that functional connectivity dynamically changes along with the input stimulus, but does so in a way that preserves the motif frequencies. Secondly, we found that the degree to which functional modules are contained within a single brain region (as opposed to being distributed between regions) increases with increasing stimulus complexity. This suggests a mechanism for how the brain could dynamically alter its computations based on its inputs. Altogether, our work reveals unexpected stimulus-dependence to the way groups of neurons interact to process incoming sensory information.

20

1 Introduction

21 Visual information is processed by networks of neurons spanning multiple regions of the neocortex. The interactions
22 between these neurons determine the sensory information extracted by the brain and used to guide behavior. For this
23 reason, much prior work has investigated properties of the networks that define the interactions between neurons in
24 visual cortex. For example, some work has focused on the patterns of anatomical connectivity between individual
25 neurons [1, 2, 3, 4], or between larger voxels of cortical tissue [5, 6, 7, 8]. At the same time, functional connectivity
26 networks – which describe the interactions between neurons – can differ substantially from anatomical networks
27 [9, 10, 11]. Notably, while anatomical connectivity is relatively fixed on the timescale of sensory processing, functional
28 connectivity can vary as the neurons within the network adapt quickly to different stimuli [12]. This motivated us

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

29 to ask whether and how different stimuli might engage different functional networks with single-neuron resolution
30 within the visual cortex. Despite the clear importance of this question for understanding visual processing, and the
31 substantial literature on functional and anatomical neural network structures (reviewed below), we are unaware of any
32 prior work that addressed how the topological structure of functional connectivity networks between individual neurons
33 spanning multiple regions varies as the stimulus changes. To fill this knowledge gap, we applied network analyses to
34 simultaneous recordings from hundreds of neurons in mouse visual cortex. Our results indicate a surprising degree of
35 stimulus-dependence to the topological structure of functional networks between individual neurons in visual cortex.

36 Previous work investigated anatomical connectivity between cortical neurons and regions using electron microscopy
37 [13, 4], paired intracellular electrophysiology recordings [1, 2], viral tracing [5, 14], and diffusion tensor imaging
38 [15]. These studies revealed many interesting features of anatomical neuronal connectivity networks, like their
39 modular organization and small-worldness [14, 16, 5, 17], and their hierarchical structure [14]. While anatomical
40 connectivity (e.g., synaptic connections between neurons) remains relatively static over the timescale of processing
41 visual inputs, functional connectivity can be much more dynamic, thus motivating efforts to understand the relation
42 between functional and anatomical connectivity [18, 19, 11, 7, 20]. These efforts are complicated by the fact that
43 different types of stimuli lead to different dynamical patterns of neural activity and to different degrees of correlation
44 between neurons [21, 22, 23, 24, 25]. Because functional connectivity depends on these properties – e.g., on the
45 time-lagged correlation between the activities of neuron pairs [26, 27] – the functional connectivity can depend on the
46 stimulus presented in the experiment.

47 Despite this potential complication, stimulus- and task-related functional connectivity patterns obtained at a coarse
48 scale using non-invasive functional magnetic resonance imaging (fMRI) have been reported to resemble resting-state
49 functional connectivity patterns [28, 29, 30], while resting-state connectivity in turn resembles anatomical connectivity
50 patterns [31]. In other reports – again, derived from fMRI experiments – stimulus-evoked functional interactions were
51 found to vary with tasks or cognitive states [32, 33, 34, 35, 36]. These fMRI studies raised the important question of
52 whether and how the functional connectivity of the underlying neuronal networks (i.e., at a finer single-neuron scale)
53 might change with stimulus or task conditions.

54 Functional connectivity at this finer scale is less well-studied due to challenges in simultaneous recordings from
55 large populations of neurons with high spatial and temporal resolution. Despite these limitations, prior work has
56 shown that functional connectivity: 1) is much more stimulus-dependent for high-frequency oscillatory activity than for
57 low-frequency [37]; 2) varies by cell type with the cortex [38]; 3) depends on the contrast of a visual stimulus [39]; and
58 4) reflects the existence of two main groups of neurons, one whose activities follow those of the rest of the population,
59 and one whose activities do not [24]. While these studies have revealed much about the stimulus-dependence of
60 functional networks at single-neuron resolution, they have not included detailed analyses of networks spanning multiple
61 brain regions. On the other hand, the previous reports of network analysis applied to single-neuron resolution functional
62 connectivity networks, focused on responses mainly to drifting grating stimulus with spontaneous activity as a baseline
63 comparison [26, 27], thus precluding an assessment of stimulus-dependent network structure. Therefore, it is still
64 unclear whether and how the topological organization of these functional networks (either within a brain region, or
65 spanning multiple regions) depends on stimulus properties or other context-defining variables [40].

66 To fill this gap, we used network analysis methods (similar to those of [26, 27]) to analyze the functional connectivity
67 networks measured in response to 6 different types of stimuli, of varying degrees of complexity, ranging from full-field
68 flashes up to natural movies. These networks were obtained from the simultaneously recorded activities of hundreds of
69 neurons in 6 different cortical regions with implanted Neuropixels probes [26]. Thus, we were able to identify functional
70 networks for each stimulus type, which spanned multiple brain regions. Note that to focus on between-stimulus analyses,
71 we constructed one network based on all conditions for each stimulus, hence the functional networks embody total
72 correlations rather than signal or noise correlations. By studying the structures of these networks and how they varied
73 with stimulus type, we identified several surprising features of the functional networks. First, while the distribution
74 of different types of 3-neuron connectivity motifs were quite similar for the different stimuli, the specific identities
75 of the neurons within those motifs depended on the stimulus. This means that the cortical network is dynamically
76 reorganized as the stimulus changes, but does so in a manner that preserves the motif frequencies. This finding points to
77 a potentially fundamental role for these motif distributions in maintaining the function of the cortical networks [41, 42].
78 Secondly, we identified highly-interacting *modules* [43, 44] and found that these modules were much more localized
79 to a single brain region (as opposed to being distributed between regions) for stimuli with higher complexity, such as
80 natural movies. Our results thus reveal distinct stimulus-dependent topology of cortical functional networks, and imply
81 a key organizational principle underlying that stimulus-dependence: preserved relative motif frequencies.

82 2 Results

83 To determine whether and how visual cortical functional connectivity networks depend on the stimulus presented
84 to the animal, we analyzed data from Neuropixels probes inserted into six visual regions of mouse cortex (Fig. 1A:
85 V1, LM, RL, AL, PM, AM), which is previously released by Allen Institute [26]. These probes simultaneously
86 recorded neural activity from each of these six regions while the mice were presented with visual stimuli of varying
87 degrees of complexity (Fig. 1B): flashes, drifting gratings, static gratings, natural scenes and movies, and gray screen
88 (approximation for resting state, or spontaneous activity). From the responses to each stimulus, we extracted the
89 directed functional connectivity using cross-correlograms (CCGs) between the spiking responses of pairs of neurons
90 (Fig. 1C). In order to take polysynaptic connections into consideration [45], we examined ‘sharp intervals’ instead of
91 the ‘sharp peaks’ that might be used to identify monosynaptic connections[46, 47, 26]. These sharp intervals were
92 defined to have a short latency and potentially multiple time lags, and were detected by searching for statistically
93 significantly outlying values in the CCG. Identification of bidirectional connections was made possible by limiting lag
94 τ to be non-negative, and each significant connection was defined as excitatory or inhibitory depending on the sign
95 of the significantly outlying CCG value (see Fig. 1C and Methods), similar to the definition used in previous work
96 [48]. Intuitively, if the spiking of the source neuron is statistically strongly correlated with the firing or non-firing of the
97 target neuron with a short time lag, then there exists an excitatory or inhibitory functional connection between them.

98 To obtain a comprehensive understanding of the stimulus-dependent structure of the functional connectivity networks
99 (Fig. 1D), we conducted network analyses at multiple topological scales, ranging from the properties of pairwise
100 connections to the local connectivity patterns of third-order functional motifs, up to larger-scale functional modules.
101 Our control analysis on running speed (not shown) showed that our subsequent observations are indeed determined by
102 the stimulus and not by locomotion.

103 2.1 Stimulus dependency of functional connectivity networks

104 We first investigated overall patterns of functional connections between neurons across stimulus types by comparing the
105 functional connectivity matrices. We found there are some common network features observed across stimulus types.
106 Specifically, the functional networks observed during all visual stimuli exhibited heavy-tailed degree distributions
107 (Supplementary Fig. 1B). Networks with this property are known to be robust to random failures [49], however, they
108 are more vulnerable to targeted attacks on hub neurons which could lead to reduced network efficiency as observed in
109 Alzheimer’s patients [50, 51].

110 While functional networks show some shared characteristics like heavy-tailed degree distributions across stimuli,
111 we also observed network properties vary with stimulus complexity. We found that natural stimuli (natural scenes and
112 movies) tended to evoke fewer functional connections than grating stimuli (both static and drifting gratings) while
113 full field flashes drive the least correlated neural activities, on the same level as resting state activity (Fig. 1E). These
114 findings are consistent with previous reports that natural stimuli decorrelate neurons in primary visual cortex (V1)
115 [52, 53]. While these previous works focus on V1, our results suggest that decorrelation by natural stimuli is a general
116 property of cortical circuits: it is found in higher visual cortical areas as well.

117 The differences in network density mainly originate from differences in excitatory connections (Fig. 1E), which
118 results in the strong correlation between the fraction of excitatory connections and the network density (Fig. 1F, middle).
119 Even though natural stimuli do not evoke the densest functional networks, the fraction of within-area connections is
120 largest for static gratings and natural stimuli (Fig. 1F, left, and Supplementary Fig. 1A; $p < 10^{-3}$, Kolmogorov-Smirnov
121 test). This is closely related to the stimulus-dependent differences in modular network structure, which we analyzed in
122 more details later in this paper.

123 To determine how the stimulus-dependence of the network density affects the network’s topological structure, we
124 measured the tendency for triplets of neurons to form closed triangles (e.g., three-neuron motifs 6,9-13 in Fig. 2B).
125 This tendency is quantified by the clustering coefficient, and we found that it increases with increasing network density
126 regardless of stimulus type (Fig. 1F, right).

127 Motivated by previous work showing that neurons with similar preferences tend to connect with each other [2, 4, 54],
128 we compared the tuning similarity of neuronal pairs connected with excitatory and inhibitory connections. To perform
129 this comparison, we computed the kernel density estimation (KDE) for signal correlation during presentation of four
130 visual stimulus types. Signal correlation is defined as the correlation between average responses of neurons to different
131 stimulus conditions which is used to test whether two neurons have similar tuning curves [21, 55]. We computed
132 these signal correlation separately for pairs with excitatory connections, inhibitory connections, and those with no
133 connections (Fig. 1G). For natural movies, we regarded each frame as a different stimulus condition when computing
134 the signal correlation [2]. Since there are only two conditions for flashes (dark or light), the signal correlation of either 1

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

135 or -1 could be trivial and thus is not considered in this analysis. Similarly, the signal correlation is ill-defined for the
136 blank gray screen stimulus, and thus it was also omitted from this analysis.

137 For all visual stimuli, the signal correlations for connected neuron pairs tended to be larger than for disconnected
138 pairs, which had distributions centered around zero (Fig. 1G; $p < 10^{-144}$, rank-sum test). Additionally, neurons with
139 excitatory connections tended to have higher signal correlations than did pairs with inhibitory connections (Fig. 1G;
140 $p < 10^{-3}$, rank-sum test).

141 In agreement with the previous findings that neurons close in space or sharing similar tuning curves are more likely
142 to have synaptic connections [54, 2], we found the probability of functional connections decreases with increasing
143 distance and increases with their increasing signal correlation (Supplementary Fig. 2A,B; Cochran-Armitage test). In
144 addition, the probability of a functional connection being excitatory/inhibitory significantly increased/decreased with
145 signal correlation during all visual stimuli (Supplementary Fig. 2C,D; Cochran-Armitage test), indicating that even
146 though neurons with similar preferences generally tend to be connected, the sign of the connection depends on the
147 extent of their tuning similarity.

148 Collectively, these analyses show that network density, fraction of connections that are within a brain region (as
149 opposed to between regions), clustering coefficient, and the distribution of signal correlation, depend systematically on
150 the stimulus type.

151 2.2 Stimulus dependency of functional connectivity motifs

152 Having observed stimulus-dependency of the general network properties, we next turned our attention to the properties
153 of the functional motifs. Specifically, we investigated two- and three-node motifs in the functional connectivity network.
154 Similar to anatomically-defined structural motifs which form fundamental building blocks of neural circuits [56, 1, 57],
155 functional motifs, which are defined by correlated neural activities, represent elementary information processing
156 components of a functional network [58].

157 To understand the distribution of two- and three-neuron motifs in each functional network, we adopted the intensity
158 method for functional motif detection. This method computes the Z-score of the intensity for a given motif by comparing
159 the motif frequency in the empirical network and in a randomly-generated surrogate network [59]. This method thus
160 identifies how much more (or less) prevalent the motif is in the real network than would be expected by chance in a
161 reference network randomly shuffled with certain preserved properties (e.g., density, degree distribution, etc.).

162 Note that, to characterize pairwise signed connections (i.e. signed two-neuron motifs), it is necessary to preserve
163 the edge sign distribution when generating density-matched randomized surrogate networks[60]. For this reason, we
164 included edge signs in the pair-preserving model [56] that preserves the distribution of (n-1)-neuron motifs and used the
165 resultant Signed-pair-preserving model to generate the surrogate network with the preserved signed (n-1)-neuron motifs
166 for comparing the motif frequency between the true network and the randomly-generated one.

167 As for two-neuron motifs, we found that bidirectional signed functional connections are much more frequent than
168 would be expected by chance in the random network (Fig. 2A). This recapitulates the structural observations that
169 bidirectional synaptic connections are highly over-represented in cortex relative to density-matched random networks
170 [1].

171 We further studied distributions of three-neuron motifs in functional networks. There are 13 types of connected
172 three-neuron motifs (Fig. 2B), of which the Feedforward Loop (FFL) is arguably the most studied type due to its
173 ubiquitous nature in empirical networks such as gene systems and neuronal networks [61]. The lollipop plot in Fig. 2C
174 shows the intensity Z score obtained using the above intensity method for all types of signed three-neuron motifs ordered
175 by their corresponding unsigned motif types. The colors in the plot represent each unsigned type (see Supplementary
176 Fig. 4 for the full set of signed connectivity patterns). Interestingly, the most salient motifs were the same for the
177 different visual stimuli. Specifically, the top six over-represented motifs are the same for different stimuli being tested
178 here (Fig. 2C, motif ID = e6, e9, e10, e11, e12, e13). In addition to these significantly over-represented motifs,
179 the same three types of motifs were significantly under-represented for all stimuli (ID = e4, e5, e8). Interestingly,
180 all 6 over-represented motifs contain at least one excitatory FFL (eFFL) structure (ID = e6, Fig. 2C) with the only
181 difference among them being the number of mutual connections that accompany the eFFL structure. The last five of
182 these over-represented motifs were studied without edge signs in previous works as ‘mixed-feedforward-feedback loops’
183 and have been found to be correlated to memory, as well as acceleration and delay of response [62]. Here we denote all
184 six of the over-represented signed motifs as excitatory-feedforward-loop-based (eFFLb) motifs.

