Cell assemblies at multiple time scales with arbitrary lag distributions
Eleonora Russo (1)* and Daniel Durstewitz (1)*

Author affiliation: (1) Dept. Theoretical Neuroscience, Bernstein Center for Computational Neuroscience, Central Institute for Mental Health, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany.

* Corresponding author: eleonora.russo@zi-mannheim.de, daniel.durstewitz@zi-mannheim.de

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Abstract
Hebb's idea of a cell assembly as the fundamental unit of neural information processing has dominated neuroscience like no other theoretical concept within the past 60 years. A range of different physiological phenomena, from precisely synchronized spiking to broadly simultaneous rate increases, has been subsumed under this term. Yet progress in this area is hampered by the lack of statistical tools that would enable to extract assemblies with arbitrary constellations of time lags, and at multiple temporal scales, partly due to the severe computational burden. Here we present such a unifying methodological and conceptual framework which detects assembly structure at many different time scales, levels of precision, and with arbitrary internal organization. Applying this methodology to multiple single unit recordings from various cortical areas, we find that there is no universal cortical coding scheme, but that assembly structure and precision significantly depends on brain area recorded and ongoing task demands.

Introduction
Even more than six decades after its conception, Hebb's (1949) fundamental idea of a cell assembly continues to play a key role in our understanding of how neural physiology may link up to cognitive function. Loosely, a cell assembly refers to a group of neurons which, by functionally organizing into a temporally coherent set, come to represent mental or perceptual entities, thereby forming the basis of neural coding and computation (1). However, the term lacks a stringent and universally accepted definition, and has been used to denote anything from the precise zero-phase-lag spike synchronization in a defined subset of neurons (2–4) to temporally coherent changes in average firing rates on larger time scales (5, 6). Often the term is meant to imply precise millisecond coordination of spike times for a 'volley' of activity which repeats at regular or irregular intervals in relation to specific perceptual or motor events (Fig. 1A, I; e.g. (2, 3, 7, 8)). Precise sequential patterns of spiking times (i.e., with time lags ≠ 0) have been reported as well (Fig. 1A, II), most commonly in the hippocampal formation where they may correspond to sequential orders of places (9, 10). More generally, neurons may contribute several spikes in any order to a fixed spatio-temporal pattern (Fig. 1A, III), as reported and linked to putative synaptic input motifs in vitro and in vivo (11, 12). At a coarser temporal scale, neurons could fire with a specific temporal patterning to which each
neuron may contribute “bursts” of variable length (Fig. 1A, IV). Such temporally ordered transitions among coherent firing rate patterns across sets of simultaneously recorded neurons have been described in different cognitive tasks and systems (13–16). At a still broader temporal scale, sets of neurons jointly increasing their average rates for some period of time (Fig 1A, V), as during persistent activity in a working memory task, have also been linked to the cell assembly idea (5).

There is indeed an ongoing, sometimes heated, controversy about the degree of temporal precision and coordination present in neural activity and its relevance for neural coding, partly based on empirical (17, 18), partly on statistical arguments (19). Based on this discussion, it seems at present premature and limiting to focus on a single specific assembly concept, theoretical idea, or particular time-scale. Here we develop a novel statistical approach for multi-cell recordings that treats the temporal scale, precision, and internal organization of coherent activity patterns as free parameters, to be determined from the data, and is thus open to a large family of possible assembly definitions (Fig. 1A). By deriving a fast parametric test statistic for pairwise dependencies that automatically corrects for non-stationarity locally, computationally costly bootstrapping and sliding window analyses are avoided, reducing computational burden by factors of 100-1000 (see SI Methods). This considerable speed-up enables a computationally efficient agglomeration algorithm that checks for assembly structure at all possible lag constellations and temporal scales, not accomplished (to this extent) by previous algorithms to our knowledge (see SI Methods). We then apply this methodology to examine in multiple single-unit (MSU) recordings from different cortical areas whether these employ a kind of universal temporal coding scheme, or whether and how properties of the assembly code are adapted to area-specific computations and task demands.

![Fig. 1. Detection of assemblies defined by different degrees of temporal precision, scale, and internal structure.](image)

**Results**

**Theoretical framework for assembly detection.** From a statistical perspective, any of the assemblies from Fig. 1A should reveal itself through recurring activity patterns in a set of simultaneously recorded spike trains, where a pattern can be any supra-chance constellation of unit activities with a specific distribution of time lags l among them. The idea is to capture the multiple temporal scales introduced above through the width \( \Delta \) used for binning the spike time series. We start from the relatively old notion of assessing the
departure of the joint spike count distribution \( p(A, B) \) of two units (or sets) \( A \) and \( B \) from independence (20, 21). For two independent units with stationary spike trains, the joint distribution of spike occurrences at a specified time lag \( l \) would factor into the single unit (‘marginal’) distributions, \( p(A, B) = p(A)p(B) \). Assume each recorded spike time series has been converted into a series \( \{c_t\} \) of spike counts of length \( T \) at bin width \( \Delta \), with \( #_A \) and \( #_B \) denoting the total numbers of spikes emitted by units \( A \) and \( B \), respectively. If \( \Delta \) is small enough such that \( c_t \in \{0,1\} \) (binary counts), then, under the null hypothesis (\( H_0 \)) of independence, the joint spike count \( #_{AB,l} \) at time lag \( l \) follows a hypergeometric distribution with mean \( \mu_{AB,l} = \frac{#_A #_B}{T - l} \) and variance \( \hat{\sigma}_{AB,l}^2 \). If the binning is such that spike counts \( c_t \) larger than one occur, the hypergeometric distribution is no longer directly applicable. We then split the series into several (mutually dependent) binary series (Fig. S1A) for which we obtain a joint mean and variance as derived in the SI Methods.

The estimated mean \( \hat{\mu}_{AB,l} \) and variance \( \hat{\sigma}_{AB,l}^2 \) could, in principle, be used to check for deviation from the \( H_0 \) of independence at lag \( l \), but in practice such a statistic would be corrupted by non-stationarities like (coupled) changes in the underlying firing rate (see SI Methods; Fig. S2). Sliding window (22) or bootstrap-based (21, 23, 24) analyses have most commonly been used to deal with this issue, but come at the price of considerable data loss or computational burden. Here we suggest a simple remedy which corrects for non-stationarity locally by using the difference statistic \( #_{ABBA,l} = #_{AB,l} - #_{AB,-l} \) (Fig. S1B).

The idea is that this way non-stationarities in firing rates would cancel out locally, on a comparatively fine time scale (\( \approx \Delta \)), since they would affect \( #_{AB,l} \) and \( #_{AB,-l} \) alike (for assessment of synchronous spiking, we use \( #_{ABBA,0} = #_{AB,0} - #_{AB,-1} \), with \( l^* \neq 0 \)). The statistic \( Q_l \equiv \frac{\hat{\mu}_{ABBA,l}^2}{\hat{\sigma}_{ABBA,l}^2} \) finally is approximately \( F \)-distributed with \( 1, 2 \) degrees of freedom and can be used for fast parametric assessment of the \( H_0 \) (see SI Methods and Figs. S2-S4 for derivation and empirical confirmation using non-stationary

**Fig. 2.** Assemblies in recordings from anterior cingulate cortex (ACC) during delayed alternation. (A) Assembly-assignment matrix for one ACC data set, with average firing rate of units indicated on the left. (B) Examples of detected assembly patterns at relatively precise (top; 50 ms) and broader (bottom; 200 ms) time scales. Insets on the right zoom in on detected assemblies with optimal binning \( \Delta \) indicated by vertical lines. See SI Methods for computation of assembly activation scores (‘activity’) as shown in the lower panels. (C) Two examples of selective assembly activity discriminating between left (blue curves) and right (red curves) lever presses during actual lever press (top) or during delay (bottom). Times of lever press and nose poke are indicated by vertical red and black dashed lines, respectively. Periods of significant differentiation indicated by black bars above curves (two-tailed, paired t-test, \( *p < .05 \), Bonferroni-corrected for number of bins tested). Shaded areas = SEM.
Having derived a fast, non-stationarity-corrected parametric test statistic for assessing independence of pairs, we designed an agglomerative, heuristic clustering algorithm for fusing significant pairs into higher-order assemblies (see Fig. S6 and SI Methods for full derivation and pseudo-code). In essence, at each agglomeration step the algorithm treats each set of units fused in an earlier step just like a single unit with activation times defined through one of its member units. This allows for the same pair-wise test procedure on sets of units as defined for single units above, while at the same time effectively testing for higher-order dependencies based on the joint (set) distributions (see SI Methods). Each pair is tested at all possible lags \( l \in \{-l_{\text{max}}, \ldots, l_{\text{max}}\} \) (with \( l_{\text{max}} \) provided by the user), which is a reasonably fast process given the parametric evaluation introduced above. Should a pair of unit-sets prove significant at several lags \( l \) at any step, only the one associated with the minimum \( p \)-value is retained. The recursive set-fusing scheme stops if no more significant relationships among agglomerated sets and single units are detected. All subsets nested within larger sets are then discarded. This whole procedure is repeated for a set of user-provided bin widths \( \Delta \in \{\Delta_{\text{min}}, \ldots, \Delta_{\text{max}}\} \). For each formed assembly, the width \( \Delta^* \) associated with the lowest \( p \)-value may then be defined as its characteristic temporal precision. All tests are performed at a user-specified, strictly Bonferroni-corrected \( \alpha \)-level (always set to 0.05 here; see SI Methods for details).

