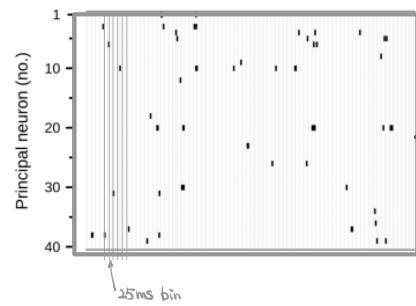


# Cell assembly identification

• raster plot  
with 25ms bins



spike count matrix  $X$   
(each 25ms bin)

neuron	1	2	3	...	$b$	...	B
1							
2							
3							
...							
i					$X_{i,b}$		
...							
40						mean $\mu_{xi}$	var $\sigma^2_{xi}$

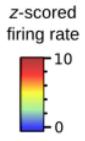
$$z_{i,b}$$

$$z\text{-score} = \frac{x_{i,b} - \mu_{xi}}{\sigma_{xi}}$$

沿行进行  
Z-score

$n \times B$

$Z$ -scored spike  
count matrix,  $Z$



$n \times B$

PCA

例如 (2 4 0 5) 沿行进行  
(200 130 350 160) Z-score 得到 ( )

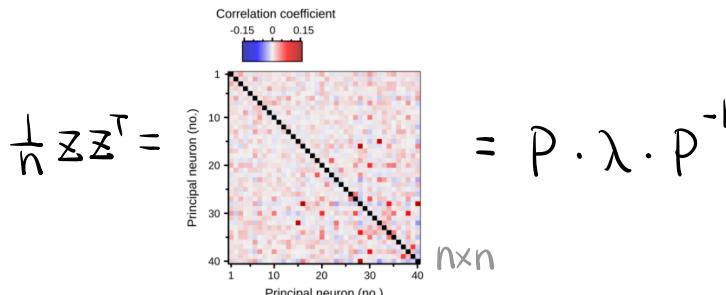
对于各特征 (neuron), 去均值,  
并将其特征的 scale 统一

也就是说,  $Z$  的每一行不再是绝对的 spike count, 而是  $Z$ -Score 归一化的 spike count, 考虑  
了在 bin 中的 spike count 与该 neuron 的总 spike count 的关系。

我们是否可以认为: 由于  $Z$ -Score 已经将各 neuron 的 spike count 的 scale 统一,  
因此即使有 interneuron 在, 也不会影响到 cell assembly 的真实情况?

例如 pyramidal (2 4 0 5) pyramidal 所有的 spike  
interneuron (200 130 350 160) 都被误认为是 INN 的 co-firing, Z-score 为 ( )  
INN 不再与 PYN  
co-firing

## ① 理解角存 1



Real symmetric matrix  $\rightarrow$  orthogonal decomposition

$$\frac{1}{n} Z Z^T = P \cdot \Lambda \cdot P^{-1}$$

$$\begin{bmatrix} P_{1,1} & P_{1,2} & \dots & P_{1,40} \\ P_{2,1} & P_{2,2} & \dots & P_{2,40} \\ \vdots & \ddots & \ddots & \vdots \\ P_{40,1} & P_{40,2} & \dots & P_{40,40} \end{bmatrix} \begin{bmatrix} \lambda_1 & & & \\ & \lambda_2 & & \\ & & \ddots & \\ & & & \lambda_{40} \end{bmatrix} \begin{bmatrix} P_{1,1} & P_{1,2} & \dots & P_{1,40} \\ P_{2,1} & P_{2,2} & \dots & P_{2,40} \\ \vdots & \ddots & \ddots & \vdots \\ P_{40,1} & P_{40,2} & \dots & P_{40,40} \end{bmatrix}^{-1}$$

$$= \begin{bmatrix} P_{1,1} & P_{1,2} & \dots & P_{1,40} \\ P_{2,1} & P_{2,2} & \dots & P_{2,40} \\ \vdots & \ddots & \ddots & \vdots \\ P_{40,1} & P_{40,2} & \dots & P_{40,40} \end{bmatrix} \begin{bmatrix} \lambda_1 & & & \\ & \lambda_2 & & \\ & & \ddots & \\ & & & \lambda_{40} \end{bmatrix} \begin{bmatrix} P_{1,1} & P_{1,2} & \dots & P_{1,40} \\ P_{2,1} & P_{2,2} & \dots & P_{2,40} \\ \vdots & \ddots & \ddots & \vdots \\ P_{40,1} & P_{40,2} & \dots & P_{40,40} \end{bmatrix}$$