185 In addition to the three highly under-represented motifs (ID = e4, e5, e8), the set of under-represented motif patterns
186 consists largely of ‘unclosed’ eFFLb motifs, suggesting that neurons tend to form pairwise-connected triplets. This
187 under-representation of unclosed motifs appears to be more pronounced with higher network density. Interestingly, the

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

188 motifs' average absolute intensity Z scores (deviation from the frequency expected by chance) increases significantly
189 with increasing network density (Fig. 3A). With more connections, the empirical functional network deviates more
190 strongly from randomized surrogate networks, highlighting the fundamental non-randomness of local functional
191 connectivity.

192 Note that it is important to examine the whole significance distribution of motifs instead of focusing on merely the
193 most striking ones. Varying the threshold on significant motifs naturally changes their total count, but importantly, it
194 does not substantially change the relationship between the functional connectivity patterns observed for the different
195 stimuli (Supplementary Fig. 5B). Our control analysis has led us to the conclusion that the presence of non-random
196 local topology of functional networks is contingent upon the density of the network(Fig. 3A,B), which is, in turn,
197 modulated by sensory input (Fig. 1E). Meanwhile, the same sets of two- and three-neuron connectivity motifs are over-
198 and under-represented for all 6 visual stimuli.

199 Our analysis of the connectivity motifs relies on comparing motif frequencies in the observed network to those
200 reference networks that are similar in some way to the observed network but are otherwise randomized. Notably,
201 there are many different ways to define random networks by preserving certain network properties. For example, the
202 commonly used Erdős-Rényi reference network preserves the network density. Other reference models can preserve the
203 degree distribution, the neuron pair distribution, or the signed neuron pair distribution (see Methods). To understand
204 how our motif analysis depends on the choice of reference model, we computed the three-neuron motif intensities for
205 the natural movies stimulus for 4 different reference models with increasingly more preserved network properties (from
206 left to right in Fig. 3F). As a result, the overall significance level for all motif types roughly decreases in the same order,
207 and the most strictly conserved reference model, the signed pair-preserving reference model, is better in identifying
208 the small subset of motifs for which the observed network is most truly non-random. Therefore, we use the signed
209 pair-preserving reference model as our default reference model throughout this study.

210 To test the robustness of our results, we separately calculated the motif distribution patterns on distinct halves of the
211 trials from our natural scene stimulus data. The high consistency between the motif distributions on the two data splits
212 (Fig.S11) suggests that noise within the dataset is unlikely to be a key factor in our results. Moreover, eFFLb motifs
213 remain to be the most salient motifs across different stimuli on various significance levels (Fig.S12). Taken together,
214 eFFLb motifs seem to be reliably the most significant patterns among three-neuron subgraphs.

215 **2.3 Properties of over-represented three-neuron motifs**

216 While the three-neuron functional connectivity motifs that were most over- or under-represented were the same for
217 all stimuli (Fig. 3B), those motifs were composed of different neurons for different stimuli (Fig. 3D,E). Specifically,
218 while most eFFLb motifs contained at least one neuron from V1, the fraction containing all 3 neurons within V1 varied
219 substantially as the stimulus changed. (Fig. 3D).

220 To further quantify the extent to which the same neurons constitute eFFLb motifs across stimuli, we computed
221 the number of eFFLb motifs sharing exactly the same neurons for all pairs of stimuli (intersection sizes), as shown in
222 Fig. 3E for one example over-represented eFFLb motif (ID=e6; results for all of the over-represented eFFLb motifs
223 are shown in Supplementary Fig. 6). We also compared motif overlapping detected using half the trials of the same
224 stimulus and using different stimuli (Supplementary Fig. 11). Notably, eFFLb motifs were more likely to be composed
225 of the same neurons during the same stimulus than for different stimuli, indicating that the identities of the neurons
226 within the over-represented motifs change as the stimulus changes.

227 Taken together, these results and those in Fig. 2C, indicate that, as the stimulus changes, the same three-neuron
228 motifs are over- or under-represented in the cortical networks, but the identities of the neurons within those motifs
229 change. This suggests that these specific motifs might have strong functional importance for the cortical microcircuit
230 because even as different stimuli dynamically alter the functional connectivity, they do so in a way that preserves these
231 motif patterns.

232 The fact that the eFFLb motifs were over-represented for all stimuli even with different constituent neurons
233 suggested that they might play an important role for the cortical microcircuit. To further investigate this question, we
234 analyzed the tuning similarity of these motifs. We found the signal correlation between pairs of connected neurons
235 within the same eFFLb motif were significantly higher than those not within the same eFFLb motif (Fig. 3C; * $p < 0.05$,
236 *** $p < 0.0001$, Student's t-test), and neurons within the same eFFLb motif are spatially closer to each other than
237 otherwise (Supplementary Fig. 5A, right; ** $p < 10^{-4}$, Student's t-test). Furthermore, neuron pairs within eFFLb
238 motifs show stronger functional connection strengths and higher CCG Z-scores than other connected neuron pairs
239 (Supplementary Fig. 5A; *** $p < 10^{-4}$, Student's t-test). Thus, the eFFLb motifs tend to consist of functionally-
240 similar neurons. These observations are consistent with previous reports of synaptic connectivity patterns in visual
241 cortex [1].

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

242 Overall, these analyses indicate that neuron pairs within the over-represented eFFLb motifs tend to be spatially near
243 each other, and to have higher functional similarity compared to other pairs. Coupled with the fact that these eFFLb
244 motifs are preserved across stimuli, this highlights the potential functional importance of these motifs within the cortical
245 microcircuit.

246 **2.4 Spatial and functional organization of network modules depends on the stimulus**

247 Having identified the fundamental motif structures as computational building blocks regardless of stimulus types, we
248 next asked how the topology of larger groups of neurons, or modules, depends on stimulus properties. Those *modules*
249 [63, 64] are thought to impart added robustness [65], efficiency [66], and functional specialization [44] to networks. We
250 thus sought to identify modules within our networks, and to determine how their properties depend on the stimulus
251 presented to the animal.

252 To achieve this goal, we revised the Louvain method [67] to optimize the Modularity estimation from previous
253 work [68, 69] so as to take into account the signs of the connections in our networks: this modified Louvain method
254 searched for sets of modules with many excitatory connections inside the same module and inhibitory connections
255 between different modules (see Methods). Thus, the method identifies sets of modules whose neurons are internally
256 correlated and externally anti- or un-correlated. This greedy optimization yields the groupings of neurons into modules
257 by maximizing the score of modified Modularity (see Methods). For comparison, we also identified modules with
258 the original Modularity algorithm that does not take into account the edge signs [65]. Although our adapted method
259 revealed results qualitatively similar to the original one (Supplementary Fig. 10), the identified module size using our
260 method is relatively smaller, suggesting a finer scale module detection with our method.

261 Unless otherwise stated, in the rest of this paper Modularity means the modified Modularity for signed module
262 detection. By maximizing the two-dimensional Modularity difference map whose dimensions correspond to resolution
263 parameters for excitatory and inhibitory links, we determined the optimal resolution parameters that control the scale of
264 identified modular structure so that the empirical network deviates most from the null model [70, 14] (see Methods;
265 Supplementary Fig. 7A). In light of the potential limitations posed by a fixed resolution parameter, we analyzed
266 multi-resolution module partitioning and found consistent results (Supplementary Fig. 7B,C,D).

267 After identifying the best parameters, we applied our module detection algorithm to the observed functional
268 networks from each stimulus, and compared the results between stimuli. During gratings and natural stimuli, functional
269 networks tend to exhibit stronger modular structures, characterized by larger deviations in Modularity from expectation
270 (Fig. 4A, bottom z-score, 53.94 ± 8.81). On the contrary, the networks obtained from flashes and in the resting state
271 were less modular (6.22 ± 4.44 ; $p = 3.4 \times 10^{-9}$, rank-sum test).

272 Anatomically parcellated brain regions are thought to work as natural modules with specialized functions [71, 72,
273 73, 74]. We thus wanted to understand how our functionally-defined modules relate to the anatomically-defined brain
274 regions. To achieve this goal, we analyzed the identified functional modules to understand the extent to which their
275 spatial organization coincided with the anatomically-defined brain regions, and the extent to which that depended on
276 the stimulus. To do this, we computed three measures from the modules for each stimulus (Fig. 4B, left). First, the
277 *coverage* quantifies the maximum extent to which a given module covers all of the neurons in any single brain region.
278 Second, the *purity* quantifies the maximum extent to which a given module is contained within any single brain region.
279 These two quantities are computed for each module, and the results in Fig. 4 show their weighted average (averaged
280 over modules, weighted by module size). A more detailed module-by-module analysis is presented in Fig. 5, below.
281 Finally, the *adjusted rand index (ARI)* quantifies the similarity between how the modules partition the set of neurons,
282 and how the brain regions partition the set of neurons. Intuitively, these measures revealed the properties of modular
283 structure from different perspectives: high coverage means at least one visual area is covered by the module, high purity
284 means that a module consists of neurons from the same visual area, and high ARI means the overall module partitioning
285 highly resembles the areal organization.

286 These three measures all show variation in the module organization for different stimuli (Fig. 4C). For natural
287 images and movies, the modules have a higher propensity to cover only a subset of a brain region than for drifting and
288 static grating stimuli. This is reflected by lower coverage scores and higher purity scores for the natural image and
289 movie networks than for the drifting and static grating networks (Fig. 4C; $p < 10^{-310}$, $p < 10^{-310}$, rank-sum test).

290 It is important to note that with increasing module size, coverage tends to increase while purity tends to decrease,
291 and the module size does depend on the stimulus (Fig. 5B). As a result, it is important to ask whether the variations in
292 coverage and purity with visual stimuli could be explained simply by stimulus dependence of module size. To address
293 this question, we compared module purity and coverage to module size (Supplementary Fig. 8). Consistently across
294 module sizes, the modules in the flashes stimulus and resting state networks had lower coverage than did the networks
295 for the other stimuli. The network from the flashes stimulus also had consistently lower purity.

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

To further probe the relationships between module sizes and coverage or purity, we analyzed the number of modules obtained for each stimulus that were above a given threshold of module size, threshold of module coverage, or threshold of module purity. Repeating this for many threshold values (Fig. 5B), we found that natural images and movies had the largest numbers of high-purity modules even though their numbers of small modules were not appreciably different from the other stimuli. These findings emphasize that the stimulus-dependent module properties we report in Fig. 4C cannot entirely be attributed to stimulus-dependent module sizes.

In general, the similarity between functional module partitioning and the anatomical areal organization is higher during gratings and natural stimuli and lower during resting state and flashes ($p < 10^{-310}$, rank-sum test), suggesting that functional connectivity tends to be more constrained by the anatomical structure and more spatially compact during complex and natural stimuli. This is reflected in the lower ARI values for the flashes and spontaneous activity (Fig. 4C; $p < 10^{-310}$, rank-sum test).

Consistent with previous work [58, 75], functional motifs seem to be more pronounced in more modular networks (Supplementary Fig. 9 F), suggesting their shared organizational principles. Similar to motifs, we also tested the functional similarity of nodes within and across modules by measuring the signal correlations of connected neuron pairs. Neuron pairs within the same module had higher signal correlations than did neuron pairs in different modules (Fig. 5A), and the probability of any two connected neurons being in the same module also increases with increasing signal correlation (Supplementary Fig. 9A; Cochran-Armitage test). These findings were consistent for the 4 visual stimuli for which the signal correlations are well-defined, and were consistent across brain regions when modules were assigned to the brain region from which most of their neurons came (Fig. 5D,E). These observations emphasize that the modular structure promotes functional specialization [44].

Our analyses of the modular organization of the functional connectivity networks reveal that the modules tend to contain neurons with similar stimulus tuning, and that their spatial organization and alignment with anatomical brain regions depend on the stimulus presented to the animal. This emphasizes that a functional module is not strictly the same as an anatomical brain region: the relationship between these concepts depends on the stimulus-defined context.

3 Discussion

We studied the topology of micro-scale functional networks measured with single-neuron spiking activity in the mouse visual cortex. These data were collected while the mice were exposed to different types of visual stimuli, and we separately analyzed the functional networks observed in the responses to each stimulus type. Thus generated functional networks could differ from the underlying anatomical connectivity, and this disparity warrants caution when interpreting the connectivity graphs. However, our science question, concerning stimulus-dependent interactions between neurons cannot be answered with the standard anatomical connection methods. For this reason, we used functional connectivity measures for this study.

We found that functional networks display stimulus-dependent network properties such as varying density, clustering coefficient and fraction of excitatory connections. Furthermore, we provide evidence that the distribution of low-order connectivity patterns (motifs with 2 or 3 nodes) remains stable, characterized by over-representation of a specific group of 3-neuron motifs, eFFLb motifs. This over-representation was preserved across the wide range of stimuli we investigated. Notably, while this motif was over-represented in all cases, the constituent neurons within that motif changed. Finally, we observed that the module-level network architecture depends significantly on the stimulus.

The consistent over-representation of eFFLb motifs suggests that they are key information-processing components of neural circuits. While these motifs were over-represented for all stimuli, the identity and areal distribution of neurons constituting the eFFLb motifs differed between stimuli. Thus it is the three-neuron *patterns* rather than the triadic interactions of specific neurons that are preserved. This observation suggests that an important computational role might arise at the motif level[76, 77, 78, 79, 80], where neurons can dynamically reorganize to form these relevant structures. These local computations, organized by motifs, could remain robust to changes like the loss of individual neurons because other neurons could be recruited into the motifs to replace any that are lost. For this reason, motif-level computational organization could provide substantial robustness to cortical computation.

The abundance of FFL motifs has been observed in numerous types of networks including gene regulatory networks [61], transportation networks [81], engineered networks [56] and neuronal networks[1, 56, 81]. Motif ID = e6 (FFL) is proven to be a sign-sensitive filter that responds only to persistent stimuli in transcriptional regulation networks [61] and multi-input FFL generalization is found to store memory as well as reject transient input fluctuations in neuronal networks [41]. For anatomical networks of neurons, there have been modeling works showing that certain eFFLb motifs (e9, e10) can function as long-term memories of the input, thus playing an important role in most cognitive tasks [62].

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

348 Therefore, the eFFLb motifs that we found to be consistently over-represented in cortical functional networks may have
349 important functional roles in cortical computation.

350 On the global scale, however, more complex visual stimuli tend to drive networks into more modular structures
351 with stronger segregation and stronger agreement between structural and functional parcellation. This suggests that
352 functional modules with more spatially segregated structure could be required in more demanding cognitive tasks.
353 Nevertheless, major functional modules observed when the animal was viewing natural scenes and movies are highly
354 overlapping at all spatial scales with almost the same neurons. These probably arise from the common subtasks required
355 by the visual processing of similar stimuli. One advantage of having shared modular components is that it allows a
356 faster adaptation and possibly a lower switching cost of functional networks to various tasks [75]. This reduction in
357 functional reorganization costs could be especially important given our observation that different neurons are organized
358 into the over-represented eFFLb motifs in the presence of different stimuli.

359 Anatomical structure has been known to stay relatively stable given different types of sensory inputs[82]. In
360 comparison, functional connectivity changes in such a fast and dramatic manner that some even try to model its
361 temporality within a single trial [83]. There are various reasons for this rapid functional adaptation, which could be
362 a change of task [32, 33, 34, 35], perceptual states [84], visual stimuli [85, 22, 23], etc. However, most prior studies
363 were either restricted to the primary visual cortex, or to voxel-level recordings obtained through fMRI, or both. It thus
364 remains unclear whether the functional interactions on a single-neuron scale across multiple cortical regions are also
365 dynamically adapted to the visual inputs. One major aim of our study was to fill this knowledge gap. By studying
366 interneuronal functional connections, our work could help improve our understanding of neuron-to-neuron connections
367 (e.g., at the synaptic scale). In contrast, studies of voxel-scale functional connectivity based on fMRI data might be less
368 informative about these finer-scale interactions.