**Performance evaluation on simulated data.** The agglomerative scheme described above is a fast heuristic proxy, similar in spirit to the apriori algorithm in machine learning (24–26), for evaluation of all possible unit and lag combinations. To illustrate and evaluate its performance, synthetic data with known ground truth were created. Cell assembly structures with the different levels of temporal precision and internal organization (i.e., lag distributions) shown in Fig. 1A were simultaneously embedded within inhomogenous (i.e., non-stationary) Poisson spike trains, with mean rate following an auto-regressive process (see SI Methods). The assembly-assignment matrix in Fig. 1B demonstrates that all five different types of assemblies (and only these, no false detections) were correctly identified with their associated temporal precision and lag distributions. Fig. 1C illustrates the quality of ‘assembly retrieval’ (measured as fraction of assembly units correctly assigned) as a function of bin width \( \Delta \). As expected, the retrieval quality steeply declines for the temporally precise assemblies as the bin width increases (types I and II), while it rises up to the appropriate temporal scale for the more broadly defined assemblies (types IV and V). For assembly-type III, defined by precise temporal relationships, yet extended across time without strictly sequential structure, both these time scales are revealed (leading to the local peak at \( \sim300 \) ms). Also note that the correlated rate increases which define assemblies of types IV and V naturally can be discovered already at lower bin widths than the one which corresponds to the temporal extent of the whole pattern. In the SI Methods (Fig. S7) we investigated more systematically how assembly retrieval varies as a function of sample size and potential spike sorting errors. Assembly detection starts to significantly degrade only when their relative contribution to the spike series drops below \( \sim4\% \) (Fig. S7A), or when more than \( \sim30\% \) of all spike times were corrupted by spike sorting errors (Fig. S7B). More importantly, across a whole range of sample sizes, spike assignment errors, and assembly structures tested, the fraction of units *false*ly ascribed to any one assembly stayed
uniformly low at about 0.5% (Figs. S7C, D), indicating that our procedure is very conservative and rarely returns false positives in the simulated scenarios.

Area- and task-specific assembly configurations and time scales. We next examined assembly structure in different brain regions from which multiple single-unit recordings were obtained in previously published experiments, including the rat anterior cingulate cortex (ACC; (27, 28)), hippocampal CA1 region, and entorhinal cortex (EC, (29–31)) (see SI Methods for further specification). Fig. 2A presents the assembly-assignment matrix from one of these data sets. Detected assemblies span a large range of temporal precisions, from ~10 ms to about 1.5 s, with a variety of lag distributions, and are composed of about 10% (ACC) to 16% (CA1, EC) of the recorded neurons. Note that different from the clear-cut hypothetical examples (Fig. 1B) which were strictly disjoint by design, many of the experimentally recorded assemblies partially overlap (i.e., share units; see also SI Methods). Fig. 2B also gives specific examples of assemblies with relatively high (top) and with lower (bottom) temporal precision. Finally, many of the unraveled assemblies were highly selective for specific task events as illustrated in Fig. 2C.

A specific question one might ask is whether different brain areas host different types of assembly structures, and how these may depend on the behavioral task. These aspects are quantified in Fig. 3A by plotting the distribution of all significant unit pairs as a function of bin width $\Delta$ and time lag $l$. Several features are noteworthy here: First, the joint ($\Delta, l$)-distribution changes dramatically as the animals move from unstructured, completely self-paced, little demanding environmental exploration (Fig. 3A, left column) to a highly structured, demanding delayed alternation task (Fig. 3A, right column). During the latter, a much larger number and richer repertoire of assembly structures turned out, as also indicated by the ‘marginal’ distributions of

Fig. 3. Assembly structure in different brain areas and behavioral tasks. (A) Relative frequency histograms (color-coded) of all significant unit pairs, pooled across all detected assemblies, as a function of characteristic time scale $\Delta \in \{0.015 \ldots 1.5\, \text{sec}\}$ and lag $l \in \{-10 \ldots 10\}$, for the anterior cingulate cortex (ACC, top), CA1 region (center), and entorhinal cortex (EC, bottom) during environmental exploration (left) and delayed alternation (right). Note that the aggregation at larger lags for ACC is partly due to the fact that the algorithm considered lags up to $l_{\text{max}} = 10$, such that significant pairs with optimal lag $l > 10$ may have been assigned to $l_{\text{max}}$. (B) Marginal distributions of significant assembly-unit pairs across temporal scales $\Delta$ for ACC (top), CA1 (center), and EC (bottom). Blue curves = environmental exploration. Red curves = delayed alternation. Shaded areas = SEM.
significant unit pairs across time scales \(\Delta\) in Fig. 3B. However, secondly, while this is true for ACC and CA1, much less of such task-dependent changes are noticeable in EC (Fig. 3A, bottom; Fig. 3B, bottom). Third, assemblies remarkably differ among the three brain regions in the range of temporal scales they occupy: While EC mainly harbors fine temporal structure with a precision of about 15-50 ms, in ACC broad rate change patterns in the 120-700 ms range appear to dominate (Fig. 3B). CA1, in contrast, expresses both temporal scales, or in fact a wide spectrum from about 30-1500 ms, of which the broader scales (>100 ms) only surface during delayed alternation. Overall, a much larger number of units engaged in any one assembly in CA1 (>90%) than in ACC (30-50%; Fig. 3B). These observations indicate that the temporal composition and precision of assembly activity strongly depends on both the brain area and the behavioral setting.

On closer inspection, some of the temporally more precise 30-50 ms assemblies in CA1 were found to code for specific place fields (‘place assemblies’) in the rat’s environment (Figs. 4A, S4). These assemblies mainly consisted of synchronous (lag-0) spiking units (Fig. 3A). Meanwhile, the more broadly tuned assemblies in CA1 tended to code for temporally extended events which often appeared to have a specific behavioral meaning in the task context: For instance, these assemblies may become active during the reward event irrespective of its spatial location (Fig. 4B), or for the whole correct choice path after a behavioral decision was made (Fig. 4D). These temporally broader assemblies commonly also followed a more sequential (lag≠0) layout (Fig. 3A). Interestingly, the single cells constituting CA1 assemblies did not necessarily share the same place preference with their ‘parent’ assembly (Fig. S11). Similar as in CA1, broader assemblies in ACC were tuned to specific task phases and events (lever presses, delays, stimulus conditions) and reflected the task’s sequential structure (Fig. 2C).

Fig. 4. Assembly coding at different time scales in CA1. Color-coded activity maps for four CA1 assemblies during an environmental exploration task (A) and a delayed alternation task (B, C, D). Below x-axis in each panel: Identities of neurons assigned to the assembly, associated time lags within assembly, and temporal scale of assembly.

Discussion

Here we introduced a novel theoretical and statistical framework, based on fast parametric testing and computationally efficient agglomerative algorithms, which detects assembly structure at many different temporal scales, and with arbitrary internal organization, while at the same time accounting for non-stationarity on a fine time scale. This enables to readdress fundamental questions about the temporal structure and nature of neural representations in an unbiased way. Illustrating this methodology on multiple single-unit recordings from ACC, CA1, and EC, it appeared as if the temporal structure and precision of the revealed assemblies were closely related to the computations performed by these brain areas: While the CA1 region processes precise spatial (2, 32, 33) and temporal (34)
environmental structure, the ACC is much less concerned with finely-granulated details of the spatial world (27). Rather, activity in ACC reflects behavioral organization, behavioral monitoring, overall context, and task structure, processes which typically unfold on much slower temporal scales (13, 27). Likewise, in addition to spatial coding, the hippocampal CA1 region has also been reported to represent aspects of higher-order decision making, like paths to a defined goal state or choice outcomes (35, 36). These capacities may become relevant only when an animal is transferred from unstructured environmental exploration to a task which involves clearly defined goal states, reward-related choices, and possibly time delays between them. Consequently, sequential organization of assemblies at broader time scales was only observed in the latter but not the former task context.

Numerous other statistical procedures for detecting assemblies or sequential patterns have been proposed previously (20–22, 24), but most of these adhere to one or the other theoretical conceptualization of a cell assembly (cf. Fig. 1A), or become computationally impractical for larger cell numbers or multiple lags (see SI Methods for further discussion). Also, none of these, to our knowledge, combines all of the features presented here. The statistical tools developed here may allow addressing questions about the nature of neural coding in different brain areas, without requiring the researcher to commit to any particular assembly concept or theoretical framework a priori. Indeed, we observed that there may be not just one type of cortical assembly code, but that the temporal precision, scale, and sequential composition with which cortical neurons organize into coherent patterns strongly depends on the brain area and task context investigated. We further note that our methods are not specific to the neuroscientific domain, but could be used more widely in other scientific areas to detect structure at multiple temporal scales in multivariate event count series.

Materials and Methods

**Statistical test for pairwise interactions.** Given $N$ recorded units and a user-defined range of bin widths $\Delta \in \{ \Delta_{\text{min}} \ldots \Delta_{\text{max}} \}$, each spike train $K$ is first divided into $T$ bins of equal width $\Delta$, resulting in $N$ spike count series $\{c_{K,t}\}$, $t = 1 \ldots T$, $K = 1 \ldots N$. For each pair of units $A$ and $B$, across a pre-specified set of time lags $l \in \{-l_{\text{max}} \ldots l_{\text{max}}\}$, the lag $\bar{l} = \arg \max_{l} \left(\#_{AB,l}\right)$ is then determined for which the maximum joint spike count $\#_{AB,l}$ is obtained. This count is tested for significance using the (stationarity-corrected) difference statistic $\#_{AB\bar{l}} = \#_{AB,\bar{l}} - \#_{BA,\bar{l}}$. A parametric distribution for this statistic is derived by splitting each spike count series into a set of $a = 1 \ldots M$ binary processes (see Fig. S1A), with $M_k \equiv \max(c_{K,t})$, based on which the total mean $\mu_{AB\bar{l}} \equiv E[\#_{AB\bar{l}}]$ and variance $\sigma^2_{AB\bar{l}}$ for $\#_{AB\bar{l}}$ can be computed (see eq. 8-10 in SI Methods). As derived and shown (Figs. S2, S3, S4) in the SI Methods, the test statistic $Q_{\bar{l}} \equiv \hat{\mu}_{AB\bar{l}}/\hat{\sigma}_{AB\bar{l}}$ is approximately $F$-distributed with $\{1, 2(T - \bar{l})M - 1\}$ degrees of freedom under the $H_0(\Delta)$ that the two processes $A$ and $B$ are independent on the specified scale $\Delta$, while they are allowed to be coupled and/or non-stationary on slower temporal scales of at least $\bar{l}\Delta$.