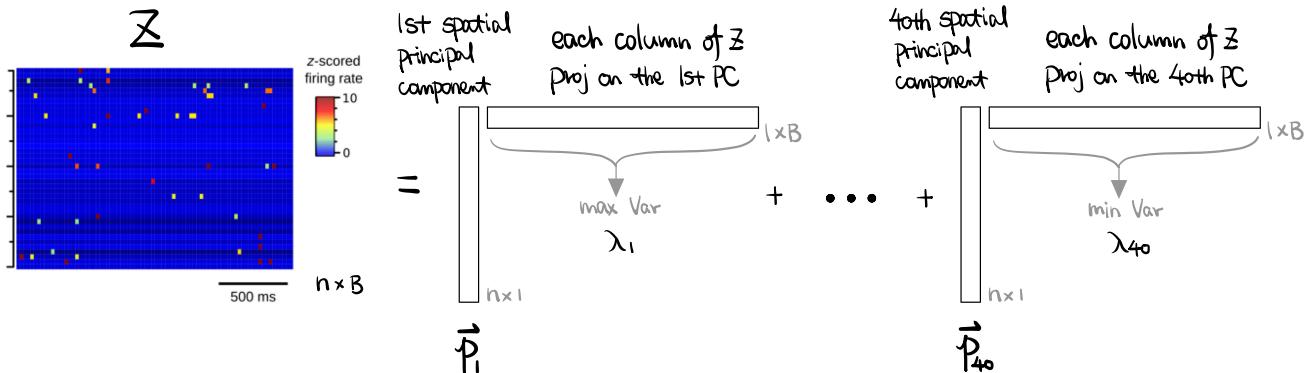
$$= \begin{bmatrix} \vec{P}_1 & \vec{P}_2 & \dots & \vec{P}_{40} \end{bmatrix} \begin{bmatrix} \lambda_1 & & & \\ & \lambda_2 & & \\ & & \ddots & \\ & & & \lambda_{40} \end{bmatrix} \begin{bmatrix} \vec{P}_1 & \vec{P}_2 & \dots & \vec{P}_{40} \end{bmatrix}^T$$

orthogonal to  
each other

$$= \lambda_1 \vec{P}_1 \vec{P}_1^T + \lambda_2 \vec{P}_2 \vec{P}_2^T + \dots + \lambda_{40} \vec{P}_{40} \vec{P}_{40}^T$$

$$= \sum_{j=1} \lambda_j \vec{p}_j \vec{p}_j^\top$$

## ② 理解兩步：



利用PCA得到五中各个显著的，且正交的 spatial patterns ( co-activation patterns within 25ms ? )

有多少 spatial principal components  
"capture" co-activation patterns ?

To estimate the number of significant patterns embedded within the data, we used the Marčenko-Pastur law (Marčenko and Pastur, 1967; Götze et al., 2004). This law states that for a  $n \times B$  matrix whose elements are independent and identically distributed random variables with zero mean and unit variance, all eigenvalues are asymptotically (i.e., when  $n, B \rightarrow \infty$  such that  $B/n$  converges to a finite positive value) bounded to the interval  $\left[ \left(1 - \sqrt{n/B}\right)^2, \left(1 + \sqrt{n/B}\right)^2 \right]$ . This suggests that if the firing activity of the neurons is independent from each other, then none of the eigenvalues is expected to exceed  $\lambda_{\max} = \left(1 + \sqrt{n/B}\right)^2$ . Using simulated spike trains, this was indeed shown to be the case even for values of  $n$  and  $B$  considerably smaller than in our data-set (Peyrache et al., 2010; Lopes-dos-Santos et al., 2011, 2013). An eigenvalue above  $\lambda_{\max}$  thus indicates that the pattern given by the corresponding principal component captures more correlation than any pattern would be expected to capture if the firing activity of all neurons was independent of each other. The number of eigenvalues above  $\lambda_{\max}$  (denoted by  $N_A$ ) therefore represents the number of distinct significant patterns.

the principal components capturing significant patterns

是否代表各 ground truth co-firing cell assemblies 的 co-firing patterns ?