369 One of the major challenges of studying functional connectivity lies in the existence of inhibitory correlations
370 and anti-correlated patterns: there is a lack of strong theoretical tools for analyzing networks with both positive and
371 negative edges [86]. While many works on functional connectivity disregard inhibition and only focus on excitatory
372 connections for simplicity, inhibitory connections play a crucial functional role in visual processing [87, 88]. This
373 highlights the need for a network analysis framework that possesses the ability to handle both positive and negative
374 edges. We address this problem by adopting and modifying motif and module detection methods for signed networks,
375 and used these methods to investigate how (signed) functional networks vary on local and global scales. The inclusion
376 of edge sign in motif analysis enables us to further distinguish motifs, since the same unsigned connectivity could
377 correspond to different functions depending on the edge signs [61]. The definition of functional modules can be
378 subjective regarding whether to keep inhibitory connections inside or between modules. Nonetheless, we showed that
379 even though qualitatively similar conclusions can be drawn without distinguishing functional inhibition from excitation
380 (Supplementary Fig. 11), ignoring edge sign (as in prior works) could lead to a less detailedunderstanding of the exact
381 pattern of functional segregation and specialization.

382 While our detailed methods thus provide a more comprehensive analysis of how functional connectivity flexibly
383 adapts to the statistics of visual input, we recognize some limitations to our analyses. First, we do not distinguish
384 neurons according to their cell types. This limits our ability to relate our functional connectivity results to the growing
385 literature on microcircuit architectures. In addition, due to incomplete recording, we observe only a subset of the
386 neurons in each brain area. Furthermore, correlation-based network inference can potentially lead to false direct
387 edge identification via high-order connections. These limitations are inherent to neural activity-based construction
388 of functional connectivity, and not just to our study. Nevertheless, we do not believe that these common limitations
389 constitute serious flaws in our analysis. Here we are not trying to find functional networks that topologically resemble
390 the anatomical network (in which case the incomplete recording issue would be quite detrimental). Instead, our focus is
391 on the adaptation of inter-neuronal interaction patterns to different visual stimuli. These interactions can be identified
392 even in incomplete recordings. On the other hand, incomplete observation might explain the presence of nonconforming
393 edges in some of our analyses, such as the presence of both excitatory and inhibitory edges emanating from a single
394 neuron. At first glance, these neurons are at odds with Dale's principle, which suggests that such bivalent neurons are
395 very uncommon in the neocortex. However, given the incomplete recordings, there could be unobserved inhibitory
396 neurons that mediate the effective inhibitory impact of an excitatory neuron on some other neurons in the circuit.

397 Additional limitations of our study arise from experimental constraints and the nature of the Neuropixels dataset
398 collected from extracellular electrophysiology probes. Kilosort2 was used to identify spike times and assign spikes to
399 individuals [89], however, no current spike sorting algorithm can ensure a completely accurate assignment of observed
400 spikes to individual neurons. This means some certain nodes in our network could correspond to more than one neuron,
401 or that there could be multiple nodes corresponding to the same neuron [90]. Finally, the limited set of visual stimuli
402 used in our experiments could introduce bias into our analysis since we do not have multiple different sets of stimuli
403 within the same stimulus type. This limitation prevents us from comparing the functional connectivity driven by distinct

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

404 stimuli within the same category (i.e., more different clips of natural movies). On the other hand, we use a relatively
 405 wide range of natural image and natural movie stimuli, and sampled multiple stimulus types of varying complexity.
 406 While these laboratory conditions are much more controlled than natural viewing conditions, we nevertheless have
 407 determined functional connectivity under a wide range of stimulus conditions.

408 On the timescale of sensory processing, neuronal networks have relatively fixed anatomical connectivity. Their
 409 functional connectivity, however, can and does vary quite substantially. Our work revealed striking patterns to this
 410 functional reorganization. These patterns suggest potentially important principles governing cortical computation,
 411 such as the dynamical organization of groups of neurons into feedforward loop motifs, and the adjustment of network
 412 modularity based on stimulus complexity. Beyond their relevance for basic neuroscience, these findings may provide
 413 guidance for how to engineer dynamically robust information processing systems.

414 4 Methods

415 4.1 Dataset

416 We analyzed the Neuropixels dataset from Allen Institute [26]. The Neuropixels project uses high-density extracellular
 417 electrophysiology probes to record spikes from multiple regions in the mouse brain. Data used to construct functional
 418 networks are recordings of the neural activity by 6 Neuropixels probes in 6 visual cortical areas (V1, LM, RL, AL, PM,
 419 AM) from 7 mice while the mice passively viewed a visual stimulus set that contains 6 types of visual stimuli with
 420 multiple repeats: grey screen (simulation for resting-state activity), flashes, drifting gratings, static gratings, natural
 421 scenes and natural movies. On average, there are 668 ± 131 units simultaneously recorded for each mouse. In order to
 422 make a fair comparison across different visual stimuli, only neurons with a firing rate of at least 2 Hz during all stimuli
 423 are included in our analysis, thus the number of neurons (size of the functional network) is the same for each mouse
 424 given different stimuli. As a result, there are 176 ± 44 units on average for each mouse.

425 4.2 Cross-correlogram and significant functional connection

426 Functional connectivity is measured through Cross-correlograms (CCGs) [91]. For each stimulus type, the average
 427 CCGs across all stimulus presentations is calculated. In order to focus on the change in connectivity driven by different
 428 stimulus types, we dismissed stimulus conditions and used all presentations as trials. CCG for lagged correlation from
 429 neurons A to B is defined as

$$CCG_{AB}(\tau) = \frac{\frac{1}{M} \sum_{i=1}^M \sum_{t=1}^N x_A^i(t)x_B^i(t+\tau)}{\theta(\tau)\sqrt{\lambda_A\lambda_B}} \quad (1)$$

430 where M is the number of trials, N is the number of time bins, x_A^i and x_B^i are the spike trains for neuron A and neuron
 431 B , $\tau \geq 0$ is the time lag between the spike trains, $\theta(\tau) = M - \tau$ represents a triangle function that corrects for the
 432 overlap time bins, λ_A and λ_B are the mean firing rates for the two neurons. It is worth noting that we only allow for
 433 non-negative time lag for the sake of bidirectional connections. We used the jitter correction method to remove slow
 434 temporal correlations [92]. The jitter-corrected CCG is obtained as the difference between CCGs of the original and
 435 jittered spike trains

$$CCG_{jc} = CCG - CCG_{jittered} \quad (2)$$

436 Apart from using ‘sharp peaks’ to define significant functional connections, we also included ‘sharp intervals’ to take
 437 into consideration the polysynaptic connections between neuron pairs with potentially multiple time lags. Specifically,
 438 for a given duration $D \in [1, \tau_{max} + 1]$, where $\tau_{max} = 12$ ms similar to the 13 ms window in previous work [27], the
 439 set of moving average CCG is obtained by

$$C(D) = \left\{ \frac{1}{D} \sum_{\tau=t_1}^{t_1+D-1} CCG_{jc}(\tau), t_1 \in [0, T - D + 1] \right\} \quad (3)$$

440 where T is the total length of spike trains. Therefore, there is an excitatory connection if

$$\frac{1}{D} \sum_{\tau=t_0}^{t_0+D-1} CCG_{jc}(\tau) > \mu_{C(D)} + n\sigma_{C(D)} \quad (4)$$

441 and an inhibitory connection if

$$\frac{1}{D} \sum_{\tau=t_0}^{t_0+D-1} CCG_{jc}(\tau) < \mu_{C(D)} - n\sigma_{C(D)} \quad (5)$$

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

442 where $t_0 \in [0, \tau_{max} - D + 1]$ is the starting time lag of the ‘sharp peak/interval’, $\mu_{C(D)}, \sigma_{C(D)}$ are the mean and
 443 standard deviation of $C(D)$, $n = 4$ denotes the 4-fold significance level in our experiment. It is straightforward that
 444 $D = 1$ indicates a ‘sharp peak’ while $D > 1$ denotes a ‘sharp interval’. If equation (4) or (5) is true on multiple
 445 durations $D \in \{D_1, D_2, \dots, D_s\}, D_1 < D_2 < \dots < D_s$, we assume the smallest duration $D = D_1$ since it always
 446 leads to the highest significance level.
 447
 448 For bidirectional connections with zero time lag for both directions ($\tau_{AB} = \tau_{BA} = 0$), we only kept the
 449 direction with the higher significance level and removed the other direction unless its second highest sharp interval is
 450 also significant.
 451 Therefore, each connection was characterized by its lag, the duration of the significant interval in the CCG, and its
 452 significance value. Lag τ is the delay between spike trains of source neuron and target neuron, and the sign of the lag
 453 determines the direction of the connection. The duration D measures how long the significant peak/interval lasts, and
 454 the connection significance signals the Z score of the ‘sharp peak/interval’. Lags τ of across-area connections are
 455 higher than within-area connections (Supplementary Fig. 1C), which is as expected since it takes more time for a signal
 456 to travel between areas than within an area.
 457 In order to eliminate the bias brought by the lack of enough spikes or trials, we used normalized entropy for each trial
 458 to measure its statistical significance. For each neuron pair, we only keep trials in which spike trains of both neurons
 459 have a normalized entropy of at least 0.9.

460 4.3 Reference model and signed motif analysis

461 Since functional networks are constructed as signed networks, signed motif analysis needs to be defined. Similar
 462 to unsigned motif detection, to examine the statistical significance of signed n-neuron motifs in the networks, we
 463 generated random networks using various reference models as the baseline and conducted a comparative analysis of
 464 motif frequency between the empirical network and random networks.
 465
 466 Three types of commonly used reference models are adopted in this work: Erdős-Rényi model, Degree-preserving
 467 model and Pair-preserving model. However, they are all defined on unsigned networks. In order to tailor these models
 468 for analysis in the context of signed networks, we randomly assigned original edge signs to reference networks
 469 randomized using Erdős-Rényi model, Degree-preserving model and Pair-preserving model. Furthermore, we defined
 470 the Signed-pair-preserving model by preserving the edge signs for each neuron pair in the Pair-preserving model during
 471 shuffling (Supplementary Fig. 3B). Therefore, surrogate networks generated using all four reference models have the
 472 same number of positive/negative connections as the real network.
 473
 474 Table 1 lists the comparison between all four reference models. Erdős-Rényi model randomly shuffles connec-
 475 tions while preserving network size, density and weight distribution [60], Degree-preserving model generates
 476 random networks while preserving size, density, weight distribution and degree distribution [93], Pair-preserving model
 477 randomizes the network while keeping size, density, weight distribution, degree distribution and neuron pair distribution
 478 [56] while the Signed-pair-preserving model preserves the signed pair distribution (Fig. 2A) in addition to the first three
 479 properties. We use Signed-pair-preserving model for signed motif analysis. For all analyses including a reference
 480 model, we randomly generated 200 surrogate networks.
 481
 482 For two-neuron motif analysis, we adopted Erdős-Rényi model as the reference model and computed the rel-
 483 ative count for each type of two-neuron connection by dividing the count of the empirical network and the average
 484 count of surrogate networks. For simplicity, we only focused on three-neuron subnetworks apart from two-neuron
 485 subnetworks during motif analysis. We used the Z score of intensity compared with reference models to determine
 486 motif significance [59]. The intensity of a certain motif M is defined as the summation over the intensities of all
 487 subgraphs g that have the structure of M

$$I(M) = \sum_{g \in M} I(g) \quad (6)$$

488 where the intensity of a certain subgraph is defined as the geometric mean of all its connection strengths

$$I(g) = \left(\prod_{ij \in l_g} w_{ij} \right)^{\frac{1}{|l_g|}} \quad (7)$$

Table 1 | Reference models

Reference model	size & density & weight	degree distribution	pair distribution	signed pair distribution
Erdős-Rényi model	✓	✗	✗	✗
Degree-preserving model	✓	✓	✗	✗
Pair-preserving model	✓	✓	✓	✗
Signed-pair-preserving model	✓	✓	✓	✓

489 where l_g denotes the set of connections in g and w_{ij} is the strength of the connection from neuron i to j . Then the Z
 490 score of intensity for motif M can be computed as

$$Z_M = \frac{I_M - \langle i_M \rangle}{\sqrt{\langle i_M^2 \rangle - \langle i_M \rangle^2}} \quad (8)$$

491 where i_M is the total intensity of motif M in one realization of the reference model.

492 4.4 Signed module detection

493 The original Modularity used to detect community structure for directed networks [94] is defined as $\hat{Q} = \frac{1}{m} \sum_{ij} \left[A_{ij} - \right.$

494 $\left. \frac{k_i^{in} k_j^{out}}{m} \right] \delta(\sigma_i, \sigma_j)$, where A is the adjacency matrix of the network, m is the number of links, k^{in}, k^{out} represent the
 495 in-degree and out-degree, respectively. δ is the Kronecker delta function and σ_i denotes the community label that node
 496 i is assigned to. In the presence of negative links, we denote $A_{ij}^+ = A_{ij}$ if $A_{ij} \geq 0$ and zero otherwise, $A_{ij}^- = -A_{ij}$ if
 497 $A_{ij} \leq 0$ and zero otherwise, so that $A = A^+ - A^-$. In order to cluster nodes towards social balance, [95] proposed a
 498 frustration metric $\sum_{ij} (\lambda A_{ij}^- - (1 - \lambda) A_{ij}^+) \delta(\sigma_i, \sigma_j)$. However, neither is suitable for partitioning signed networks. In
 499 this work we adopted modified Modularity for community detection of the signed, weighted and directed CCG network.
 500 Modified Modularity of a certain partition σ is defined as the weighted combination of the positive and negative parts
 501 [68, 69]

$$Q(\sigma) = \frac{m^+}{m^+ + m^-} Q^+(\sigma) - \frac{m^-}{m^+ + m^-} Q^-(\sigma) \quad (9)$$

502 where

$$Q^+(\sigma) = \frac{1}{m^+} \sum_{ij} (A_{ij}^+ - \gamma^+ p_{ij}^+) \delta(\sigma_i, \sigma_j) \quad (10)$$

$$Q^-(\sigma) = \frac{1}{m^-} \sum_{ij} (A_{ij}^- - \gamma^- p_{ij}^-) \delta(\sigma_i, \sigma_j) \quad (11)$$

504 γ^+ and γ^- are the resolution parameters, m^+ and m^- are the number of positive and negative connections, respectively,
 505 p^+ and p^- are the connection probabilities for positive and negative links, respectively. Here we take into consideration
 506 degree distribution by defining the probabilities as $p^\pm = \frac{\pm k_i^{out} \pm k_j^{in}}{m^\pm}$, where $\pm k_i^{out}$ is the positive/negative out-degree of
 507 neuron i and $\pm k_j^{in}$ is the positive/negative in-degree of neuron j . Therefore, equation (9) can be rewritten as

$$Q(\sigma) = \frac{1}{m^+ + m^-} \sum_{ij} [A_{ij} - (\gamma^+ p_{ij}^+ - \gamma^- p_{ij}^-)] \delta(\sigma_i, \sigma_j) \quad (12)$$

508 The Louvain method is a module (community) detection algorithm for partitioning networks into groups of nodes
 509 with dense connections within groups and sparse connections between groups [67]. The algorithm uses the original
 510 Modularity \hat{Q} as a quality function to optimize the partitioning of the network. The Louvain method operates through
 511 a series of iterative steps that merge neighboring modules to maximize the Modularity gain until a locally optimal
 512 partition is reached. The algorithm uses a bottom-up approach, starting from single-node modules, and iteratively
 513 merges modules to form larger ones. To take into consideration edge signs, we revised the quality function in the
 514 Louvain method from original Modularity \hat{Q} to modified Modularity Q . Therefore, the modified Louvain method
 515 aims to find an optimal partition of nodes such that positive connections are placed within modules while negative

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

	\mathcal{A}_1	\mathcal{A}_2	\cdots	\mathcal{A}_s	sums
\mathcal{M}_1	n_{11}	n_{12}	\cdots	n_{1s}	a_1
\mathcal{M}_2	n_{21}	n_{22}	\cdots	n_{2s}	a_2
\vdots	\vdots	\vdots	\ddots	\vdots	\vdots
\mathcal{M}_r	n_{r1}	n_{r2}	\cdots	n_{rs}	a_r
sums	b_1	b_2	\cdots	b_s	

Table 2 | Contingency table for module partition $\mathcal{M} = \{\mathcal{M}_1, \mathcal{M}_2, \dots, \mathcal{M}_r\}$ and visual areal organization $\mathcal{A} = \{\mathcal{A}_1, \mathcal{A}_2, \dots, \mathcal{A}_s\}$. \mathcal{M}_i denotes the set of neurons in the i -th module while \mathcal{A}_j represents the set of neurons in the j -th visual area. Since we only focus on six cortical areas, thus $s = 6$. Each entry n_{ij} denotes the number of neurons that are assigned into module \mathcal{M}_i are from visual area \mathcal{A}_j : $n_{ij} = |\mathcal{M}_i \cap \mathcal{A}_j|$.