**Recursive assembly agglomeration algorithm.** Once all significant pairs $(AB, \bar{l})$ with their associated time lags have been identified, larger sets are recursively built up by treating already formed sets like single units, and pairing them in turn with all single units $C$ which had entertained a significant
relationship with either A or B in the previous step. The algorithm then proceeds with the resulting composite (triple) groups \((AB, \tilde{I}_{AB})C, \tilde{I}_{(AB)C}\) in the very same manner, and likewise with all higher-order structures iteratively constructed this way, until finally no more units engage in significant relationships anymore with the already agglomerated sets. At any step, unit-sets with the same elementary units but different lag distributions may result, from which only the one associated with the lowest \(p\)-value will be retained. Further pruning steps are described in the SI Methods, along with pseudo-code for the algorithm. An instance of assembly activation in the multivariate spike time series was registered whenever spikes in the elementary assembly units occurred in the order prescribed by the associated pattern of time lags. The total assembly activation score (as given in Figs. 2B-C) is then defined as the number of such activation instances within a given time bin of size \(\Delta\). A Matlab (MathWorks) implementation of the whole procedure is provided at www.bccn-heidelberg-mannheim.de.

Construction of synthetical ‘ground-truth’ data. As described in detail in the SI Methods, we have evaluated our approach on a variety of ‘ground truth’ artificial data sets (Figs. 1C, S2-S4, S7). For these, single spike trains were simulated according to inhomogeneous (non-stationary) Poisson processes with time-varying rates, where the rate fluctuations were implemented either as large step-like changes (‘stimuli’) or drawn from slowly varying auto-regressive processes with Gaussian noise. Rate correlations among units on different time scales were realized either by directly coupling the step-like changes in different units (Fig. S3) or through the covariance matrix of the linear Gaussian rate process (Fig. S4). Different assembly structures as illustrated in Fig. 1 were then embedded within these simulated multivariate spike time series. These simulated data were used to systematically examine both the ability of our algorithm to retrieve embedded assemblies (Figs. S7A, B), as well as its false discovery rate (Figs. S7C, D), under different experimental scenarios (low sample size, spike sorting errors).

Brief description of experimental data sets. The in-vivo recordings from the rat (Long-Evans) anterior cingulate cortex (ACC) were taken from two studies by Hyman et al. (27, 28). Simultaneous recordings from about 30-35 isolated, artefact-free single units were obtained through a set of 16 tetrodes on a task where animals were free to explore novel environments (27), and on an alternation task within a Skinner-box with a 10 sec delay between alternating lever presses. Simultaneous hippocampal and entorhinal cortex (EC) recordings from two Long-Evans rats implanted with multi-shank (32 or 64 sites) silicon probes came from Pastalkova et al. (30) and Mizuseki et al. (31). Recordings were performed while animals freely explored a 180 cm x 180 cm arena with water or Froot Loop items randomly dispersed throughout, or while alternating between two arms of a figure-eight shaped maze to obtain reward at water spouts located at the rear of the arms. A delay of 10 sec or 20 sec, respectively, spent in a running wheel, was inserted between trials for the two animals tested. See original articles (27–31) and SI Methods for further details.

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Supplementary material

**Statistical test for pairwise interactions**

Assume we have recorded $N$ spike trains, each divided into $T$ bins of equal bin width $\Delta$, resulting in a spike count series $\{c_{K,t}\}$, $t = 1 \ldots T$, for each recorded unit $K$. Bin width $\Delta$ sets the temporal precision at which unit interactions are to be detected. We would like to test for a range of time lags $l \in \{-l_{\text{max}} \ldots l_{\text{max}}\}$ whether the joint spike count $#_{AB,l}$ of units $A$ and $B$ at lag $l$ significantly exceeds of what would have been expected under the null hypothesis ($H_0$) of independence of the two spiking processes. For clarity, note that $#_{AB,l}$ is computed by counting the number of times we have a spike in unit $A$ and a corresponding spike in unit $B$ $l$ time bins later. From the range of all considered lags, we select the one which corresponds to the highest count $#_{AB,l}^\dagger$, i.e. $\hat{l} \equiv \arg\max_l(\#_{AB,l})$. For deriving the proper distributional assumptions under the $H_0$, spike count series $\{c_{K,t}\}$ are often thresholded (20, 24, 37, 38) such that binary $\{0,1\}$-series are obtained, presumably since multivariate extensions of the binomial or hypergeometric distribution are not yet commonplace (see (39, 40)). Especially for larger bin widths $\Delta$ this implies a serious loss of information, however. Therefore we sought a different approach to the problem that makes use of the full spike counts, based on the first two moments of a multivariate hypergeometric distribution. Instead of thresholding, we split each spike count series into a set of $M = 1 \ldots M_K$ binary processes as indicated in Fig. S1A, where $M_K \equiv \max(c_{K,t})$ is the maximum spike count observed for unit $K$ for the specified bin width. The first binary process is defined by having a ‘1’ in all time bins for which spike count $c_K, \geq 1$ (and ‘0’ otherwise), the second process by having a ‘1’ only in those time bins for which $c_K, \geq 2$, and so on. For any two units $A$ and $B$, defining $M \equiv \min(M_A, M_B)$, the total joint count $#_{AB,\hat{l}}$ at selected lag $\hat{l}$ is now simply given by the sum of joint counts $#_{AB,\alpha}^{\hat{l}}$ across all pairs of binary subprocesses $\alpha = 1 \ldots M$,

\[
#_{AB,\hat{l}} = \sum_{\alpha=1}^{M} #_{AB,\hat{l}}^\alpha
\]

Since each of the $M$ subprocesses is binary, each number $#_{AB,\alpha}^{\hat{l}}$ follows a hypergeometric distribution under the $H_0$ (since marginal counts $#_A^\alpha$ and $#_B^\alpha$ are fixed by the observed data, a binomial distribution is not appropriate (41)). From this, and noting that the $M$ binary processes are not independent since by construction each higher-rank process $\gamma > \alpha$ can only have ‘1s’ where the lower-rank processes $\alpha$ had as well (but not necessarily vice versa), one can derive the expectancy and value and variance of $#_{AB,\hat{l}}$, respectively, under the $H_0$ as

\[
\mu_{AB,\hat{l}} = E[\#_{AB,\hat{l}}] = \sum_{\alpha=1}^{M} \frac{#_{A,\alpha}^{\hat{l}} #_{B,\alpha}^{\hat{l}}}{T - \hat{l}}
\]


\[
\sigma_{AB,t}^2 = E \left[ \left( \#_{AB,t} - E [\#_{AB,t}] \right)^2 \right] = \sum_{\alpha=1}^{M} \text{var}(\#_{AB,t}^{\alpha}) + 2 \sum_{\alpha=1}^{M-1} \sum_{\gamma > \alpha}^{M} \text{cov}(\#_{AB,t}^{\alpha}, \#_{AB,t}^{\gamma}) \\
= \sum_{\alpha=1}^{M} \frac{\#_{A,t}^{\alpha} \#_{B,t}^{\alpha} (\bar{T} - \#_{A,t}^{\alpha} (\bar{T} - \#_{B,t}^{\alpha}))}{\bar{T}(\bar{T} - 1)} + 2 \sum_{\alpha=1}^{M-1} \sum_{\gamma > \alpha}^{M} \frac{\#_{A,t}^{\alpha} \#_{B,t}^{\gamma} (\bar{T} - \#_{A,t}^{\alpha} (\bar{T} - \#_{B,t}^{\gamma}))}{\bar{T}(\bar{T} - 1)} \\
\text{with } \bar{T} = T - \bar{l}.
\]

A parametric test statistic, \( S_{T} \equiv (\#_{AB,t} - \hat{\mu}_{AB,t})/\hat{\sigma}_{AB,t} \) (\( t \)-distributed with \( 2(T - \bar{l})M - 1 \) degrees of freedom), could be based directly on these moments under the null hypothesis of independence on all time scales (for all choices of \( \Delta \)), and if the data were truly stationary (by which we mean here that the joint probability distribution \( \pi_{AB,t}(u,v) \equiv pr(c_{A,t} = u \land c_{B,t+l} = v) \) is time-invariant, i.e. \( \pi_{AB,t}(u,v) = \pi_{AB,t}(u,v) \) for all \( t \)). This will commonly not be the case with electrophysiological time series. Rather, there will be rate fluctuations on different temporal scales, as for instance induced by oscillatory drive or external stimuli (42). Under these conditions, variance estimator eq. 3 will usually be highly biased, often underestimating the true variation. Furthermore, the joint count \( \#_{AB,t} \) may not factor into the product of the marginal counts as in eq. 2 anymore, since, in general, \( E[\#_{A}]E[\#_{B}] \neq (T - l) \sum_{t=1}^{T-l} E[\#_{A,t}]E[\#_{B,t+l}] \) for any fixed non-stationary set \( \{\pi_{AB,t}(u,v), t = 1 \ldots T - l\} \). Finally, we are interested in testing for independence at a defined time scale \( \Delta \), but may want to permit the processes to be coupled at other temporal scales, like for instance with common external or oscillatory drive. In this case the simple test statistic defined above \( (S_{T}) \) breaks down (Fig. S2 left column). Hence, we would like to test against a stronger \( H_{0} \) which allows for uncoupled or coupled rate changes on broader temporal scales without corrupting our assessment of independence on finer scales.

In the time series literature, the most common remedies for non-stationarity issues are bootstrap-based techniques (21, 23, 24, 43) and sliding window analyses (22). These two methods have, however, severe limitations. Bootstrap-based approaches are computationally quite demanding since essential steps of the algorithm may have to be repeated for a 100-1000 bootstrap replications. This may become outright prohibitive especially when multiple lag distributions and constellations are to be considered as in the present work. Sliding window analysis, on the other hand, uses only small fragments of the data set for estimation in each window, thus can be seriously plagued by low sample size issues (resulting in weak statistical power). Sometimes this is (partly) addressed by pooling across many trials, but this in turn requires a) a sufficient number of trials, b) stationarity across trials, and c) clear external timestamps such that windows across trials are indeed comparable and can be aligned. In many tasks probing higher cognition, where just a handful of trials are not rare (e.g. (13, 27)), or in self-paced tasks, these methods are thus not applicable.