NO!

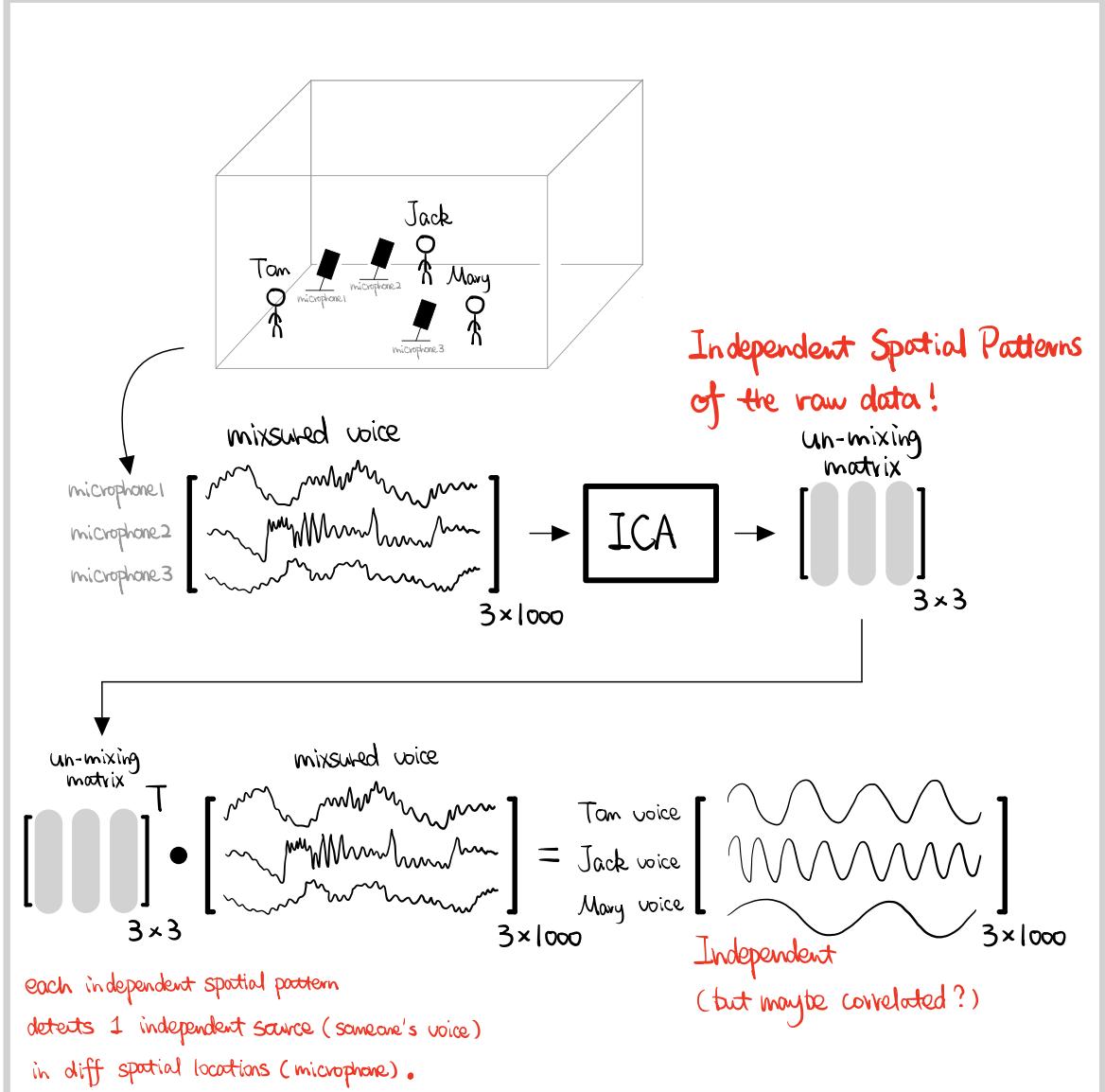
the first  $N_A$  principal components each capture a significant amount of correlation between the firing activities of the neurons. However, principal components are restricted to be orthogonal to each other, while cell assemblies do not need to be (e.g., they can contain overlapping neurons). Principal components are also extracted from the data sequentially, which usually causes the first principal component to seemingly be a mixture of multiple assemblies (Laubach et al., 1999; Lopes-dos-Santos et al., 2011). Moreover, ③ PCA is solely based on pairwise correlations, but higher-order correlations could also inform assembly identification.