516 connections are between modules.

517
518 In order to determine the resolution parameters for module analysis, we obtained a Modularity difference
519 heatmap by varying γ^+ and γ^- and computing the difference between Modularities of empirical and surrogate
520 networks generated by the Signed-pair-preserving model, then looked for the γ^+ and γ^- that maximize the difference
521 [14]. This way we obtained the modular partitioning that is the least random. We used the Z score of Modularity to
522 show how modular a functional network is through comparison with a reference model (Signed-pair-preserving model).
523 The Z score of Modularity is defined as

$$Z_Q = \frac{Q - \langle q \rangle}{\sqrt{\langle q^2 \rangle - \langle q \rangle^2}} \quad (13)$$

524 where q is the Modularity in one realization of the reference model. Only modules with a size of at least four neurons
525 are included in subsequent analysis to eliminate the noise influence of isolated single neurons, pairs and triplets. Note
526 that we included connection strength in both motif and module analyses. The CCG peak values represent connection
527 strengths, i.e., we use absolute sum of positive/negative connection weights instead of number of positive/negative
528 connections and the positive/negative degree of a neuron is replaced by total positive/negative connection weights.
529 Unless otherwise stated, Q represents the modified Modularity for signed networks. When visualizing modular structure,
530 the location of each node is determined by applying the Fruchterman-Reingold Layout recursively on the hypergraph
531 and then the subgraph of each community (python package Netgraph).

532 4.5 Analysis of modular structure

533 To measure the fundamental properties of modular structure, we used (weighted average) coverage, (weighted average)
534 purity and Adjusted Rand Index (ARI) to show how neurons from different visual areas are clustered together. Coverage,
535 defined as $\max_j \frac{|\mathcal{M}_i \cap \mathcal{A}_j|}{|\mathcal{A}_j|}$ and purity, defined as $\max_j \frac{|\mathcal{M}_i \cap \mathcal{A}_j|}{|\mathcal{M}_i|}$, are neuron-level metrics, while their weighted averages
536 (WA) with module size as weight are network-level metrics. The WA coverage is

$$\frac{\sum_i \max_j \frac{|\mathcal{M}_i||\mathcal{M}_i \cap \mathcal{A}_j|}{|\mathcal{A}_j|}}{\sum_i |\mathcal{M}_i|} \quad (14)$$

537 whereas the WA purity is

$$\frac{\sum_i \max_j |\mathcal{M}_i \cap \mathcal{A}_j|}{\sum_i |\mathcal{M}_i|} \quad (15)$$

538 For each module partition, we also used Adjusted Rand Index (ARI) to measure its similarity to areal organization for
539 each network. Based on the contingency table 2, ARI is defined as

$$ARI = \frac{\sum_{ij} \binom{n_{ij}}{2} - \left[\sum_i \binom{a_i}{2} \sum_j \binom{b_j}{2} \right] / \binom{n}{2}}{\frac{1}{2} \left[\sum_i \binom{a_i}{2} + \sum_j \binom{b_j}{2} \right] - \left[\sum_i \binom{a_i}{2} \sum_j \binom{b_j}{2} \right] / \binom{n}{2}} \quad (16)$$

540 **4.6 Multi-resolution module partition**

541 To reduce noise in module partition, we focused on the most active neurons that have at least 1 connection during all
542 stimuli when examining how module partition changes with resolution parameters. Since the module partition method
543 (modified Louvain method) is stochastic, 200 independent runs were carried out for partitioning any empirical network.
544 To compare module partitioning results across resolution parameters, we combined multiple partitioning results based
545 on a voting mechanism that keeps frequent modules. We first looped over each run of the modified Louvain algorithm
546 and for each module in the run, updated the module assignment count for each node in the module. Next, we initialized
547 a list of unassigned nodes and assigned them to the module with the highest vote count that it is partitioned into during
548 at least one run. Each node was only assigned to one module. In each step, we removed all the nodes assigned to the
549 most frequent module from the list of unassigned nodes and continued until all nodes were assigned to a module.
550

551 Due to the significantly greater abundance of excitatory connections compared to inhibitory connections, the
552 parameter γ^+ exerts a substantially more pronounced effect on the outcomes of module partitioning than γ^- .
553 Consequently, we limited the range of variation for γ^- while placing greater emphasis on the alignment and comparison
554 of module identity with γ^+ across a broader range of values.
555

556 To compare module partitions across multiple resolutions, we assigned module IDs to modules across resolu-
557 tions based on their hierarchical structure and produced a visualization of the resulting heatmap. To accomplish this, we
558 started from the highest resolution, and traversed through the resolutions in reverse order. For the highest resolution, we
559 assigned each module a unique ID.
560

561 Then for subsequent resolutions, we identified the largest submodule from the previous resolution and deter-
562 mined that its ID is inherited from previous module. To achieve this, we consider the modules from the previous
563 resolution and calculated their overlaps with the current module. We select the submodule(s) with the maximum
564 overlap and retrieve the corresponding ID(s) assigned to it. These ID(s) are assigned to the current module, ensuring
565 consistency and preserving the hierarchical relationship across resolutions. For other modules that are not the largest
566 submodule of any previous module, we assigned a new module ID to it.
567

568 Once the module IDs are assigned to the modules for all resolutions and stimuli, we sorted the nodes within
569 each area based on their combined similarity across all stimuli to ensure an intuitive visualization. Specifically, we
570 employed a two-opt optimization algorithm to determine an optimal node order that maximizes the similarity between
571 module IDs of adjacent nodes across resolutions. The object is to minimize the hamming distance between 10 adjacent
572 nodes, making neighboring nodes more likely to have similar module IDs across resolutions.
573

4.7 Statistical analysis

574 Since module partitioning and the generation of surrogate networks are both stochastic, each analysis involving
575 modular structure or surrogate networks is performed with 200 independent runs. We adopt Cochran-Armitage trend
576 test to assess the association between variables. Student's t-test is used for the significance level between Gaussian
577 distributions, and Shapiro-Wilk test is used for normality test. If at least one distribution is not normal, Wilcoxon
578 rank sum test is used. Kolmogorov-Smirnov test is used to test whether a distribution is the largest among a set of
579 distributions. Benjamini/Hochberg method is used to correct p value for false discovery rate in multiple tests.
580

581 For data with a limited number of samples, the non-parametric bootstrap method was used to calculate the
582 confidence interval for a given sample of data. The confidence interval is based on the distribution of the medians of the
583 bootstrap samples, which is an approximation of the sampling distribution of the median of the population from which
584 the data were drawn. The percentile method is used to calculate the confidence interval, which involves finding the
585 upper and lower bounds of the interval based on the percentiles of the bootstrap distribution. Specifically, we used a
586 95% confidence level for all the confidence intervals, the sample size was 10000.

587 **5 Data availability**

588 All the data analyzed in this manuscript is part of the Allen Brain Observatory introduced in [26]. The
589 data used to generate main text Figs. 1–5 is available for download in Neurodata Without Borders
590 (NWB) format via the AllenSDK. Example Jupyter Notebooks for accessing the data can be found at
591 https://allensdk.readthedocs.io/en/latest/visual_coding_neuropixels.html.

592 6 Code availability

593 Code for analyses in the manuscript and generation of figures are available from the repository:
594 <https://github.com/HChoiLab/functional-network>.

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598 8 Author contributions

599 Conceptualization (DT, JZ, XJ, HC); Methodology and Software (DT); Formal Analysis (DT); Investigation (DT, JZ,
600 XJ, HC); Writing (DT, JZ, XJ, HC); Visualization (DT); Supervision (JZ, XJ, HC)

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607 10 Competing interests

608 The authors declare no competing interests.

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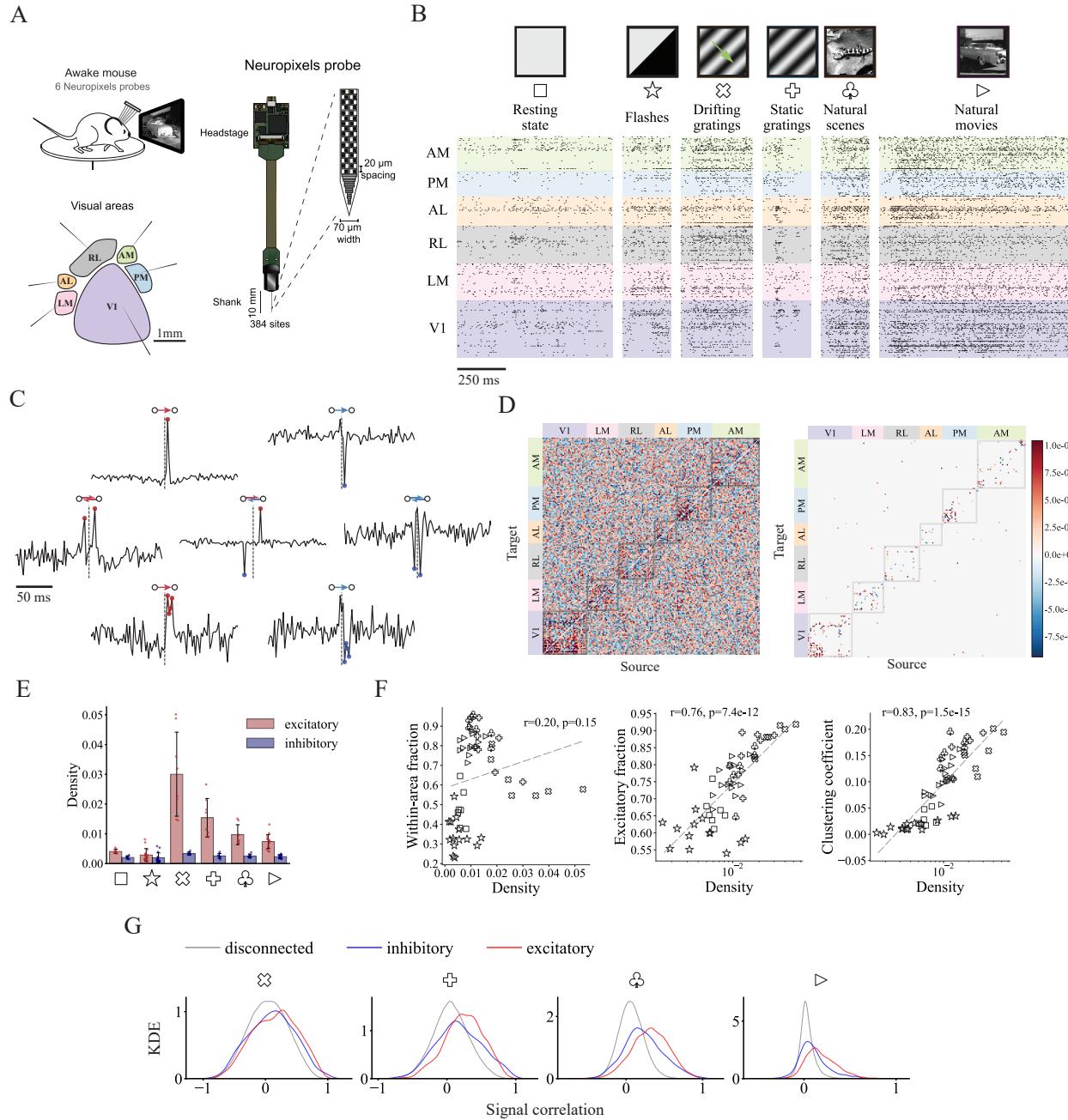


Fig. 1 | From spike trains to functional connectivity for mouse visual cortex. (A) Schematic of data collection with Neuropixels probes inserted through six visual cortical areas (AM, PM, AL, RL, LM and V1). (B) Example spike trains of 193 units from the visual cortex of a mouse during six different types of stimuli. For brevity, each stimulus is denoted using a unique symbol in all figures. (C) Example CCGs (cross-correlograms) of excitatory (red)/inhibitory (blue), unidirectional/bidirectional and monosynaptic ('sharp peak')/polysynaptic ('sharp intervals') connections. (D) (left) Example matrix of CCG with units ordered by area during natural movie stimuli. (right) Connectivity matrix with only significant connections ($|Z| > 4$). (E) Density of excitatory and inhibitory connections during all visual stimuli. Density is defined as the number of connections normalized by total possible number of connections. (F) Fraction of within-area connections, fraction of excitatory connections and clustering coefficient against network density. Each visual stimulus is characterized by a symbol, consistent with (B). (G) Kernel density estimation (KDE) of signal correlation distributions for disconnected neuron pairs and pairs with inhibitory/excitatory connections during presentations of four types of visual stimuli.