We therefore propose a new approach to non-stationarity here. Rather than testing \( \#_{AB,t} \) directly for significance, our approach focuses on the asymmetry between the occurrence rates of patterns \((AB,l)\) and \((BA,l) = (AB,-l)\), respectively, defined through

\[
\#_{ABBA,l} = \#_{AB,t} - \#_{AB,-l}.
\]

The idea is that non-stationarity on slower time scales \((> l, \text{if } \Delta \text{ is the time scale considered})\), e.g. in the form of correlated rate changes, will, on average, cancel out in \( \#_{ABBA,l} \), since it will
affect \#_{AB,l} and \#_{AB,-l} alike (for clarity, note that \#_{AB,l} is accumulated across \( t = 1: T - \bar{l} \), while \#_{AB,l} runs across \( t = \bar{l} + 1: T \); we also note that while differencing to remove non-stationarity is, in general, a more common practice in the ‘classical’ time series literature, e.g. (44), here we use this technique in a very specific sense by forming the difference between one joint count series and its reverse). More precisely, the scenarios covered by our \( H_0(\Delta) \) should be those where the two processes \( A \) and \( B \) are independent on the specified scale \( \Delta \), in fact independent for all scales at least up to \( l\Delta \), while they may be coupled and/or non-stationary on slower temporal scales of at least \( l\Delta \). Our \( H_0 \) requires \( E[\#^\Delta_{AB,l}] = E[\#^\Delta_{AB,-l}] \) (where superscript \( \Delta \) indicates that counts were taken at that temporal resolution), which strictly holds if

\[
(5) \quad \sum_{\alpha = 1}^{M} \sum_{\tau = 1}^{T-\bar{l}} p(c_{A,t+\bar{l}}^{\Delta} | c_{B,t}^{\Delta}) p(c_{B,t}^{\Delta}) \sum_{\alpha = 1}^{M} \sum_{\tau = 1}^{T-\bar{l}} p(c_{A,t}^{\Delta} | c_{B,t+\bar{l}}^{\Delta}) p(c_{B,t+\bar{l}}^{\Delta})
\]

The \( H_0(\Delta) \) furthermore demands that this factorizes as

\[
(6) \quad \sum_{\alpha = 1}^{M} \sum_{\tau = 1}^{T-\bar{l}} p(c_{A,t+\bar{l}}^{\Delta}) p(c_{B,t}^{\Delta}) \sum_{\alpha = 1}^{M} \sum_{\tau = 1}^{T-\bar{l}} p(c_{A,t}^{\Delta}) p(c_{B,t+\bar{l}}^{\Delta}).
\]

Now, if the two processes \( A \) and \( B \) are reasonably stationary at least on scales up to \( l\Delta \), we have \( p(c_{A,t+\bar{l}}^{\Delta}) \approx p(c_{A,t}^{\Delta}) \) and \( p(c_{B,t+\bar{l}}^{\Delta}) \approx p(c_{B,t}^{\Delta}) \) and the equalities above should (approximately) hold, even if the processes are non-stationary and/or coupled on broader temporal scales. Thus, under the \( H_0 \) of independence on the time scale fixed by precision \( \Delta \), \( E[\#^\Delta_{AB,l}] = E[\#^\Delta_{AB,-l}] \) if (6) holds exactly, and approximately otherwise.

Under the alternative hypothesis of dependence on the specific scale \( \Delta \) considered, on the other hand, if pattern \((AB,l)\) occurs more frequently than expected by chance, e.g. because unit \( A \) excites unit \( B \), there is no a-priori reason why the reverse for pattern \((AB,-l)\) should hold true as well at the specified lag; hence \#_{ABBA,l} would be expected to differ from zero.

To accommodate the strictly synchronous case \((\bar{l} = 0)\), finally, we slightly modify eq. 4 to be

\[
(7) \quad \#_{ABBA,0} = \#_{AB,0} - \#_{AB,-l^*},
\]

with \( l^* \neq 0 \) (we used \( l^* = 2 \); note that \#_{ABBA,0} > 0 if synchrony is the dominant pattern, while the sequential case \( l = 2 \) is tested separately by \#_{ABBA,l}). Again, under the \( H_0 \), \( E[\#_{AB,0}] = E[\#_{AB,-l^*}] \), while under the \( H_1 \) of synchronous spiking \#_{ABBA,0} would be expected to be larger than zero (one may also define the symmetric quantity \#_{ABBA,0} = \#_{AB,0} - (\#_{AB,l^*} + \#_{AB,-l^*})/2, but it makes the computation of the variance, eq. 10 below, more cumbersome). Following the same lines laid out above in deriving eqns. 2 and 3, for these modified, stationarity-corrected statistics we thus obtain for the mean \( \mu_{ABBA,l} \) and variance \( \sigma^2_{ABBA,l} \) under the \( H_0 \), respectively,

\[
(8) \quad \mu_{ABBA,l} \equiv E[\#_{AB,l}] - E[\#_{AB,-l}] = 0
\]

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\[ \sigma_{ABBA,l}^2 \equiv E \left[ (\#_{ABBA,l} - E[\#_{ABBA,l}])^2 \right] = 2\sigma_{AB,l}^2 - 2\text{cov}(\#_{AB,l}, \#_{AB,-l}), \]

where we use \( \hat{\mu}_{ABBA,l} = \frac{\#_{ABBA,l}}{(T - l)} \) as an estimator for \( \mu_{ABBA,l} \) that will remain approximately unbiased even under non-stationarity (see above). If the process is strongly non-stationary, however, evaluating eq. 9 directly across the whole time series may still give an inaccurate estimate of variance. In general, we therefore divide the binned spike train into \( C \) segments of \( k \) time bins each, and combine the local, segment-wise variance estimates into the global estimate \( \hat{\sigma}_{ABBA,l}^2 \) with

\[ \begin{align*}
(10a) \quad \hat{\sigma}_{AB,l}^2 &= \text{var}(\#_{AB,l}) = \text{var} \left( \sum_{c=1}^{C} \#_{AB,l}^c \right) = \sum_{c=1}^{C} \text{var}(\#_{AB,l}^c) + 2 \sum_{c=1}^{C-1} \sum_{\zeta>c} \text{cov}(\#_{AB,l}^c, \#_{AB,l}^\zeta), \\
(10b) \quad \text{cov}(\#_{AB,l}^c, \#_{AB,-l}^c) &= \sum_{c=1}^{C} \text{cov}(\#_{AB,l}^c, \#_{AB,-l}^c) + 2 \sum_{c=1}^{C-1} \sum_{\zeta>c} \text{cov}(\#_{AB,l}^c, \#_{AB,-l}^\zeta), \text{ and} \\
(10c) \quad \text{cov}(\#_{AB,l}^c, \#_{AB,-l}^c) &= \sum_{\alpha=1}^{M} \frac{\#_{AB,l}^c \#_{AB,-l}^c}{k} (k - \#_{AB,l}^c)(k - \#_{AB,-l}^c) + 2 \sum_{\alpha=1}^{M} \sum_{\gamma>\alpha} \frac{\#_{AB,l}^c \#_{AB,-l}^c}{k} (k - \#_{AB,l}^c)(k - \#_{AB,-l}^c). \end{align*} \]

For smaller segment length \( k \) (here we used \( k=100 \)), this approximation will become more accurate (as within each short segment the process will approach stationarity), yet at the same time computationally more demanding. In practice, covariation among segments may often be negligible compared to the within-segment variance contributions (see below; e.g. if autocorrelations decay relatively fast), so to reduce computational burden one may evaluate the quantity \( \hat{\sigma}_{ABBA,l}^2 \approx 2 \sum_{c=1}^{C} \text{var}(\#_{AB,l}^c) - 2 \sum_{c=1}^{C} \text{cov}(\#_{AB,l}^c, \#_{AB,-l}^c) \). This is in fact the estimate we have used throughout this manuscript.

Based on the estimates derived above, we can then define the following approximately \( F \)-distributed quantity which can be used for significance testing:

\[ \begin{align*}
(11) \quad Q_{l} & \equiv \frac{\hat{\mu}_{ABBA,l}^2}{\hat{\sigma}_{ABBA,l}^2} \sim F_{1, 2(T - l)M - 1} \\
\text{with 1 numerator and } 2(T - l)M - 1 \text{ denominator degrees of freedom. Finally, all } \alpha \text{-levels are Bonferroni-corrected for the number } R \text{ of tests performed (see pseudo-code below and sect. ‘Recursive assembly agglomeration algorithm’).} \end{align*} \]
Limitations of parametric testing under non-stationarity
To examine the error made by the various approximations introduced above, we empirically studied different scenarios by simulation. In one set of simulations, discrete, step-like rate-changes were used. Within a total of $T=10^6$ ‘elementary’ time bins (not to be confused with bin width $\Delta$ used for assembly detection), low-rate states were randomly interspersed with $m$ high-rate states of duration $L$ (expressed in terms of numbers of elementary bins). For each elementary time bin, spikes were drawn from a Bernoulli process with probabilities $\pi_{\text{low}}$ and $\pi_{\text{high}}$, respectively. Here, $L$ explicitly defines the time scale on which the two processes are non-stationary and, in some simulations, coupled. Simulations (with $\Delta=100$, $l\in\{0,5,10\}$) were performed with both relatively fast ($m=25$, $L=3000$, on the order of $2l_{\text{max}}\Delta$; Fig. S3, center column) and slow ($m=1$, $L=75000$; Fig. S3, right column) rate variations (both with the same number of high-rate bins), and with one independent scenario (Fig. S3, top row) and one where the rate variations were completely synchronized between the two processes (Fig. S3, bottom row). As the percentile-percentile plots in Fig. S3 reveal, in all these cases departures from the theoretical $F$ distribution were remarkably mild. We emphasize that this was the case although the difference between the low and high rate states was assumed to be rather large in our simulations ($\pi_{A,\text{low}}=0.01$ vs. $\pi_{A,\text{high}}=0.05$, and $\pi_{B,\text{low}}=0.03$ vs. $\pi_{B,\text{high}}=0.15$), a larger violation of stationarity than one may actually expect empirically.