PCs ~~can~~ ~~not~~ represent cell correlation patterns however →  
can not represent each other.

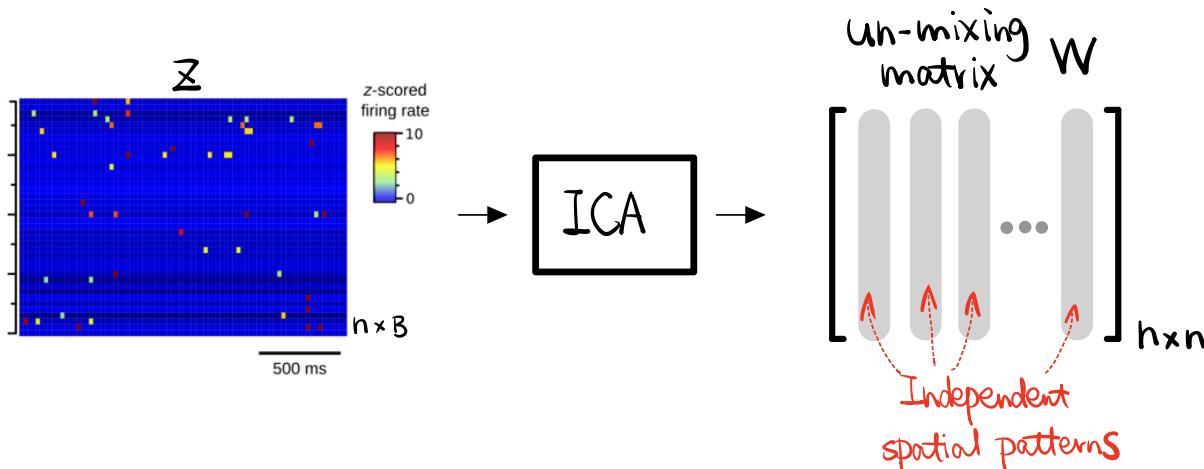
PCA is NOT an optimal solution

cell assemblies ←  
may be can partially  
represent each other.  
because there may  
be overlapping cells

what is ICA:



Can we forget about the PCA result and use ICA on  $Z$  directly (shown below)? NO



an ICA directly applied to matrix  $Z$  without prior dimension reduction would extract as many patterns as there are neurons, which leads to spurious results (Lopes-dos-Santos et al., 2013). To restrict the number of patterns identified by ICA to  $N_A$ , the data is first projected onto the subspace spanned by the first  $N_A$  principal components:

利用 PCA 得到的 Top- $N_A$  PCs, 将  $Z$  降维到 significant co-activation patterns 数量  $N_A$ ,

(correlation)

最大程度地保留了各 co-activation patterns

又将信号维度由 neuron 数量 降低到 significant co-activation pattern 的个数  $N_A$  ( $n \rightarrow N_A$ )