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

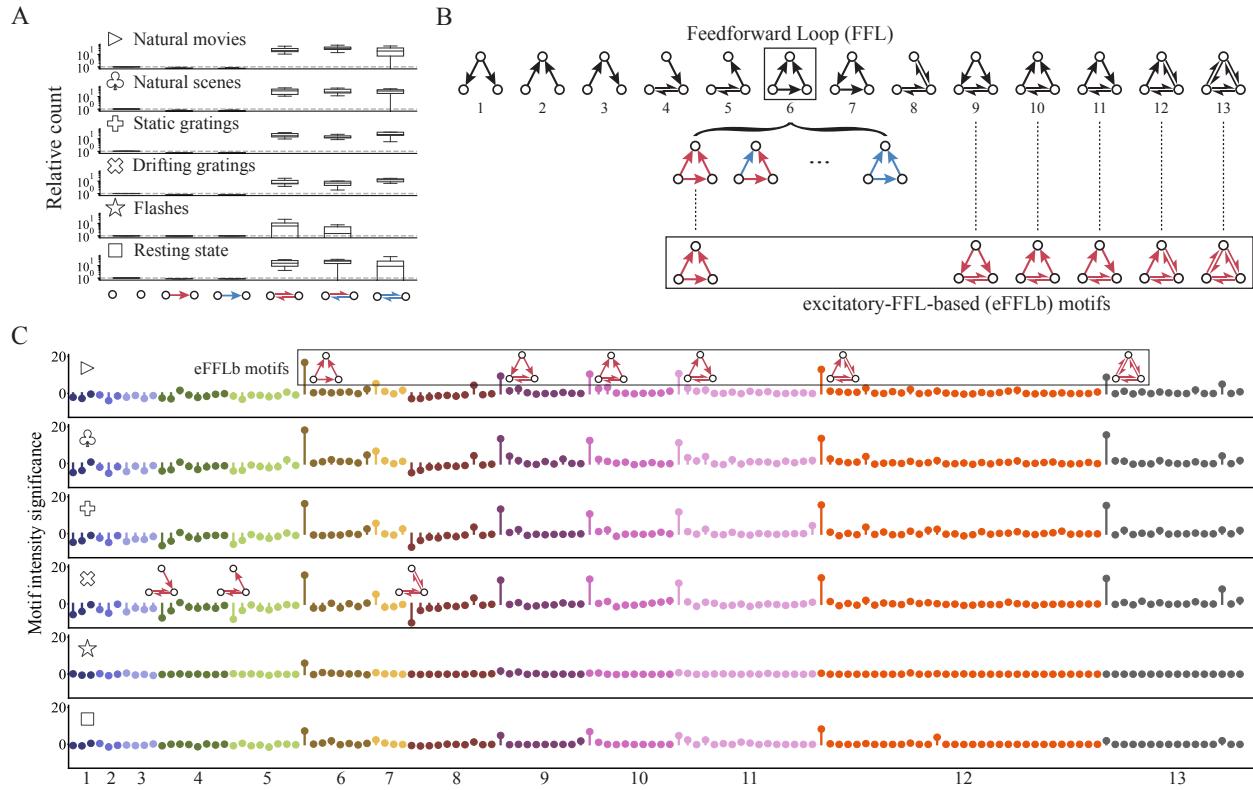


Fig. 2 | Highly preserved local structure during different types of visual stimuli. (A) Relative count of signed neuron pairs with respect to reference model (Erdős-Rényi model). All three types of bidirectional pairs are considerably over-represented. (B) 13 types of motifs without edge signs. Examples of signed motifs based on Feedforward Loop structure (motif ID = 6) are shown. Excitatory-FFL-based (eFFLb) motifs are defined as motifs with at least one FFL structure consisting of all excitatory connections. Note that eFFLb motifs can be classified into four types based on the number of mutual connections: zero (ID = 6), one (ID = 9, 10, 11), two (ID = 12) and three (ID = 13). In what follows, we use the prefix “e” or “i” to denote signed motif types with only excitatory connections or only inhibitory connections, respectively. (C) Motif intensity significance sequences of all signed motifs (signed three-neuron subnetworks). Each row shows results for a certain stimulus type and each color corresponds to a certain unsigned motif structure. Motif intensity significance for each signed motif is obtained through its intensity Z score of the empirical network with Signed-pair-preserving model as the reference. The overall sequences are remarkably similar across visual stimulus classes, with six types of over-represented motifs (eFFLb motifs) and three under-represented motifs preserved. It is worth noting that the above-mentioned nine significant motifs only contain excitatory connections.

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

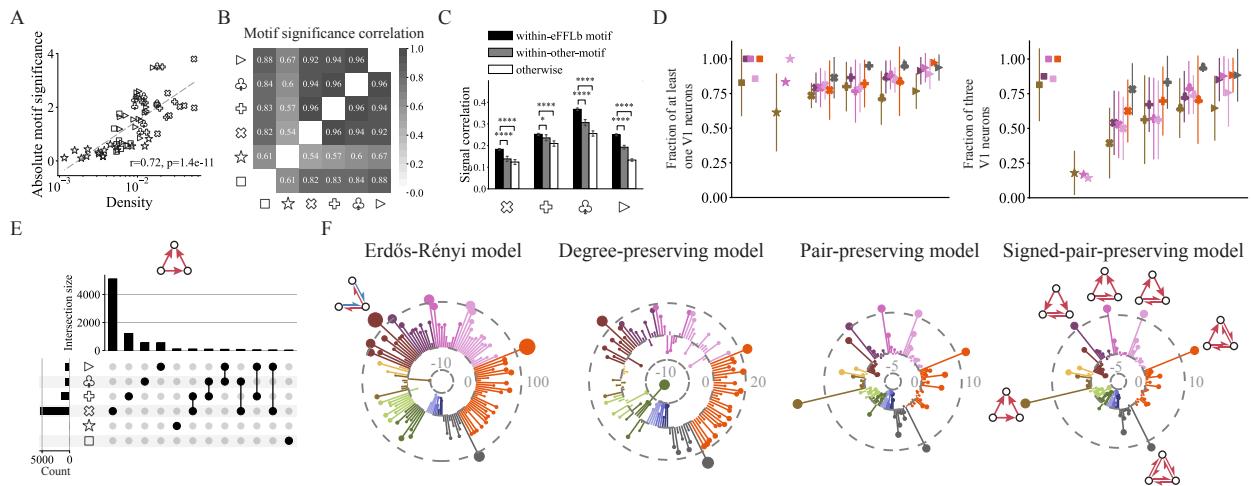


Fig. 3 | Same motifs and similar patterns are organized from different neurons. (A) Average absolute motif significance (absolute Z score of intensity) across all signed motifs against network density. Similar to within-area fraction and clustering coefficient (Fig. 1F), there is also a logarithmic relationship between motif significance and density. (B) Pairwise correlation of normalized motif intensity distribution for six visual stimuli. Extremely high correlation proves the similar motif presence during different types of stimuli. (C) Signal correlation during four visual stimuli (except for resting state) for within-eFFLb-motif, within-other-motif connections and others. Other over-represented motifs are determined using a significance level of 99%. * $p < 0.05$, *** $p < 0.0001$, rank-sum test. (D) Fraction of motifs with at least one V1 neuron or all three V1 neurons during all visual stimuli for six over-represented excitatory-feedforward-loop-based (eFFLb) motifs, consistent with Fig. 2C. From left to right, motif ID = e6, e9, e10, e11, e12, e13. (E) Intersections of unique motif sets for motif ID = e6 during six types of stimuli. Horizontal bar plot shows the size of each intersection set while vertical bar plot displays the number of signed motif ID = e6 for each type of stimulus. A unique motif is defined as a certain signed motif with three specific neurons, and intersections with less than 20 elements are removed for brevity (see Supplementary Fig. 4 for the complete results). A large number of unique motifs appear only during one type of stimuli, demonstrating that even though functional motifs are preserved across visual stimuli, component neurons are changing. (F) Multiple motif intensity significance sequences were obtained through four different reference models for natural movies as the representative stimulus type: Erdős-Rényi model, Degree-preserving model, Pair-preserving model and Signed-pair-preserving model with an increasing number of preserved network properties (Methods). Colors are consistent with (D) and Fig. 2C, and connectivity pattern is shown only once for each type of most significant signed motif for brevity. Empirical functional networks are progressively more similar to surrogate networks from left to right.

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

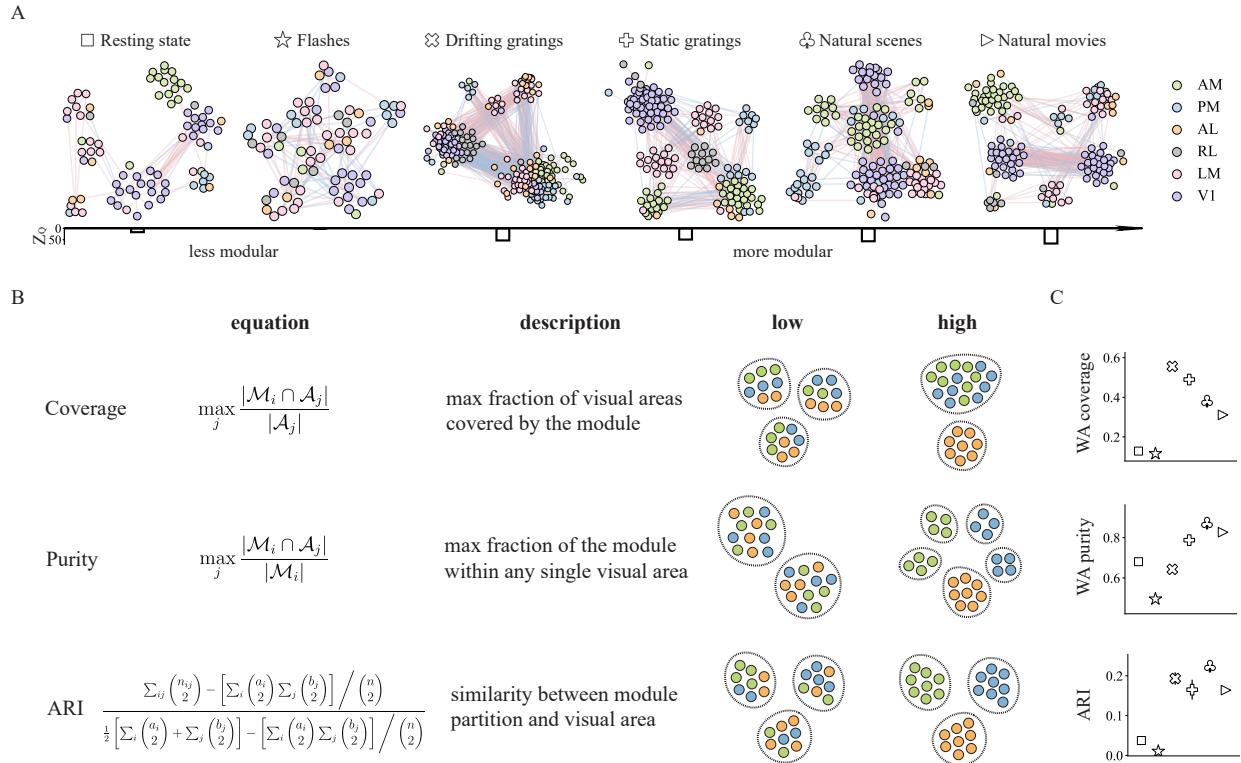


Fig. 4 | Distinct modular structures during different types of visual stimuli. (A) Topological structure of functional connectivity of a mouse during six types of visual stimuli with neurons colored by area. The color of each connection shows its sign with red denoting excitatory connection and blue representing inhibitory correlation. The community partition is obtained through modified Modularity for signed networks (see Methods). We computed the Z score of Modularity with Signed-pair-preserving model as the reference to show the degree to which functional network has a modular structure. Networks during gratings and natural stimuli show significant modular structure. (B) We used three measures to reveal the modular structure regarding visual area from different perspectives. Coverage and purity are module-level measures, where the former marks the degree to which the module covers any visual area, while the latter measures the degree to which all neurons in the module are from the same visual area. We computed the average coverage and purity weighted by module size to show the overall properties of the whole functional network (see Methods). Adjusted Rand Index (ARI), a network-level measure, was also used to quantify the difference between module partition and visual areal organization. The weighted average (WA) coverage is 0.375 and 1 (ranges from 0 to 1), WA purity is 0.333 and 1 (ranges from 0 to 1) and ARI is -0.03 and 1 (ranges from -0.5 to 1) for the corresponding two toy examples visualizing the ‘low’ and ‘high’ cases for the measure. (C) WA coverage, WA purity and ARI during six visual stimuli. The error bars show the confidence intervals over all mice obtained with non-parametric bootstrap method. In general, there tend to be fewer and larger modules with higher coverage during grating stimuli, whereas we usually find more and smaller modules with higher purity during natural stimuli. As a result, ARI is lower during resting state and flash while higher during grating and natural stimuli.

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

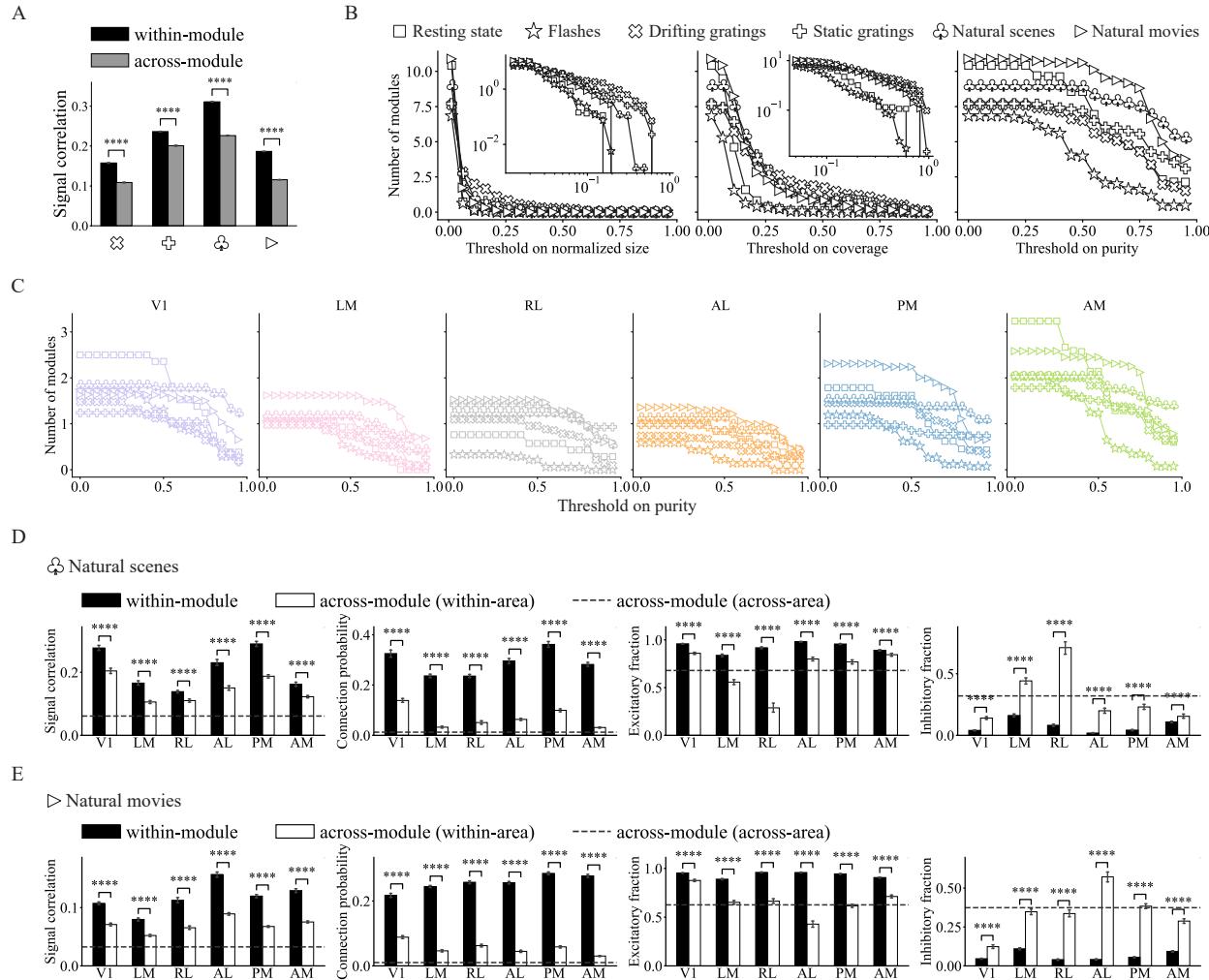


Fig. 5 | Stronger segregation during natural stimuli. (A) Signal correlation for within-module and across-module connections. $*** p < 10^{-4}$, Student's t-test. Connected neurons partitioned into the same module tend to have higher signal correlations than connected neurons from different modules, demonstrating our module partition provides insight into not only the connectivity pattern but also functional similarity to some extent. (B) Number of modules with normalized size, coverage or purity higher than the threshold. Normalized size is the size of module normalized by the total number of neurons in the network, insets show the plots on a log-log scale. (C) Number of modules with purity higher than the threshold for each visual area during all visual stimuli. (D, E) Properties of the modular structure during natural scene and natural movie presentations. We examined four different aspects of the case where neurons from a single visual area are divided into multiple modules (in which they are the dominant area), with signal correlation indicating the functional similarity along with connection probability, excitatory fraction and inhibitory fraction demonstrating the validity of our module partitioning algorithm. $*** p < 10^{-4}$, Student's t-test.