In another set of simulations, time-varying firing rates for the two neurons were drawn from a slowly varying auto-regressive process with Gaussian noise (or, equivalently, a joint multivariate Gaussian). Spike counts for each bin were then drawn from a Poisson distribution with the rate $\lambda$ determined by the auto-regressive process passed through a non-negative transform (‘link-function’, see eq. 13 below). We simulated scenarios with both somewhat faster ($\text{cov}(\lambda_{A,t},\lambda_{A,t+2l_{\text{max}}}) \approx 0.8$; Fig. S4, center column) and very slowly decaying auto-correlations ($\text{cov}(\lambda_{A,t},\lambda_{A,t+2l_{\text{max}}}) \approx 0.99$; Fig. S4, right column), and without ($\text{cov}(\lambda_{A,t},\lambda_{B,t}) = 0$; Fig. S4, top row) or with ($\text{cov}(\lambda_{A,t},\lambda_{B,t}) \approx 0.7$; Fig. S4, bottom row) correlations among the two processes present. As Fig. S4 reveals, the results for these simulation experiments were very similar to those shown in Fig. S3, with the empirical $Q_I$ distribution deviating at most only slightly from the theoretical $F$ distribution. We thus conclude that for empirically reasonable scenarios of non-stationarity and coupling on longer time scales, the parametric statistical procedure introduced above should return quite accurate results.

It is important to note that while coherent rate changes constitute a coupled non-stationarity from the viewpoint of smaller timescales $\Delta$, for which they will be removed by our difference statistic, they actually represent an assembly on their own characteristic scale (type V in Fig. 1). They are indeed correctly identified as such in both simulated scenarios when the bin width is chosen to be about the same as the time-scale of the ‘non-stationarity’, i.e. $\Delta \approx L$ ($m=75$, $L=1000$; Fig. S3 and S4, left columns, blue curves in bottom graphs). Hence, non-stationarity, in the present definition, refers to somewhat slower (co-)variations that may mislead detection of coincident events on comparatively finer time scales. Both, the relevant time scale for assembly detection and the associated time scale of non-stationarity, are strictly defined by the experimenter by fixing $\Delta$. Whether detected temporal structure is interpreted as an assembly or as non-stationarity therefore depends on the timescale chosen, and is ultimately up to the experimenter and the research question posed. From this perspective, our method basically ensures that coincidence structure is not falsely attributed to a certain temporal precision $\Delta$ while
it was really produced by slower (co-)variations, an issue that has frequently plagued the discussion about precise temporal coding in the nervous system in the past (eg., (45) vs. (17)).

**Oscillations.** Finally, as an example of a particularly common form of non-stationarity in neural data (e.g. (42)), we considered oscillations (10). (Note that oscillations constitute a type of non-stationarity from the present neurophysiological perspective, although they may be considered stationary in the classical statistical definition with access to an infinite ensemble of time series starting at random phases (see (46)). We tested two scenarios here: One in which two neurons were spiking independently at the time scale considered, but were driven by a common oscillatory drive at the same frequency and phase, and one where on top the units exhibited supra-chance coincident patterns. Specifically, for both units $A$ and $B$ the firing probability was taken to follow a Poisson distribution with rate parameter $\lambda_{(A,B)}(t) = 5(\sin(2\pi \theta t) + a_{(A,B)})$, $a_A = 1, a_B = 0.5, \theta = 4Hz$, yielding mean firing rates of $5Hz$ and $2.5Hz$, respectively. For the independent case, no structure is detected for smaller bin widths $\Delta$ up to $\approx 30$ ms (Fig. S5A), while for larger bin widths ($> 50$ ms) the algorithm picks up the coordinated rate changes. For the second scenario (finer time-scale dependence on top of oscillation), patterns are inserted on top with a spike in unit $A$ followed by one in unit $B$ 20 ms later, phase-locked to the underlying rhythm. Within a $T=1500$ sec long simulation period, a total of 90 of such patterns were placed randomly with a 20 ms delay to the oscillation peak. Figure S5B shows that these patterns are indeed correctly detected by our procedure at the smaller bin sizes ([5, 10] ms) tested. Thus, once again, this example illustrates that the detection of finer time scale spike time relations is not confounded by covariations at slower scales, while the slower covariations are flagged up as coherent structure at their own respective time scale (50-120 ms).

**Recursive assembly agglomeration algorithm**

Our assembly agglomeration scheme starts from all significant pair-wise interactions, and then adds new elements only on the basis of the structures already formed, similar in spirit to the apriori-algorithm in machine learning (25, 26, 47). This heuristic procedure drastically reduces the number of configurations to be tested, but may lose significant unit configurations with non-significant subgroups (24). For each pair of units $A$ and $B$, the spike count $\#_{AB,l}$ is obtained for each triplet $(A,B,l)$, with $l \in \{-l_{max} \ldots l_{max}\}$, and the maximum count is tested for significance (Fig. S6, step 2). Since the marginal elementary processes $\#_A^g$ and $\#_B^g$ do not change for different lags $l$ (except for the small influence from the $l$ bins cut off), this selection procedure is formally equivalent to performing an explicit significance test for each lag $l$ and retaining the one associated with the lowest $p$ value. In the next step, all significant configurations $(AB, \bar{l})$ are treated like single units, with the joint ‘spike (activation) times’ defined (arbitrarily) as those of unit $A$ whenever it matches up with a spike in unit $B$ separated by $\bar{l}$ time steps (bins). Each significant pair $(AB)$ is then paired in turn with all single units $C$ which had entertained a significant relationship with either $A$ or $B$ in the previous step (Fig. S6, step 3). Proceeding with composite pairs $((AB, \bar{l}_{AB})C, \bar{l}_{(AB)C})$ exactly as described above, higher-order structures are thus recursively built up. Note that this procedure effectively tests for higher order structure, rather than just aggregating pairwise information: After screening for pairwise relations in the first step, for instance, in the second iteration the algorithm tests for the factorization $P(A,B,C)=P(A,B)P(C)$ whenever a unit $C$ is considered for inclusion into the already formed set $(AB, \bar{l})$, rather than for, e.g., $P(A,B,C)=P(A)P(B)P(C)$, or “$P(A,B)=P(A)P(B)$ ∨
\[ P(A,C) = P(A)P(C) \lor P(B,C) = P(B)P(C) \]. Likewise, higher order joint distributions are considered in all subsequent iterations.

Significance levels \( \alpha \) at each step of the agglomeration scheme are strictly Bonferroni-corrected as \( \tilde{\alpha}_i = \alpha / R_i \) (using \( \alpha = 0.05 \) here), with \( R_i \) the total number of tests performed. Specifically, for the first step, \( R_1 = N(N - 1)(2l_{max} + 1) \), where \( N \) is the total number of single units (correcting for the total number of different pairs). For each subsequent step \( i \), \( R_{i,a} = N_{a,i}N_{u,a}(2l_{max} + 1) \), where \( N_{a,i} \) is the number of assemblies tested in iteration \( i \), and \( N_{u,a} \) the number of units tested in combination with that assembly \( a \) (hence allowing an higher \( \alpha \)-level for assemblies tested in conjunction with less different units). At any step, unit-sets with the same elementary units but different lag distributions may result. From all these, we select only the one associated with the lowest \( p \)-value, and discard all others. This whole procedure will stop when no units engage in significant relationships anymore with the already agglomerated sets (Fig. S6, step 4). All true subsets of larger sets are finally discarded (but may be retained if hierarchical nesting is of interest, see below). A pseudo-code for the agglomeration scheme is included below. To test for structure at different temporal resolutions (scales), the whole scheme is re-run for a range of user-provided bin widths \( \Delta = \{\Delta_{min} \ldots \Delta_{max}\} \). For each assembly pattern the width \( \Delta^* \) associated with the lowest \( p \)-value is defined as the characteristic time scale (or temporal precision) for that assembly.

As a final note, for very large \( \Delta \), the binned spike counts may potentially fluctuate around a high mean level and never fall below some minimum count \( c_{floor} \) considerably larger than zero for the whole time series. In our count statistics, also spikes up to that baseline rate \( c_{floor} \) would contribute to the coincidence counts \( #_{AB,I} \), although they are completely non-informative with respect to the coupled dynamics among units, thus potentially biasing the results for large \( \Delta \). In this case, since we are only interested in actual firing rate covariations, we suggest to subtract off the minima \( \min \left( \{c^A\}_t \right) \) and \( \min \left( \{c^B\}_t \right) \) from the two considered series \( A \) and \( B \), respectively, thus removing the non-informative floor count before statistical testing. This procedure would not affect the evaluation of spike coincidences at reasonably small \( \Delta s \) for which \( \min(\{c^A\}_t) = 0 \), obviously.

Further assembly pruning. Further pruning may be applied to the set of assemblies returned by the algorithm if desired. This may sometimes help interpretability and visualization, but of course depends on the exact analysis goals. If, for instance, the interest is in whether the same assemblies are replayed at a different time scale (e.g. (33)), then one may want to keep more than just the one assembly associated with the lowest \( p \)-value across time scales \( \Delta \). Here, solely for the purpose of visualization, in Fig. 2A the full set of assemblies returned by the algorithm was pruned by selecting among all assemblies (across different \( \Delta \)) with cosine distance <0.3 only the one with lowest \( p \)-value. In Fig. 1B, again for clarity and visualization, pruning was performed by discarding across scales \( \Delta \) any assembly which is a subset of another, larger assembly (by default this is always done within each time scale \( \Delta \)). No pruning was used for any of the other figures presented in here.