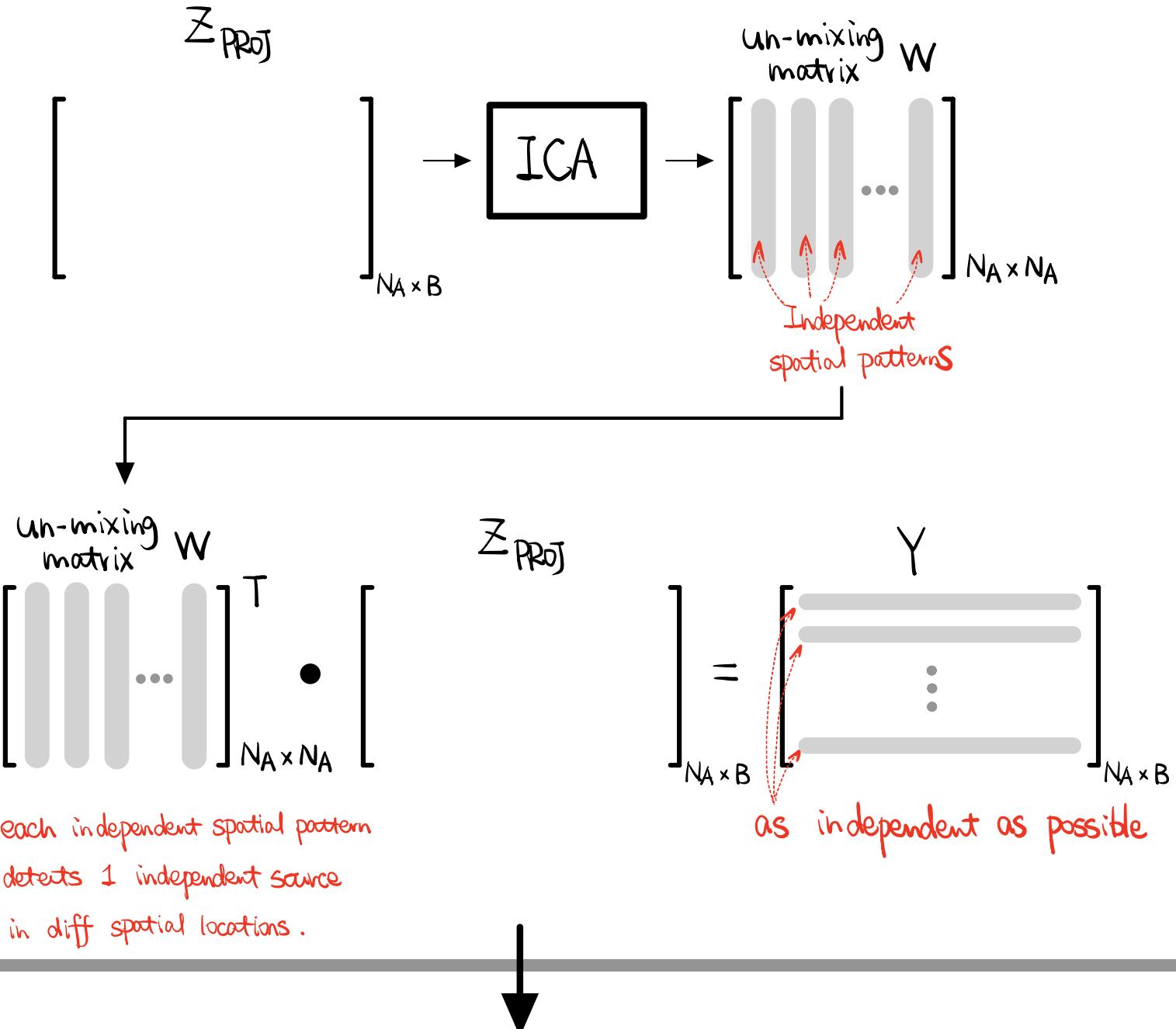
$$P_{\text{SIGN}}^T \cdot Z = Z_{\text{PROJ}}$$

Diagram illustrating the projection of matrix  $Z$  onto the subspace spanned by the first  $N_A$  principal components.

The left side shows the matrix  $P_{\text{SIGN}}^T$  with dimensions  $n \times N_A$ , where columns represent the 1st PC through the  $N_A$ th PC. The right side shows the resulting projected matrix  $Z_{\text{PROJ}}$  with dimensions  $N_A \times B$ .

The middle part shows the original matrix  $Z$  with dimensions  $n \times B$  and a color scale for z-scored firing rate from 0 to 10, with a 500 ms time scale.