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

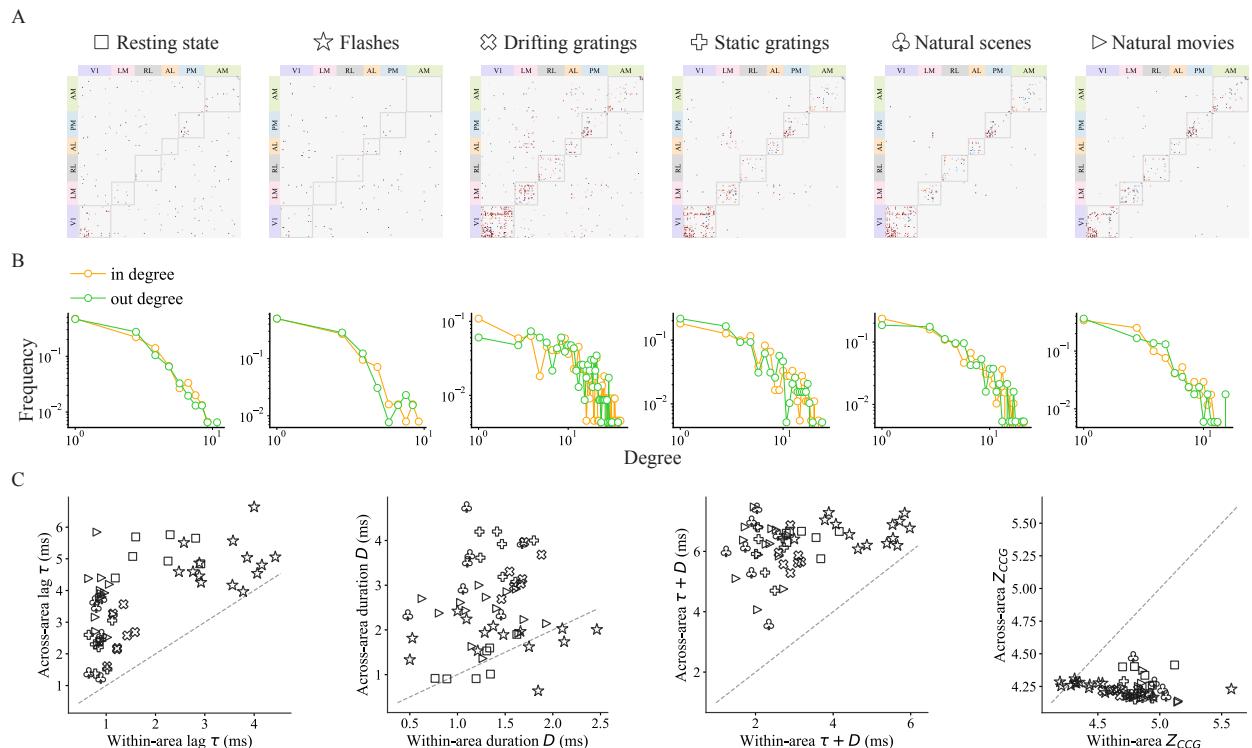


Fig. S1 | Basic properties of functional networks during all visual stimuli. (A) CCG adjacency matrices of a mouse given distinct stimuli (on the same scale as in Fig 1D). (B) Directed degree distributions of a mouse given all stimuli. (C) Across-area VS within-area comparisons for the lag τ , duration D and their sum $\tau + D$.

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

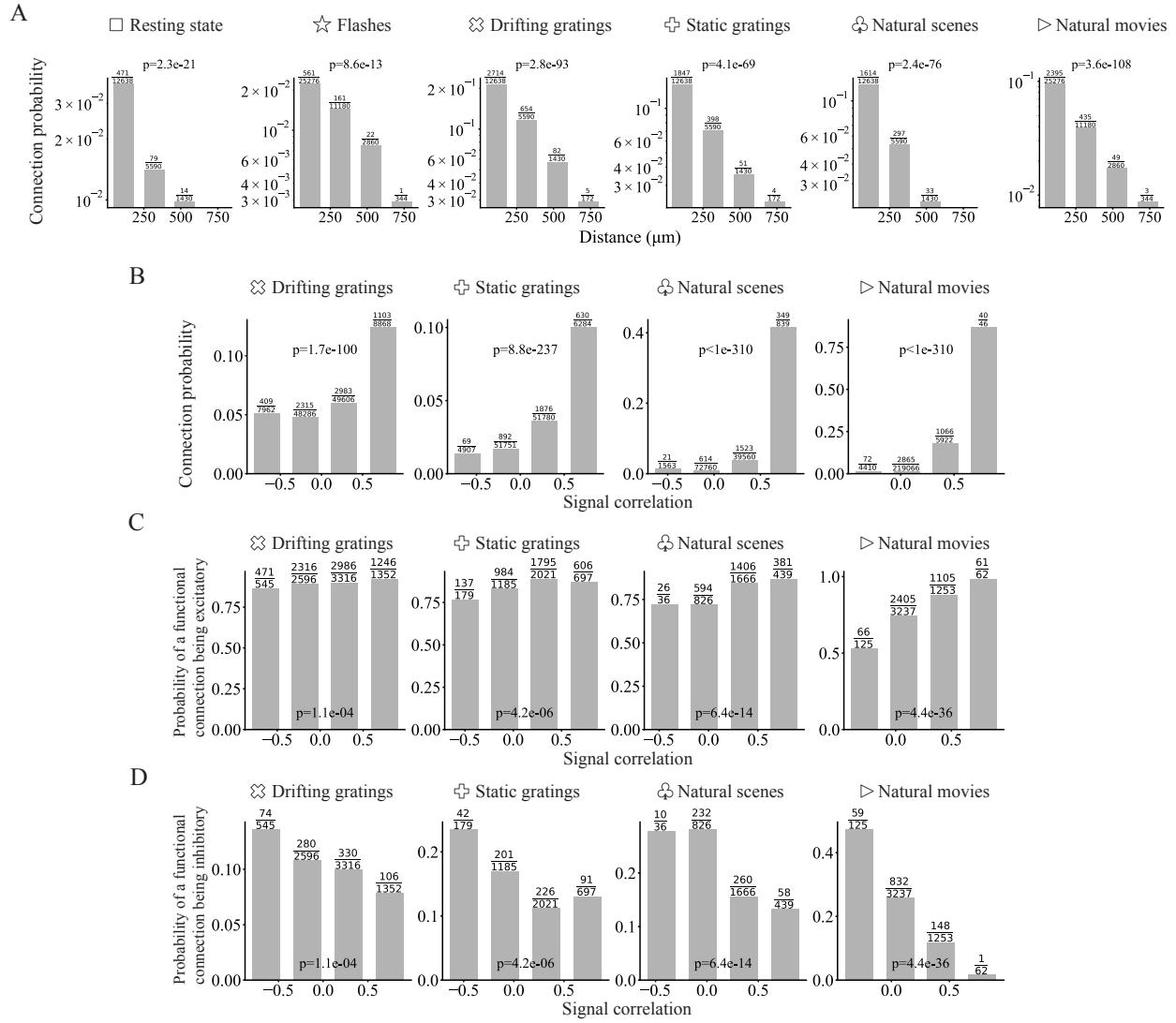


Fig. S2 | Cochran-Armitage trend test for association between (A) (functional) connection probability and distance of neurons. The rest of the plots show the Cochran-Armitage trend test for association between signal correlation and (B) (functional) connection probability, (C) probability of a functional connection being excitatory and (D) probability of a functional connection being inhibitory, under four different types of visual stimuli. Here we excluded flashes since there are only two stimulus conditions (light or dark) and signal correlation could be heavily biased and trivial.

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

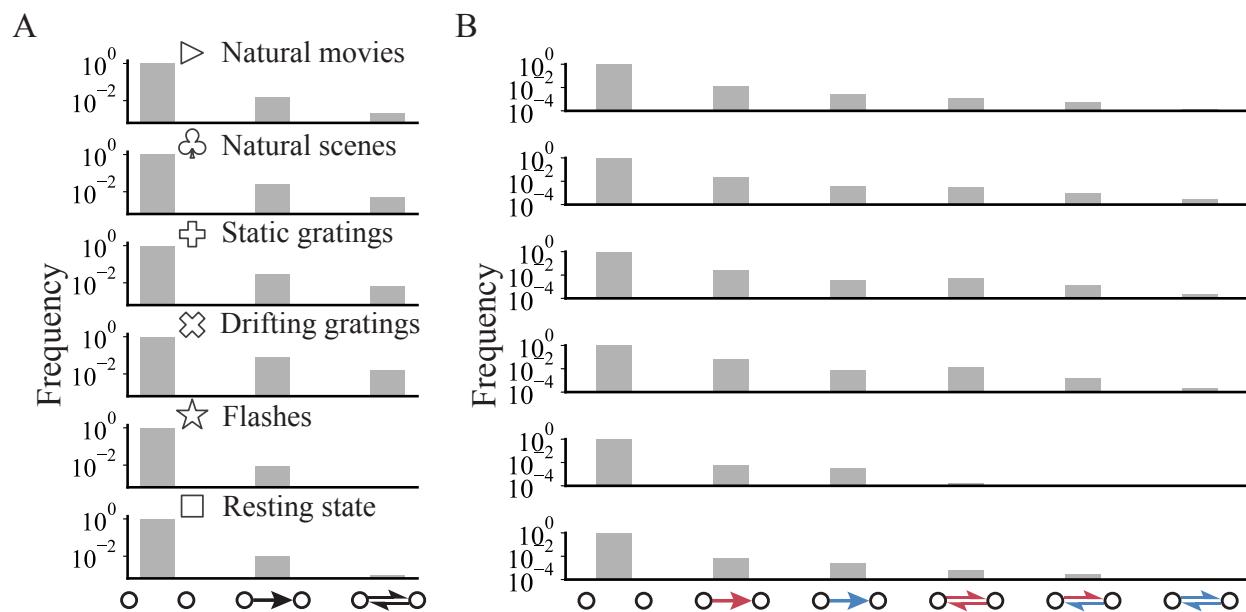


Fig. S3 | Distributions of (signed) neuron pairs. (A) Distribution of neuron pairs without edge signs. (B) Distribution of signed neuron pairs.

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

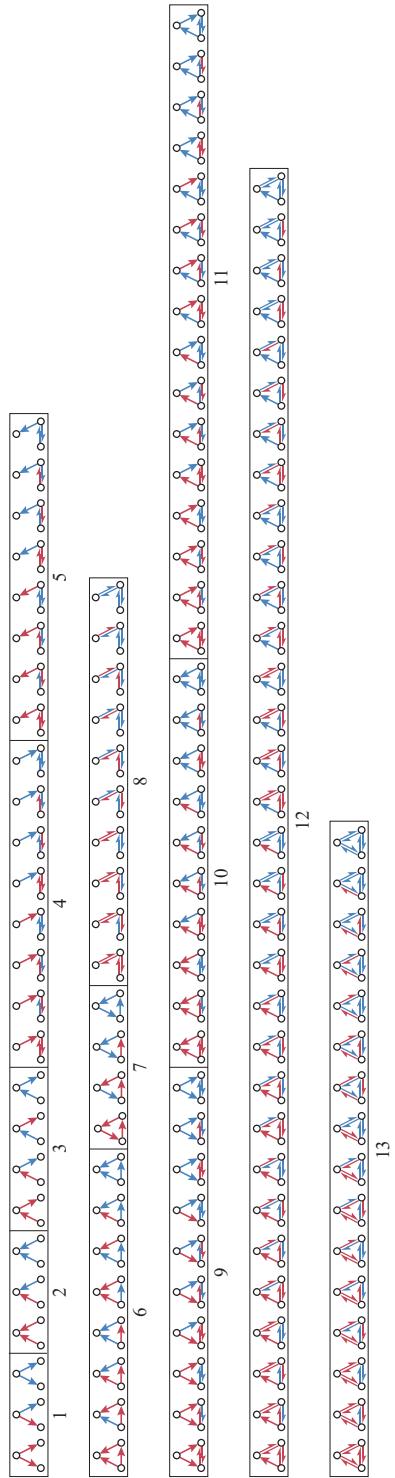


Fig. S4 | All signed motifs in the same order as Fig. 2C.

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

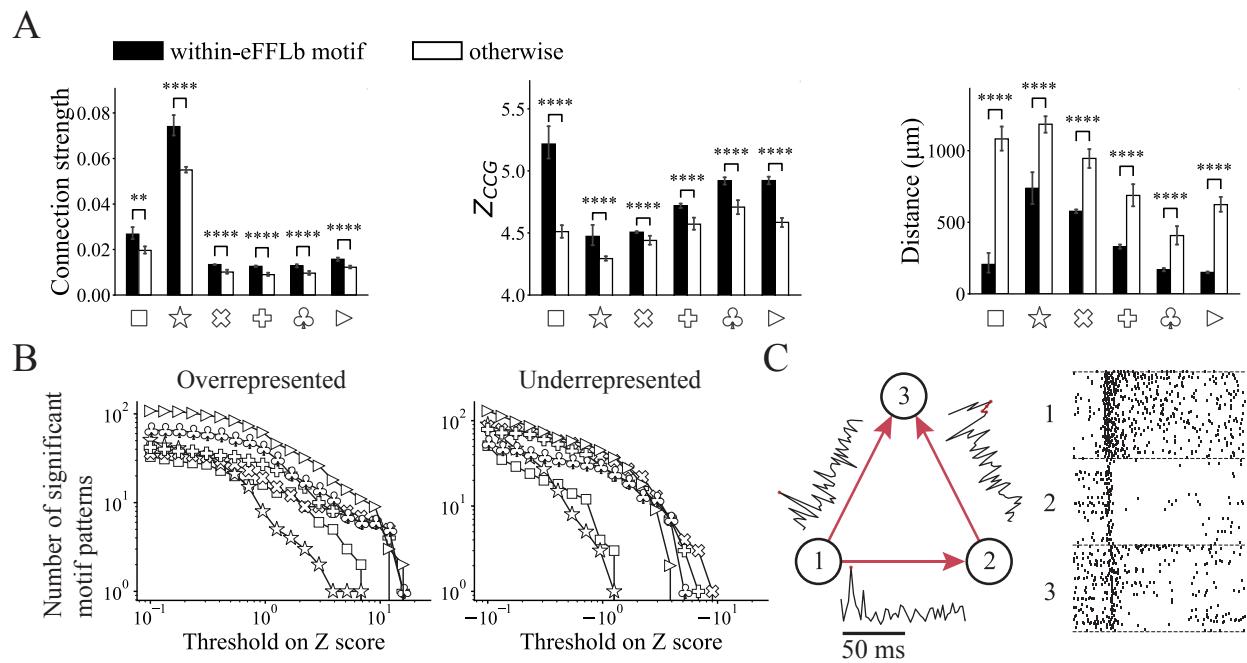


Fig. S5 | Further comparison of within-eFFLb-motif and other connections. (A) (left) Connection strength, (center) Z score of CCG and (right) physical distance for within-eFFLb-motif connections and others during all visual stimuli. ** $p < 10^{-2}$, *** $p < 10^{-4}$, Student's t-test. (B) Number of significant signed motifs with intensity Z score higher than the threshold (over-represented) or lower than the threshold (under-represented) during all visual stimuli. (C) Example eFFL motif with CCGs for each connection and spike trains for each neuron.