Assembly activation. An instance of assembly activation in the multivariate spike time series was registered whenever spikes in the elementary assembly units occurred in the order prescribed by the associated pattern of time lags, with the activation time point defined as that of
the assembly unit spiking earliest. The total assembly activation score (as given in Fig. 2B-C) is then defined as the number of such activation instances within a given time bin of size $\Delta$. This can lead to activation scores much larger than one, especially for assemblies defined through rate changes on coarser time scales, since each set of single assembly unit spikes occurring in the right order is counted. (Alternatively, one may define assembly activation through the correlation of the average assembly spike count pattern with the observed spike count patterns along the series of binned spike counts at the respective assembly resolution $\Delta$. This would result, however, in a temporally much less well resolved activation score which otherwise would essentially return the same information.)

A Matlab (MathWorks) implementation of the whole procedure is provided at www.bccn-heidelberg-mannheim.de. To give an idea of the performance speed, on a 12-core, 2.5 GHz, workstation, for a set of 50 simulated units (see below), a time series of length $T=1400$ sec, and with $\Delta = \{0.015, 0.05, 0.1, 0.15, 1\} \text{ sec}$ and $l = \{-10, ..., 10\}$, this whole procedure took $< 50$ min for five embedded assemblies with five units each (scenario from Fig. 1). Significant further improvements in performance speed may be obtained through an implementation in a more basic language like C++. 




Pseudo-code for agglomerative assembly formation

\% \( N \): total number of units
\% \( u_i, i=1\ldots N \): single units
\% \( U_m \): set of units and corresponding lags (assemblies)
\% \( r \): set counter

\[ r = N, \quad L_{old} = 0 \]

\textbf{for} \( i = 1: N \), \( U_i \leftarrow \{(u_i, 0)\} \) \% Initialize lists with single units \( u_i \)
\textbf{for} \( all \ i \leq N, \ j \leq N : Z_{ij} = FALSE \) \% Initialize all single unit pair comparisons to be ‘false’ (= ‘accept \( H_0 \)’)
\( r = N, \quad L_{old} = 0 \)

\textbf{REPEAT} \% agglomeration procedure

\( L_{new} = r \)
\textbf{for} \( m = L_{old} + 1: L_{new} \) \% move through all lists formed in previous step
\textbf{for} all \( u_s \notin U_m \) \% in first step \((m \leq N)\) probe \( U_m \) with all other single units not yet tested, or \((m > N)\) probe \( U_m \)
\textbf{with} all other single units that occur in at least one other significant pair with a unit from \( U_m \)

\[ \bar{l} \equiv \text{argmax}_l (\#(U_m, u_s, l)) \] \% test for significance at lag \( \bar{l} \) with maximum pair-wise count:
\[ \text{if } Pr(Q_{\bar{l}} \geq F_{1,(\bar{l}-1)M-1}[U_m, u_s, \bar{l}] | H_0) \leq \alpha / R \quad \text{with} \quad R = (L_{new} - L_{old}) \cdot |\{u_s\}| \cdot (2l_{\text{max}} + 1) : \]
\[ r \leftarrow r + 1, \quad U_r \leftarrow \{U_m, (u_s, \bar{l})\} = \{u_{r,1}, 0\), \( u_{r,2}, \bar{l}_2\), \ldots, \( u_{r,|U_m|+1}, \bar{l}_{|U_m|+1}\}\]
\textbf{if} \( |U_m| = 1, \quad Z_{sm} = Z_{ms} = \text{TRUE} \)

\% form new list where each \( \bar{l}_j \) is defined relative to the activation time point \( \langle 0 \rangle \) of the first unit \( u_{r,1} \) in the ordered list; set pair-wise flag to ‘true’ if single-unit comparison

\( L_{old} \leftarrow L_{new} \)

\textbf{UNTIL} \( Pr(Q_{\bar{l}} \geq F[U_m, u_s, \bar{l}] | H_0) > \alpha / R \) \% Pruning steps:

- Discard all \( U_m \) for which \( \exists \ n \neq m : U_m \subset U_n \)
- For all \( U_n, U_m \) for which \( \forall \ u_s \in U_n, u_s \in U_m : \)

\( \text{Remove } U_m \text{ if } Pr(Q_{\bar{l}} \geq F[U_m] | H_0) > Pr(Q_{\bar{l}} \geq F[U_n] | H_0), \) and \( U_n \) otherwise

where \( |\cdot| \) denotes the cardinality of a set, and all set-operations (\( \in, \subset \), etc.) are defined in terms only of the unit-elements composing a set (i.e., ignoring the associated lags with which their occur).
Alternative procedure:
In the REPEAT-loop, instead of probing all pair-wise relations among the current lists (assemblies) and all single units from significant pairs, one could also check for significant relationships among pairs of lists \( U_n, U_m \). As in classical hierarchical, agglomerative cluster-analytic procedures (48), at each step one may only fuse the pair \( (U_n, U_m) \) associated with the lowest \( p \)-value, add this to the current set of lists while removing \( U_n, U_m \), and repeat until \( Pr(Q_i \geq F[U_n, U_m, l] | H_0) > \alpha/R \) for all \( n, m \). This would yield a dendrogram-like representation and thus reveal strictly hierarchical nesting among assemblies. It comes at the cost, however, that a) many higher-order assemblies may go undetected, and b) partial overlap among assemblies, which one may expect if units in assemblies act like ‘letters in words’, would be prohibited by definition of the agglomerative procedure. Also note that hierarchical nesting, to the degree present, could also be revealed with the definition of the agglomeration scheme in the pseudo-code above if subsets of further agglomerated sets are not pruned away at the end.

Construction of synthetical ‘ground-truth’ data
To test the full assembly detection schemes developed above, artificial spike trains from 50 cells were created according to inhomogeneous Poisson processes by drawing inter-spike-intervals from an exponential distribution with rate parameter \( \lambda_{it} \) for each unit \( i \). Instantaneous firing rates \( \lambda_{it} \) were governed by an underlying stable first-order autoregressive process

\[
(12) \quad s_{t+1} = Ds_t + \epsilon_t, \quad \epsilon_t \sim N(0, \sigma_s^2 I),
\]

with coefficient matrix \( D \), and \( E[\epsilon_t \epsilon_{t'}^T] = 0 \) for all \( t \neq t' \) (white noise process). (Note that although \( D \) is set such that the autoregressive process itself is stationary, i.e. \( \max[|\text{eig}(D)|] < 1 \), it implies fluctuations in the firing rate which makes the Poisson spiking processes themselves non-stationary in our definition above). Since \( s_t \), in principle, is unbounded (in particular, can assume negative values), it was pushed through a sigmoid non-linearity

\[
(13) \quad \lambda_t = \left(1 + \text{erf} \left(\frac{s_t - \bar{s}}{\sigma_s}\right)\right) \tilde{\lambda},
\]

with \( \text{erf} \) the error function, and constant mean rate vector \( \tilde{\lambda} \). Finally, to ensure a refractory period, a constant delay \( \tau_{ref} \) is added to each inter-spike-interval. Where not indicated otherwise, parameters used for the simulations were \( \bar{\lambda} = 5Hz \) for all units, \( \tau_{ref} = 15ms \), \( D = 0.9 I \), where \( I \) denotes the identity matrix, \( v = 0.2, \sigma_s = 0.01 \).

Assemblies of all five types illustrated in Fig. 1A were embedded within the same set of 50 spike trains as disjunctive groups of 5 neurons each. Note that since our algorithm is aimed at detecting significant spike time patterns (rather than, for instance, underlying connectivity), explicit control of such patterns and spike train statistics with vivo-like characteristics is most important for a ground truth check, while adding more biophysical realism to the underlying simulation setup would not help in this case. For assembly type I, each occurrence is marked by five precisely synchronous spikes across the set of assembly neurons (e.g. (2, 49)). For assembly type II, spikes follow a precise sequential pattern across the set of assembly neurons on each
instance of activation (33, 50). Time lags between spikes were drawn from a uniform distribution [0 0.1] sec, and then fixed for each occurrence. For assembly type III, spikes across the set of assembly neurons followed a precise temporal pattern, but did not exhibit a strict temporal order, i.e. each neuron could contribute one to several spikes to the assembly pattern without strictly leading or following others (e.g. (11)). For the simulations, these patterns were generated by distributing a few spikes at a Poisson rate of 10 Hz across a period of 0.2 sec for each assembly neuron, but then keeping these patterns fixed on each occasion of assembly activation.

For the less precise assembly type IV, short windows of extra spikes for each assembly neuron were organized in a specific temporal pattern, with the exact occurrence of the extra spikes within the defined time windows determined randomly on each repetition (cf. Fig. 1A; e.g. (51–55)). Specifically, time windows of 0.3 sec with extra spikes at a Poisson rate of 10 Hz were (without loss of generality) arranged in a sequential order, with the time lag between these windows drawn from a uniform distribution, [0 0.4] sec. While this sequential ordering of time windows was fixed, within each window spikes were drawn at random on each assembly repetition. Assembly type V, finally, was simply defined by an increase of the Poisson firing rate from 5 Hz to 10 Hz for periods of 1 sec simultaneously within the set of assembly neurons, as, e.g., during the delay period of a working memory task (e.g. (56)).

For all assembly patterns, all spikes from the background process were erased within a ±15 ms window around each assembly spike to preserve the refractory period. Assembly activation times were distributed (uniformly) randomly across the whole spike time series.

**Performance evaluation: Low sample size limit and corrupted spike trains**

To evaluate the performance, statistical power, and potential biases of our assembly detection algorithm more systematically, we focused on two experimentally relevant scenarios: Low assembly occurrence rates and spike sorting errors. The Rand index (57) was used to quantify the match between predefined assemblies and those retrieved by the algorithm. The Rand index measures the agreement between two partitions, in our case of units into assemblies, and is defined as

\[
R(r, s) = \frac{r+s}{n(n-1)/2},
\]

where \(r\) is the number of unit pairs correctly assigned to the same assembly in both partitions, \(s\) the number of unit pairs correctly assigned to two different assemblies, and \(n\) the total number of detected assembly units. \(R(r,s)\) varies between 0 and 1, assuming 1 only if the assembly structure extracted by the algorithm exactly maps onto the one predefined. To obtain a clean picture on the algorithm’s statistical performance for each assembly type, unconfounded by the presence of other assembly types, in these analyses each assembly type from Fig. 1 was investigated separately (i.e., unlike the analyses described in the main text where the different assembly types were mixed in the same simulations).