An ICA was then applied to the matrix  $\mathbf{Z}_{\text{PROJ}}$ . That is, a  $N_A \times N_A$  un-mixing matrix  $\mathbf{W}$  was found such that the rows of the matrix  $\mathbf{Y} = \mathbf{W}^T \mathbf{Z}_{\text{PROJ}}$  are as independent as possible. This optimization-problem was solved using the fastICA algorithm (Hyvärinen, 1999) implemented in R (fastICA-package: Marchini et al., 2013). The resulting un-



each column of  $\mathbf{W}$  is an independent co-activation pattern  
in the projection space. Now, let's project these independent  
co-activation patterns back to the original basis spanned by  
all the neurons.

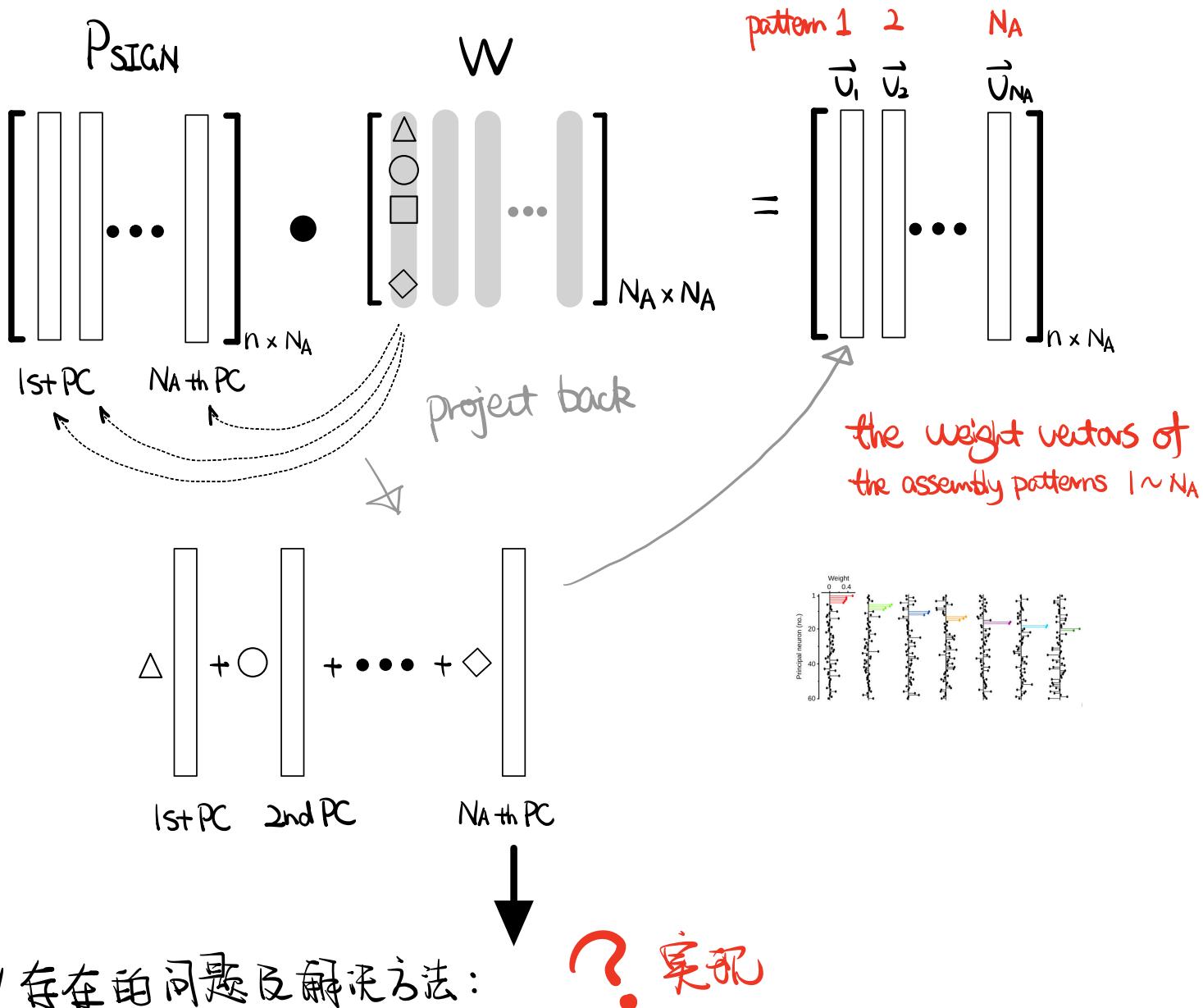
$P_{SIGN}$

• un-mixing matrix  $W$

=

$V$

where the columns of  $V$  (i.e.,  $v_1, \dots, v_{N_A}$ ) are the weight vectors of the *assembly patterns*.