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

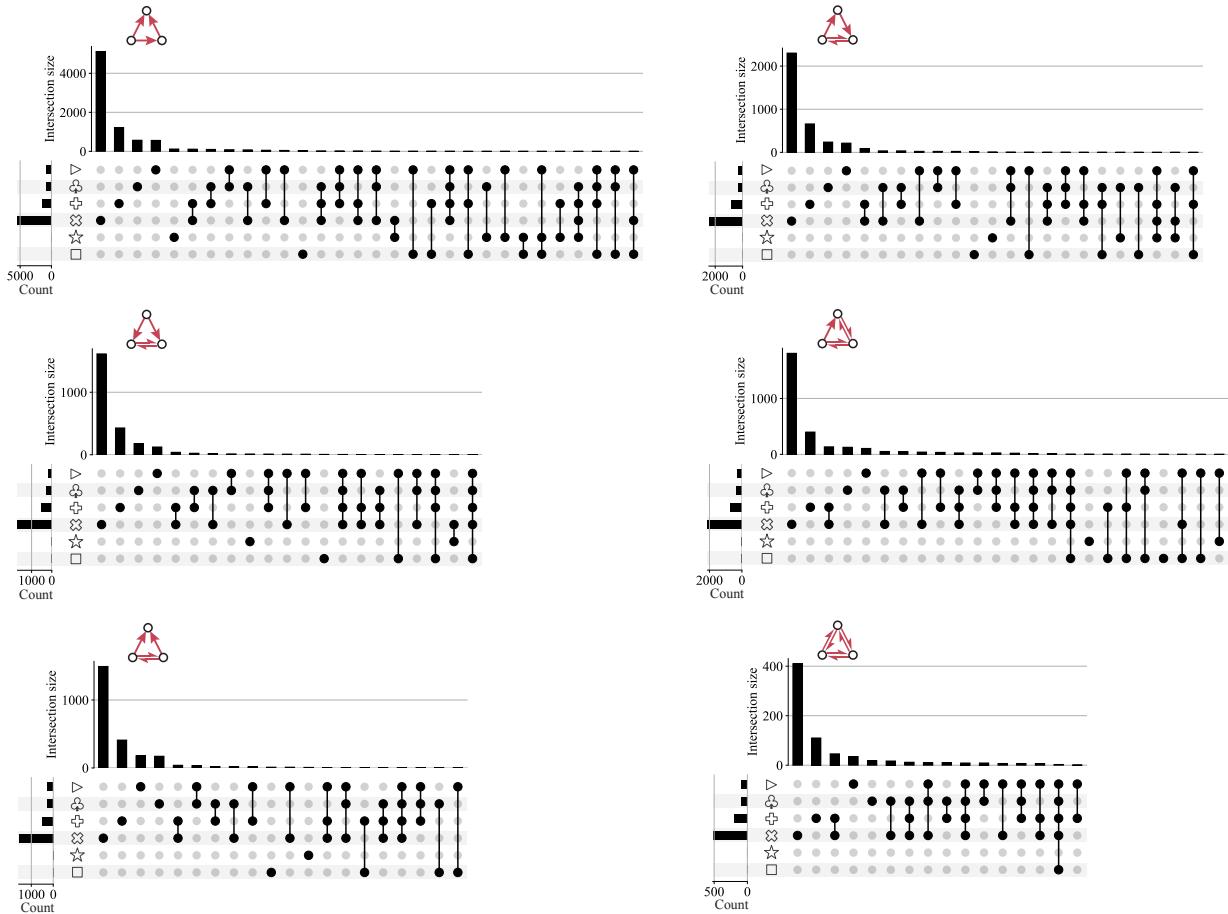


Fig. S6 | Intersections of unique motif sets for eFFLb motifs during six types of stimuli. All possible intersections with at least 1 element are shown.

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

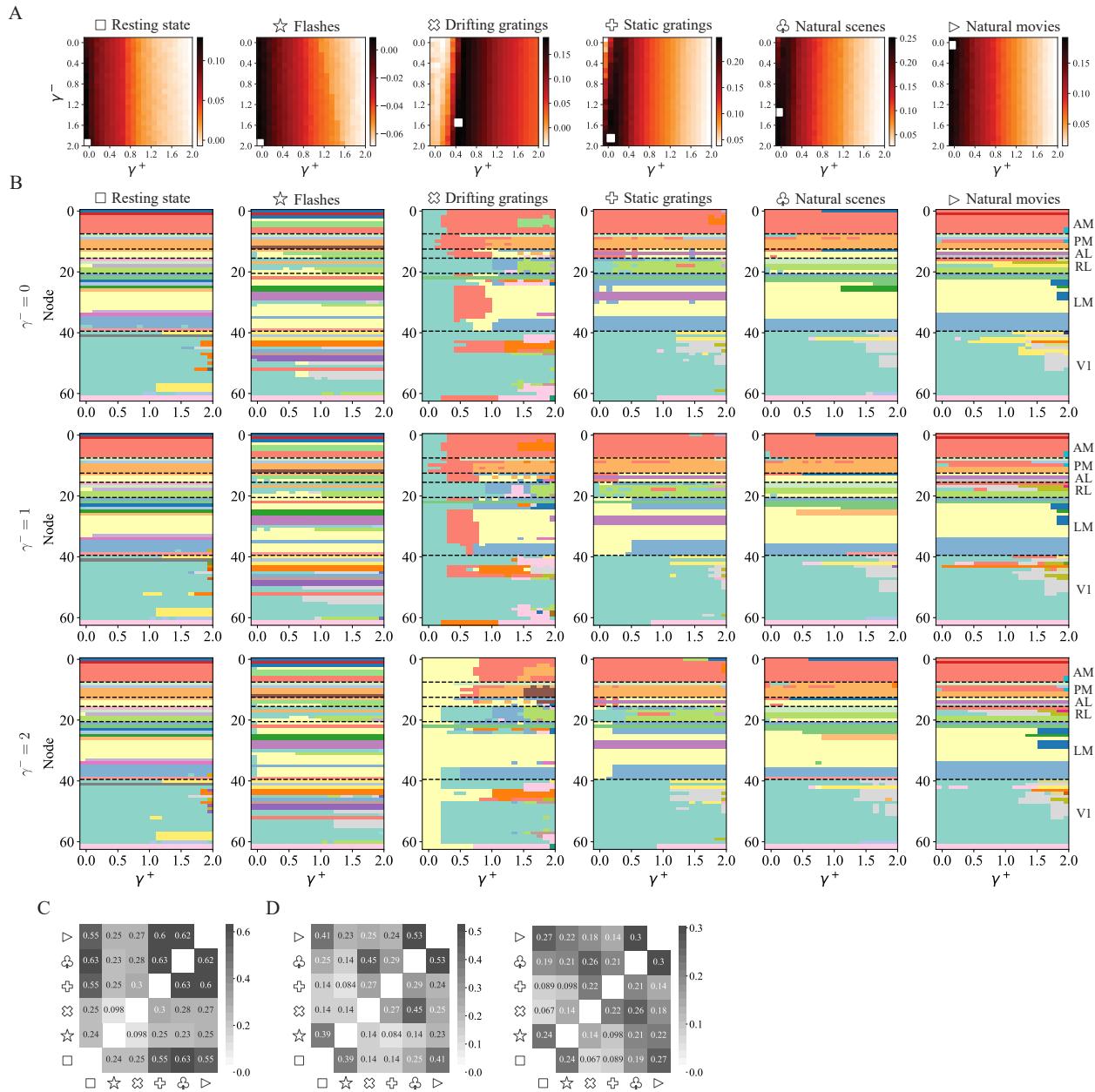


Fig. S7 | Multi-resolution modular structure. (A) The Modularity difference heatmap between the empirical network and reference model (Signed-pair-preserving model) is used to determine the resolution parameters γ^+ and γ^- , white box represents the maximum of the heatmap while its coordinates correspond to the optimal resolution parameters. (B) Modular partition at different resolution parameters for a mouse. Since empirical functional networks tend to have more excitatory than inhibitory connections, γ^+ has a larger impact than γ^- thus we only show the results obtained with three different values of γ^- . Module IDs (colors) across different γ^+ are determined by assigning the merged module the ID of its largest submodule at the previous step (larger resolution parameter), similar to the previous method [14]. Only neurons with at least one excitatory connection during any stimuli are included for brevity, and remaining neurons within each visual area are ordered based on their partition similarity while the module IDs across different multi-resolution modular partition maps are matched using a heuristic algorithm based on their similarity for visual comparison. (C) The heatmap of pairwise adjusted rand index (ARI) between visual stimuli for the same mouse in (B). Each multi-resolution modular partition map is considered a single clustering result, and ARI is used to measure the similarity between different partition maps. Note that ARI is independent of the color-matching heuristic algorithm and is thus more reliable. (D) The heatmaps of pairwise ARI between visual stimuli for two other mice. Only mice with at least one neuron in each area are shown. Despite individual differences, the similarity between natural scenes and movies is always among the highest.

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

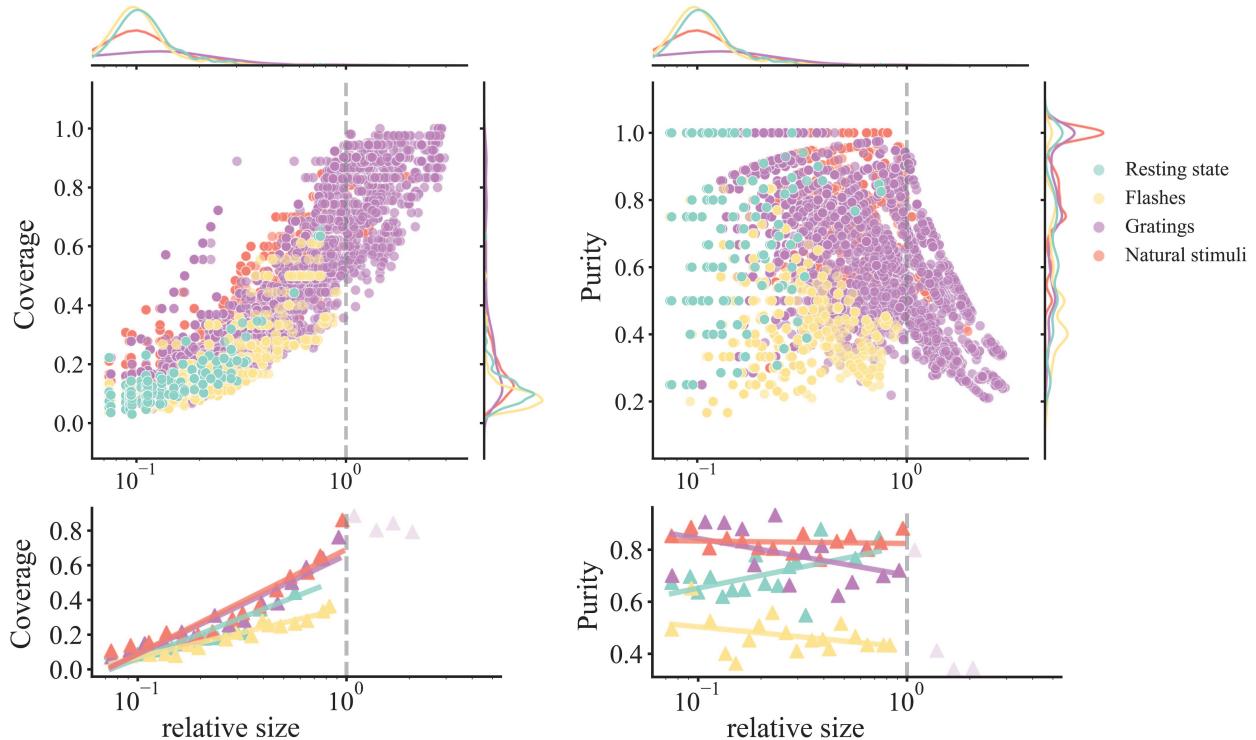


Fig. S8 | Fundamental properties of modular structure with module size. (top) Coverage/purity against relative module size for four stimulus types where each dot represents a single module; relative module size is defined as module size divided by the largest area size. (bottom) Coverage/purity against relative module size with regression after log binning. Modules with a relative size larger than 1 are excluded in regression since their coverage/purity will introduce bias.

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

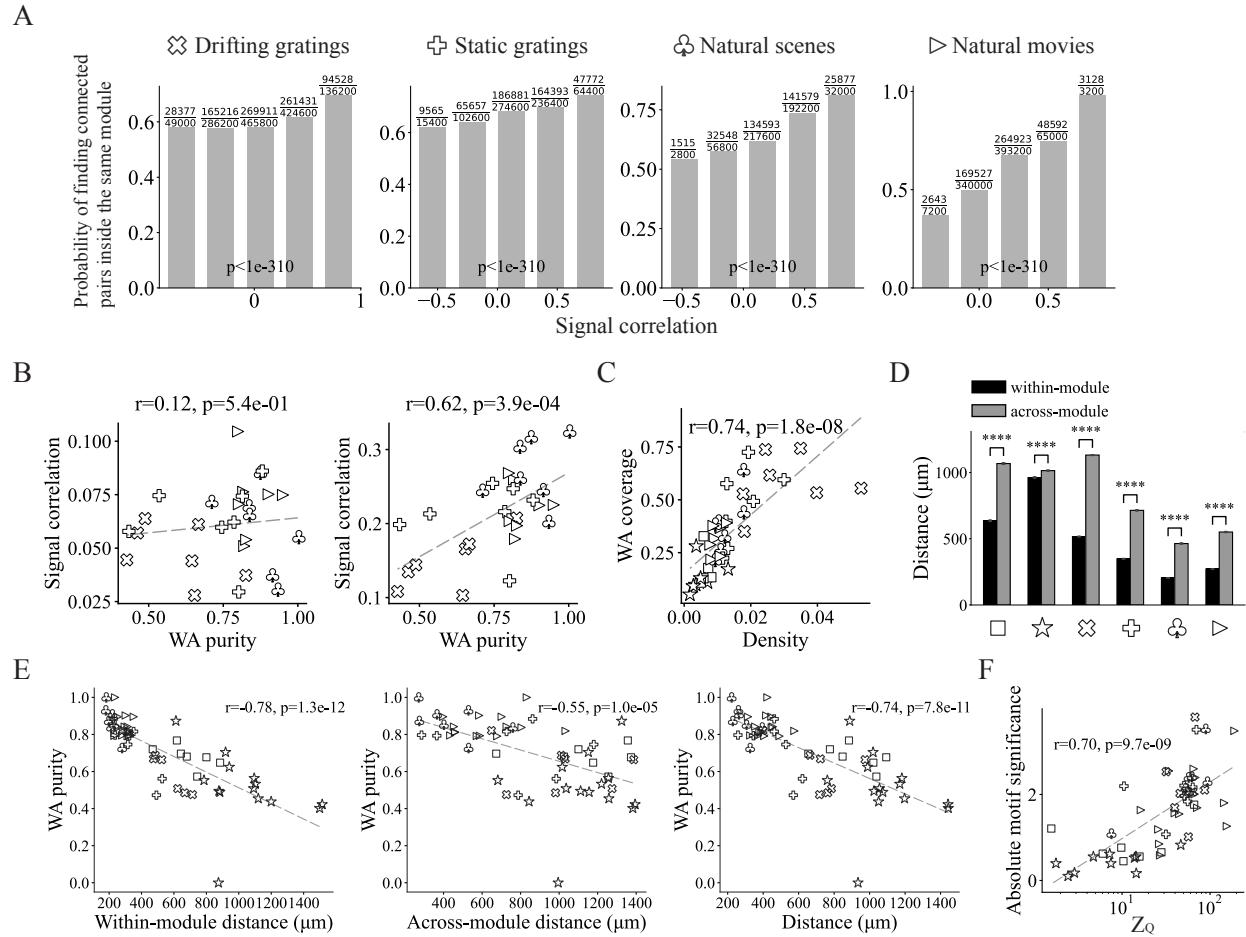


Fig. S9 | Biological interpretation of the modular structure. Wald Test is used in (B), (D) and (E). (A) Probability of finding connected neuron pairs inside the same module against their signal correlation, Cochran-Armitage trend test. (B) Linear regression results of signal correlation of chunked tuning curves with equal length against WA purity for (left) disconnected neuron pairs and (right) connected neuron pairs. Tuning curves are chunked into sequences with equal lengths for a fair comparison across stimuli, each dot represents the functional network of a mouse during certain stimulus presentations. (C) Linear regression results of WA coverage against network density. (D) Physical distance between connected neuron pairs that belong to the same or different modules. *** $p < 10^{-4}$, Student's t-test. (E) Linear regression results of WA purity against average distance between (left) within-module connected neuron pairs, (center) across-module connected neuron pairs and (right) all connected neuron pairs. Each dot represents the functional network of a mouse during certain stimulus presentations. (F) Average absolute motif significance (absolute Z score of intensity) across all signed motifs against Z score of Modularity.