Fig. S7A plots \(R(r,s)\) for all five assembly types from Fig. 1 as a function of total
assembly occurrences (in spike time series of length T=1400 sec). Obviously, assembly detection gradually degrades as these structures start to drown in the noise but, as Fig. S7A reveals, this only happens when the occurrence rates drop below ~0.18 repetitions/sec. Likewise, as shown in Fig. S7B, more than ~30% of all spike times need to be corrupted by spike sorting errors (assignment of assembly spikes to wrong units) before performance notably decays. In more detail, Figs. S7A and S7B suggest that sequential assembly patterns (types II and IV) are more vulnerable to lower sample sizes than those assemblies defined through precisely or broadly, respectively, synchronous firing (types I and V). The reason for this likely is that the binning procedure itself introduces some noise (as spikes may fall randomly into one or the other of two neighboring bins), which affects sequential assemblies more than simultaneous assemblies (for which, in the case of our simulations, it is guaranteed that aligned groups of spikes end up in the same bin). Considering just assembly types I and V, also note that assemblies defined by broader simultaneous rate increases are detected much more easily than those characterized by precise spiking. This is not surprising given that in absolute terms each incidence of an assembly of type V contributes much more spikes to the whole process than an assembly of type I, thus in turn causing assemblies of type V being detected across a much larger range of bin widths than assemblies of type I (cf. Fig. 1C).

Importantly, across the whole range of relative occurrence rates, spike assignment errors, and assembly structures tested, the fraction of units falsely ascribed to any one assembly stayed uniformly low at about 0.5% (Figs. S7C-D), indicating that our procedure is very conservative and rarely returns false positives in the simulated scenarios.

**Relation to previous methodological approaches**

Numerous other statistical procedures for detecting assemblies or sequential patterns have been previously proposed (20–22, 24, 37, 38, 43, 47, 58–69), but most of these adhere to one or the other theoretical conceptualization of a cell assembly (cf. Fig. 1A), or become (computationally) impractical for larger cell numbers or multiple lags, e.g. because they rely on time-consuming bootstrap analyses (e.g. (21, 23, 24, 43, 60, 64)). Along similar lines as our procedure, unitary event analysis scans simultaneously recorded spike trains for precise spike co-occurrences (Fig. 1A, I) that exceed the joint spike probability predicted from independent Poisson processes with the same local rate (20, 22). However, this procedure has not been extended yet to multiple lags or larger bins (with higher counts), and deals with non-stationarity through sliding windows or bootstrap analyses. In another approach to synchronous spike-cluster detection based on the cumulants of the population spike density of all simultaneously recorded neurons, Staude et al. (66, 67) developed a method and stringent statistical test for checking the presence of higher-order (lag-0) correlations among neurons, without however providing the identity of the recorded assembly units. A recent ansatz by Shimazaki et al. (37) builds on a state-space model for Poisson point processes developed by Smith and Brown (70) to extract higher-order (lag-0) precise correlation patterns from multiple simultaneously recorded spike trains (see also (21, 24, 43, 60, 63) for other recent approaches to the detection of groups of synchronous single spikes).

Smith et al. (71, 72) address the problem of testing significance of recurring spike time sequences or activity chains like those observed in hippocampal place cells (Fig. 1A, II, IV; see
also (23, 58, 64, 65, 73)). Their approach makes use only of the order information in the neural activations, neglecting exact relative timing of spikes or even the number of spikes emitted by each neuron, in order to allow for derivation of exact probabilities based on the multinomial distribution and combinatorial considerations. In a similar vein, Sastry & Unnikrishnan (47) employ data mining techniques like 'market-basket analysis' and the a-priori-algorithm to combat the combinatorial explosion problem in sequence detection, scanning first for significant sequential pairs, then based on this subset of pairs for triples, quadruples, and so on, iteratively narrowing down the search space as potential sequences become longer. Several procedures for revealing common modulations in firing rate have been proposed as well (38, 61, 62, 74).

Although some of these techniques are related in one or the other aspect to our algorithm, none of them, to our knowledge, combines all of the features presented here. Our procedure combines fast parametric (bootstrap-free) testing with a fast agglomeration algorithm. This enables to consider, in a heuristic sense, all potential cell combinations with a large range of different temporal lags. We furthermore introduce a novel way for dealing with non-stationarity which is fast and allows to utilize the complete data set for assembly estimation, rather than slicing it into sufficiently short windows or using computationally demanding bootstrapping. Finally, we showed how a parametric statistic for evaluating the deviation of joint spike distributions from independence can be obtained also for series of counts larger than one by splitting the process into several binary streams. This enables to treat processes developing on slower scales (larger bin widths) within the same statistical framework without loss of information, another novel feature introduced here. The statistical tools developed here may thus allow to readdress important questions about the nature of neural coding in different brain areas, without requiring the researcher to sign up for any particular assembly concept or theoretical framework a priori.

Comparison with linear decomposition and correlation-based methods. The most popular choices for studying pairwise interactions and (synchronous) multiple-unit structures are, respectively, (Pearson-type) cross-correlations (e.g. (75, 76)) and principal component analysis (PCA; e.g. (62, 74)), owing to their methodological simplicity. In comparison to the methods developed here, there are a number of important issues to note:

First, both standard cross-correlation and PCA are purely linear techniques. For strictly binary series, the Pearson cross-correlation is equivalent to computing the deviation of the joint spiking probability from the product of its marginals in the numerator, \(p(A, B) - p(A)p(B)\) (e.g., (42)). For larger counts or more than two units, however, cross-correlations and PCA, unlike our method, do not capture nonlinear interactions or higher-order joint probabilities. Indeed, PCA, strictly, does not even extract correlations among units but rather variance-maximizing directions from the multiple single-unit activity space (e.g. (77, 78)), which is a different objective and may lead to results different from methods aimed directly at correlations (e.g., (79)).

Second, very importantly, for either cross-correlation or PCA based methods, statistical significance of the unraveled relationships or structures needs to be properly assessed. In particular, false positives need to be avoided. This is in itself a highly nontrivial topic: Several authors have pointed out in the past that interpretation of cross-correlations may be severely plagued by the presence of (inevitable) non-stationarity (like slow rate covariances or stimulus...
responses) which may compromise ‘traditional’ testing by means of, e.g., mere bin or inter-spike-interval shuffling (42, 76, 80). Indeed, as illustrated in Fig. S8, superfluous peaks in the cross-correlation may occur, and even flagged up as significant by conventional bootstrapping, although in reality spike times for the two units were drawn independently. Hence, more sophisticated bootstrapping and sliding window analyses have to be afforded that take into account auto-correlations in the time series and non-stationarity (81). But these imply that statistical quantities need to be recalculated hundreds to thousands of times, a heavy computational burden that may severely restrict assembly assessment to a limited number of units or few possible lag constellations and temporal scales only (given that this is an NP-hard combinatorial optimization problem (66, 82)). In fact, so far researchers have focused mostly on synchronous activity (62, 74, 83, 84). Moreover, these methods come with some ad-hoc choices, e.g., the to be used window or block length (in case of block permutations; e.g. (85)), for which there is likely no globally optimal choice across the whole time series. For PCA based methods, sometimes the theoretical Marčenko-Pastur distribution has been used for assessing significance of eigenvalues (62, 74), but as illustrated in Fig. S9, this may be severely misleading especially in the case of non-stationary data.

Third, cross-correlation analysis still needs to be augmented with some agglomeration scheme that builds up higher-order structures from the pairwise interactions, again a non-trivial endeavor in its own right, especially if various time lag constellations are to be evaluated. PCA, on the other hand, in its basic and most applied formulation only recovers strictly synchronous activity. PCA also comes with a number of other inherent short-comings, a) because it is not geared toward identifying correlations but, as noted above, variance-maximizing directions (77, 78, 86), b) because it may produce assemblies of very unequal size since it places most variation on the first factor, after which variance contributions often fall off exponentially, and c) because of quite high susceptibility to noise if assembly structures are not very clear-cut (Fig. S10).

Our algorithm aims to address all these statistical and computational issues in one go, provides proper and fast statistical assessment of unit interactions, unconfounded by non-stationarity within the limits shown (Fig. S2, S3, S4), does this for all possible lag constellations (not just synchrony), and comes with an efficient, statistically anchored algorithm for detecting higher-order structures.

**Supplementary Experimental Procedures**
The in-vivo recordings from the rat (Long-Evans) anterior cingulate cortex (ACC) were taken from two studies by Hyman et al. (27, 28). In both studies multiple single unit recordings were performed with a set of 16 simultaneously implanted tetrodes, with an average of 35 and 30 isolated (and artifact-free) units per recording session, respectively (with \( n=11 \) and \( n=9 \) sessions in total). In the environmental exploration task studied in Hyman et al. (27), rats were offloaded in a novel environment which they were free to explore, with one to several transfers between two different environments. Each environment was analyzed separately by concatenating the
spike trains associated with the repeated exposures to the same environment. The delayed alternation task studied in Hyman et al. (28), a classical working memory paradigm, took place in a Skinner-box with two levers which the animals had to press in alternating fashion. A delay of 10 sec was introduced between each lever press and a nose poke the animals had to perform before continuing with the next lever press.