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

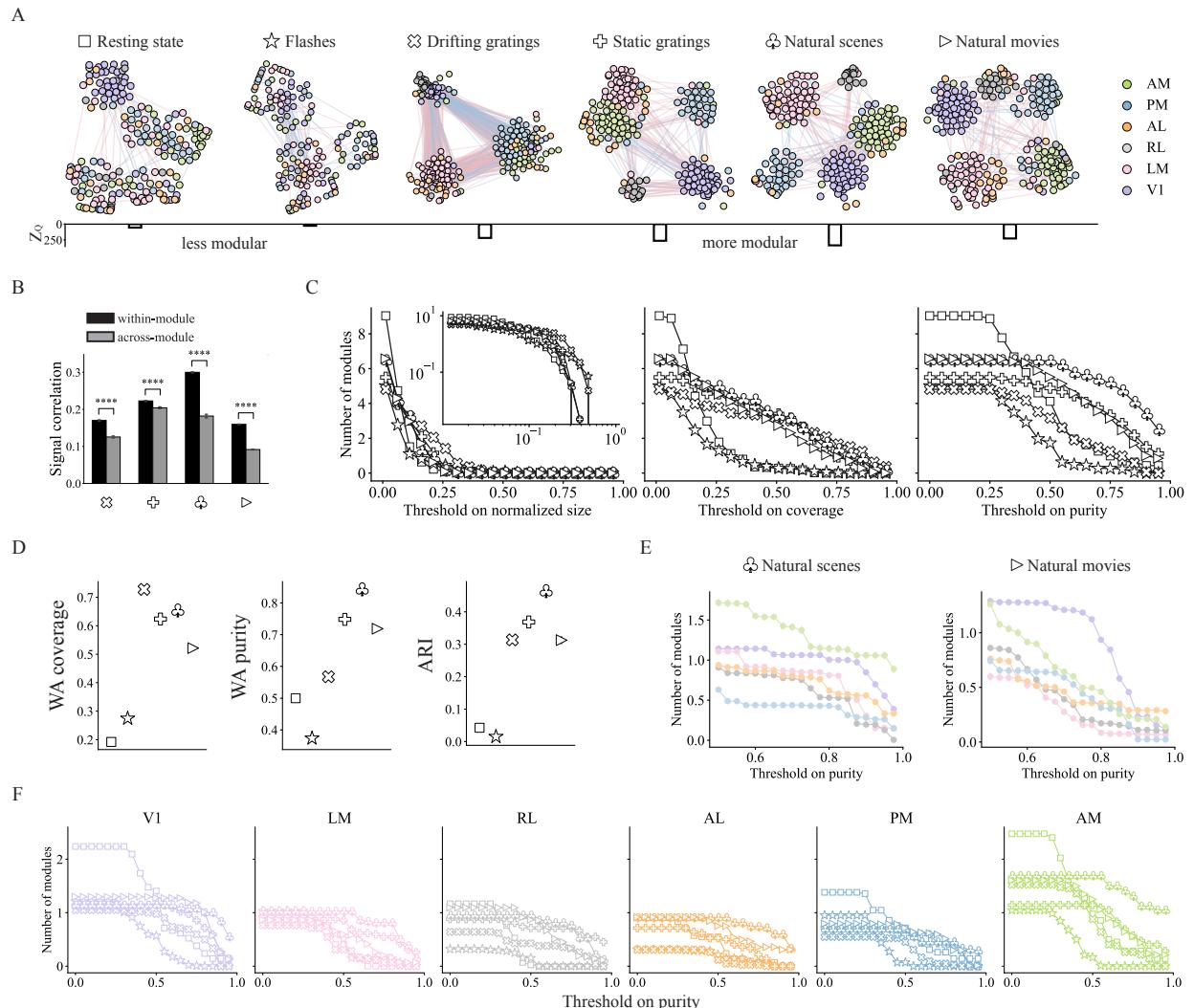


Fig. S10 | Results on modular structure with original Modularity by omitting edge signs. (A) Topological structure of functional networks during all visual stimuli. (B) Signal correlation for within-module and across-module connections. *** $p < 10^{-4}$, Student's t-test. (C) Number of modules with normalized size, coverage or purity higher than the threshold, inset shows the plot on a log-log scale. (D) WA (weighted average) coverage, WA purity and ARI during six visual stimuli, the error bars show the confidence intervals over all mice obtained with non-parametric bootstrap method. (E) Number of modules against threshold on purity for each visual area separately during natural scenes and natural movies. (F) Number of modules with purity higher than the threshold for each visual area during all visual stimuli.

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

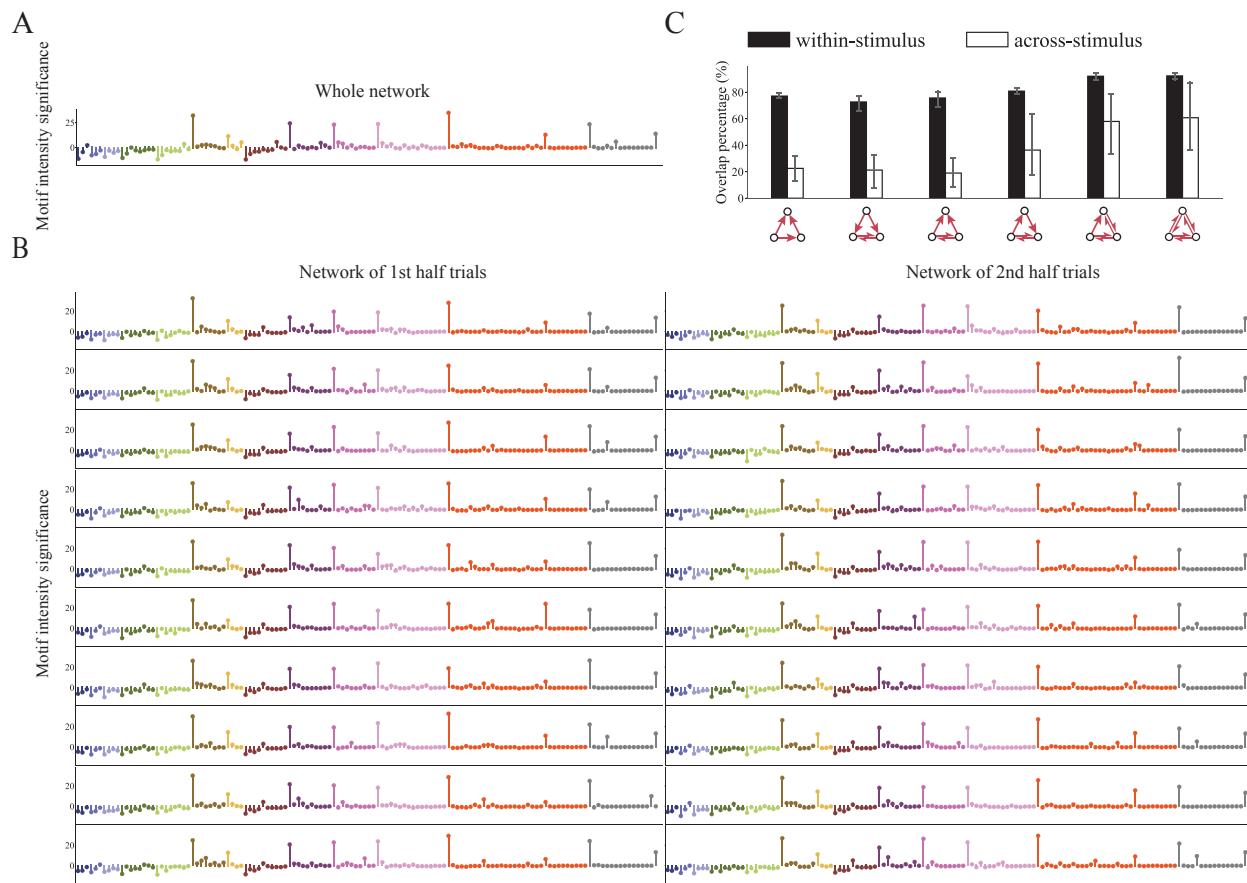
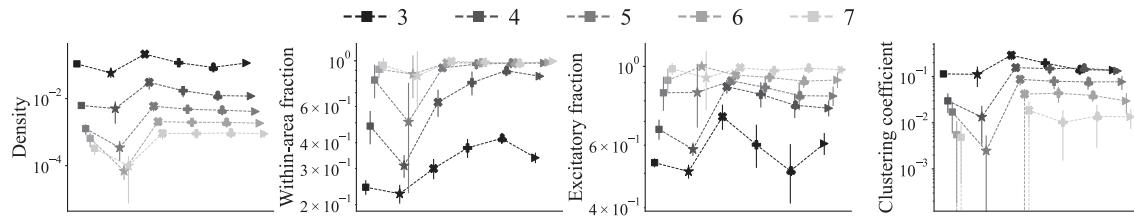


Fig. S11 | Functional networks constructed from different trials. (A) Motif sequence of the network during all trials of natural scenes. (B) Motif sequences of the networks during two halves of the trials of natural scenes. Each row corresponds to a realization of the random split. (C) Overlap percentage for eFFLb motifs. For each network, overlap percentage is defined as the percentage of motifs that are found on this network and at least one other network. Overlap percentage is higher for networks evoked by different trials from the same stimulus than different stimuli; *** $p < 0.027$, rank-sum t-test, corrected using Benjamini/Hochberg method.

STIMULUS-DEPENDENT FUNCTIONAL NETWORK TOPOLOGY IN MOUSE VISUAL CORTEX

A



B

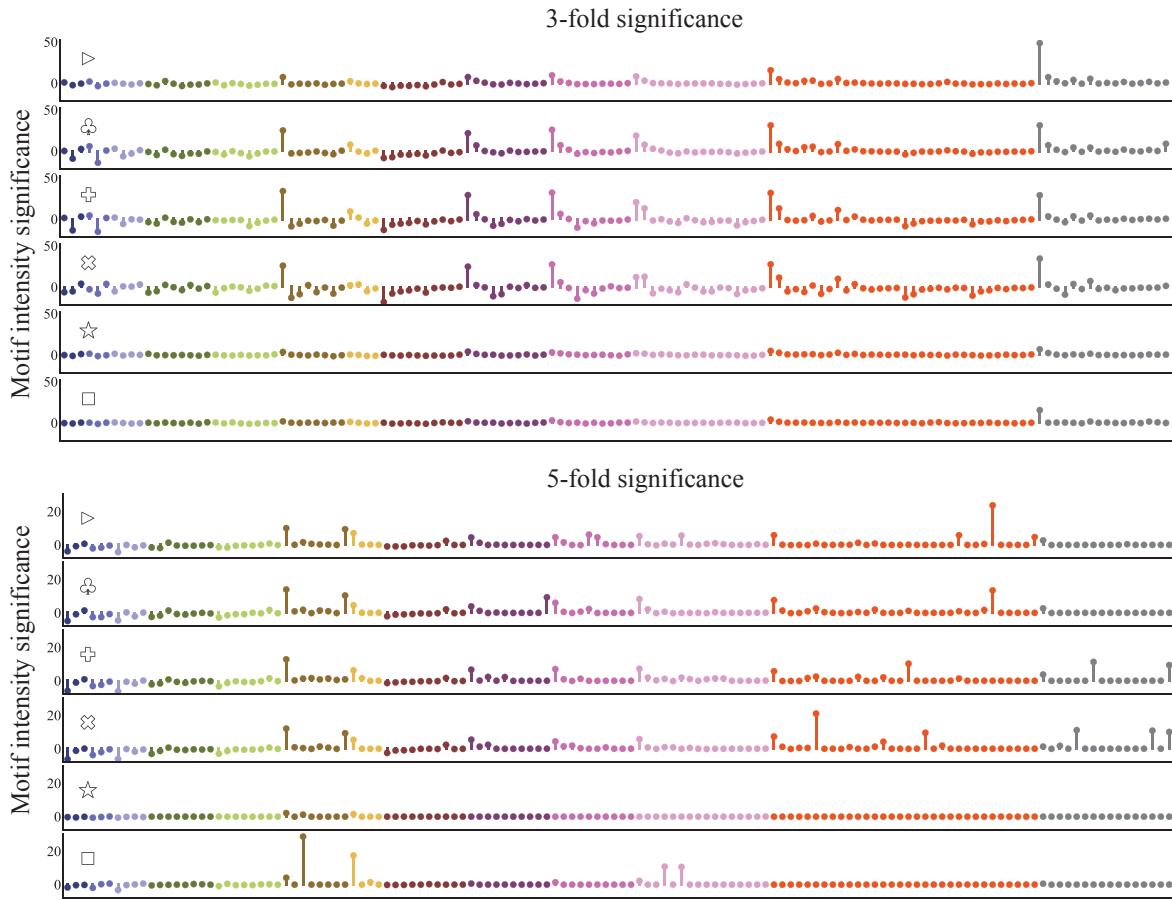


Fig. S12 | Functional networks constructed using different significance levels on functional connections. (A) Fundamental properties of the network on different significance levels (from 3-fold to 7-fold). (B) Motif sequences of the networks on different significance levels. Only 3-fold and 5-fold are shown since higher significance levels lead to extremely sparse networks. See Fig. 2C for results on 4-fold significance.