Hippocampal and entorhinal cortex (EC) recordings on the exploration task, performed simultaneously within these two areas, were borrowed from (29). Recordings were collected from two Long-Evans rats implanted with multi-shank (32 or 64 sites) silicon probes lowered into the CA1 hippocampal pyramidal layer and into layers 3-5 of entorhinal cortex. In this task (31), rats were free to explore a 180 cm x 180 cm arena with water or Froot Loop items randomly dispersed throughout. Here we analyzed $n=13$ sessions with on average 29 (CA1) and 20 (EC) artefact-free units per session, respectively. CA1 and EC recordings on the delayed alternation task come from (30), who used the same animals employed on the exploration task (31), from which we took $n=16$ (EC) and $n=13$ (CA1) sessions, respectively, with on average 25 (CA1) and 18 (EC) isolated and reasonably artefact-free units. Animals had to alternate between the two arms of a figure-eight shaped maze to obtain reward at water spouts located at the rear of the arms. A delay of 10 sec or 20 sec, respectively, spent in a running wheel, was inserted between trials for the two animals tested. For all analyses, all units with average firing rates below 0.2 Hz were excluded. Please see original publications for further details on electrode placement, unit separation, and experimental design.
**Supplemental Figures**

![Supplemental Figures](image)

**Fig. S1. Method details.** (A) For deriving a statistical test that works with any temporal bin width the spike count series were separated into an overlay of several (dependent) binary subprocesses. See Supplemental Text for further explanation. (B) Dealing with non-stationarity in the spike trains.

In the case of non-stationarity in the form of a common rate increase in two units A and B (highlighted in gray), some spike co-occurrences caused by the rate increase might be incorrectly attributed to coupled activity (mutual dependence) at the finer timescale (bin width) at which coupling is investigated (at a lag of one in the illustrated example), even if there is not really any such coupling as assumed in this example. This corruption by non-stationarity may be removed by considering the difference count $\#_{AB} - \#_{BA}$, in which spurious excess coincidences in one direction ($\#_{AB}$: red arrows) would cancel out with those in the reverse direction ($\#_{BA}$: blue arrows). It is important to note that if, on the other hand, the rate increase is on the timescale of interest, as it is the case for the ‘rate assemblies’ of type IV or V in Fig. 1), subtracting off the reverse-lag count would not prevent assembly detection on that time scale.
Fig. S2. Comparison of non-corrected ($S_l$) and stationarity-corrected ($Q_l$) pairwise test statistics. Percentile-percentile plots showing agreement between the theoretical distributions for different test statistics considered in the text ($S_l$; $Q_l$ with $C=1$ and with $C=100$ segments) and the distributions empirically obtained, for the truly stationary case (top row), independent non-stationarity (center row), and non-stationarity coupled among the two units A and B (bottom row). Overlaid are distributions derived from several simulation runs with spike time series analyzed for the three different lags $l = 0$ (blue curves), 5 (yellow) and 10 (red). $\Delta=100$ in all cases. Simulations are with non-stationarity implemented as step-type rate-changes (see Supplementary Methods) with $m=1$ and $L=75000$. Identity line (bisectrix) is marked in gray. Results for test statistic used for all data analyses in this work highlighted by light-gray box.
Fig. S3. Statistical testing under non-stationarity on different time scales: step-like rate change. Percentile-percentile plots showing agreement between the theoretical and empirical $Q_\ell$ distributions (computed based on segments of length $k=100$ bins, see Supplemental Experimental Procedures). Non-stationarity is implemented here via step-type rate changes with parameters as given in Supplemental Text. Overlaid are distributions derived from several simulation experiments with spike time series analyzed for the three different lags $\ell = 0$ (blue), 5 (red), and 10 (yellow), and $\Delta=3$. Identity line (bisectrix) is marked in gray.
Fig. S4. Statistical testing under non-stationarity on different time scales: rate covariation. Agreement in theoretical vs. empirical $Q_t$ ($C=100$) distributions (P-P-plots as in Fig. S3) under non-stationary conditions implemented through autoregressive processes (eqn. 12-13). Denoting by $C=[d, c, d]$ the coupling matrix of the auto-regressive process, parameters were: $c=0$ (top row) and $d=0.4, 0.997, 0.99999$ (from left to right); $d=0.4, c=0.5$ (bottom-left), $d=0.995, c=0.003$ (bottom-center), $d=0.9996, c=0.0004$ (bottom-right). Overlaid are distributions derived from several simulation experiments with spike time series analyzed for the three different lags $l = 0$ (blue), 5 (red), and 10 (yellow), and $\Delta=3$. Identity line (bisectrix) is marked in gray.
Fig. S5. Detecting coupling among oscillating units. Two Poisson units with different mean rates were subjected to a common oscillatory drive. (A) Illustration of the two units’ mean spike rates together with various spike trains drawn from this same process. (B) Detected fraction (from 0 to 1) of significant couplings among the two units as a function of bin width for the case where the units were just driven by the same oscillation but otherwise independent (gray curve) vs. the case where the units exhibited finer-time scale spike interactions on top (blue curve). For the independent case, coupling is only detected at the time scale of the common oscillation, but not at finer scales. Error bars = SEM.
**Fig. S6. Pipeline of assembly agglomeration algorithm.**

(1) **Binning:** Spike trains are binned at some time scale \( \Delta \) of interest.

(2) **Detection of pairwise interactions at chosen scale \( \Delta \):**
For all unit pairs, test \( \#_{AB,\bar{t}} - \#_{BA,\bar{t}} \) for significance, with \( \bar{t} \equiv \text{argmax}_t(\#_{AB,t}) \).

Significant unit pairs form elementary assemblies fed into the agglomerative recursion loop.

(3) **Assembly agglomeration:** Test pairwise combinations of previously formed assemblies (with time stamps centered on first activated assembly unit) and single units for significance, and add latter to assembly set if significant. For this step, only units are considered which already are significantly related to at least one assembly member.

(4) **Recursive loop and pruning:** All assemblies extended in the previous iteration are fed back into step 3. After each iteration, from all assemblies consisting of the same set of units but with different patterns of time lags only the one with the lowest \( p \)-value is retained. The loop is terminated if no units have been added to existing assemblies in the previous iteration. In a final pruning step all assemblies which are true subsets of larger assemblies are removed.
Fig. S7. Performance evaluation of assembly detection algorithm. (A) Rand index \( R(r, s) \), eq. 14, as function of the total number of occurrences of the assembly pattern in spike time series of length \( T=1400 \) sec, for all five types of assemblies from Fig. 1 (as indicated in the inset legend). Percentages at the half-width points of the \( R(r, s) \)-curves indicate the proportion of spikes in the time series contributed by the assembly structures at these points. (B) Rand index for all assembly types as a function of the fraction of incorrectly assigned spikes (‘sorting errors’). (C) Fraction of units incorrectly assigned to an assembly across a range of assembly occurrence rates. (D) Fraction of units incorrectly assigned to an assembly as a function of the fraction of misattributed spikes. Error bars = SEM.
Fig. S8. **Spurious peaks in the cross-correlation function due to non-stationarity.** Two units with Poisson spike trains \((T=1900\text{ sec})\) and step-like rate variations of length \(L=0.5\text{ sec}\) (as, e.g., induced by a stimulus) were simulated. The onsets of the rate steps were drawn *independently* for the two units from normal distributions \(N(t_i,\sigma^2)\) centered at randomly selected time points \(t_i\) (with \(\sigma = 2\text{ sec}\)). The Pearson cross-correlation was computed (binning \(\Delta=0.15\text{ sec}\)) and tested for significance using inter-spike-interval shuffling (3000 repetitions). Dashed line indicates 2 standard deviations from mean. For this same simulation setup, our method correctly indicated the absence of true spike time dependencies when applied with the same bin width \(\Delta\) as used for the cross-correlogram.
Fig. S9. Assembly detection with PCA under non-stationarity. For comparison with PCA-based assembly detection methods, simulations were performed with 50 non-stationary Poisson spike trains with four embedded, disjoint assemblies. Assemblies were defined as synchronous Poisson spike events (i.e., “type I”, cf. Fig. 1A; 250 activations in total) occurring at random times within a set of five units. Non-stationary events were implemented as step-like changes shared among 4 groups of 5 units each, randomly chosen from the 50 units simulated, at random timings as described in sect. “Limitations of parametric testing under non-stationarity” (parameters used here were $\Delta=0.02$ sec, $m=250$, $L=1$ sec, $T=1950$ sec, baseline rate=5 Hz, up-state rate=10 Hz). For assembly detection by PCA, based on the cross-correlation matrix indicated in C, we followed the procedure described in Lopes-dos-Santos et al. (62) using code made publically available by the authors.. (A) Examples of spike trains with assembly occurrences marked in red. (B) True unit-assembly assignment matrix. (C) Cross-correlation matrix with diagonal set to zero for better visualization. (D) Eigenvalue spectrum. Red line marks the upper limit of Marčenko-Pastur distribution. (E) ‘Loadings’ of units on the two only significant principal components, indicating assignment of units to the two assemblies detected this way. (F) Fraction of correctly detected assembly units (left), fraction of units falsely assigned to an assembly (center), and fraction of correctly detected assemblies (right) for PCA (yellow bars) and our method (blue bars). Error bars = SEM from 50 different repetitions of the whole procedure.
Figure S10. Stability of PCA solutions to degradation in assembly patterns. Even under fully stationary conditions, PCA may completely fail to detect assembly patterns if degraded by spike assignment noise. Simulation setup was as in Fig. S9, with the exception that no non-stationary step changes were included, i.e. spike trains were completely stationary. PCA methods were implemented as in Fig. S9 (see ref. (59)). Noise in the form of spike misattributions or spike failures was introduced by randomly removing a fraction of assembly spikes from each spike train. (A) Fraction of correctly detected assembly units as a function of the proportion of spike assignment errors, (B) fraction of units incorrectly assigned to an assembly (false discovery rate), and (C) fraction of correctly detected assemblies (out of the total number of embedded assemblies), for PCA (yellow curves) and our method (blue curves). Error bars = SEM.
**Fig. S11. Single-unit composition of cell assemblies.** Color-coded normalized (to area under curve) activity maps for two assemblies (top row) and their constituent single neurons (rows below) within an environmental exploration task (left) and a delayed alternation task (right). Average firing rates of the constituent single units indicated below each map.