1. 各个 weight vectors 的尺度 (scale) 不同

→ 使每个 weight vector  $\vec{v}_k$  转化成单位向量

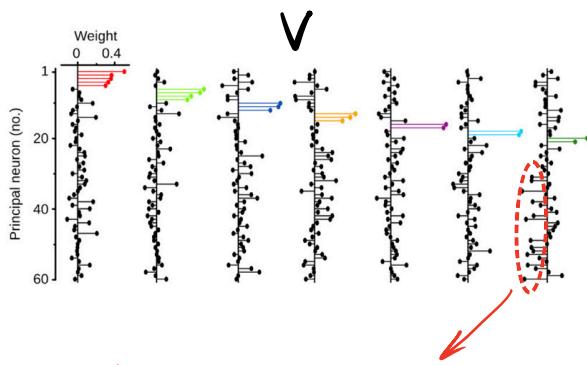
2. 各个 weight vectors 的正负号随意.

→ 每个 weight vector  $\vec{v}_k$  中, 确保绝对值最大的值的符号为正, 以此为准则.

随意的

As both the sign and the scale of the ICA output is arbitrary, all weight vectors were scaled to unit length (i.e.,  $\sum_{i=1}^n v_k(i)^2 = 1$  for  $k = 1, \dots, N_A$ ) and their sign set such that the highest absolute weight of each assembly pattern was positive.

3.



负权重意味着什么？

若从 spike count matrix 的角度来考虑，是不应该有负权重出现的。因为整个 matrix 都是正的，其 spatial patterns 也为正。但是！不要忘了 spike count matrix 经过了 Z-Score，得到的 matrix 又是有正有负的。因此，基于此得到的各 co-activation pattern weight vectors 确实可以有负值出现。但并不是所谓的负贡献，只是由于 Z 中各 cell 具有  $\text{mean} = 0$  的特性，使 weight vectors 也向负偏移了。但我们的关注点在最大且即为 weight 对应的 cells 所组成的 cell assembly。

那么，如何量化的找到 cell assembly 中的 member neurons?



Most of the detected assembly patterns consisted of a few neurons with high weights and a large group of neurons with weights around zero (e.g., see **Figures 1C and S2A**). For each assembly pattern it would therefore be possible to define a corresponding “cell assembly” as those neurons whose weight exceeds the mean weight by two standard deviation (member neurons; with both mean and standard deviation calculated from only the weights of that pattern). Note that in this study, this definition of cell assemblies is only used for an intuitive way of interpreting and discussing the presented results. Importantly, all analyses were performed directly on the assembly patterns themselves (i.e., using the weight vectors formed by the contribution of all recorded principal neurons).

综上，我们得到了 cell assembly pattern  $k$  的 weight vector  $\bar{w}_k$  及 member neurons。

Note that the detection of assembly patterns is solely based on neuronal co-activations within 25 ms time bins; the particular sequence of activations within these co-activation events is not considered here. Also note that for a co-activation pattern to be detected as significant, it needs to be active in many different time bins.

## 2. assembly pattern (weight vector) 的两个属性

### 2.1 assembly pattern (weight vector) 的稀疏性：

特征是 member neurons 对该 assembly pattern 的“统治力”

The sparsity of an assembly pattern (see **Table S1**), which reflects to what extent the weight vector of a pattern is dominated by a small group of neurons, was calculated as

$$1 - \frac{\sqrt{n} - \sum |v_i|}{\sqrt{n} - 1}$$

where  $n$  is the length of the weight vector (i.e., the number of principal neurons recorded that day) and  $v_i$  is the weight vector's  $i^{\text{th}}$  element (i.e., the contribution of neuron  $i$  to the pattern).

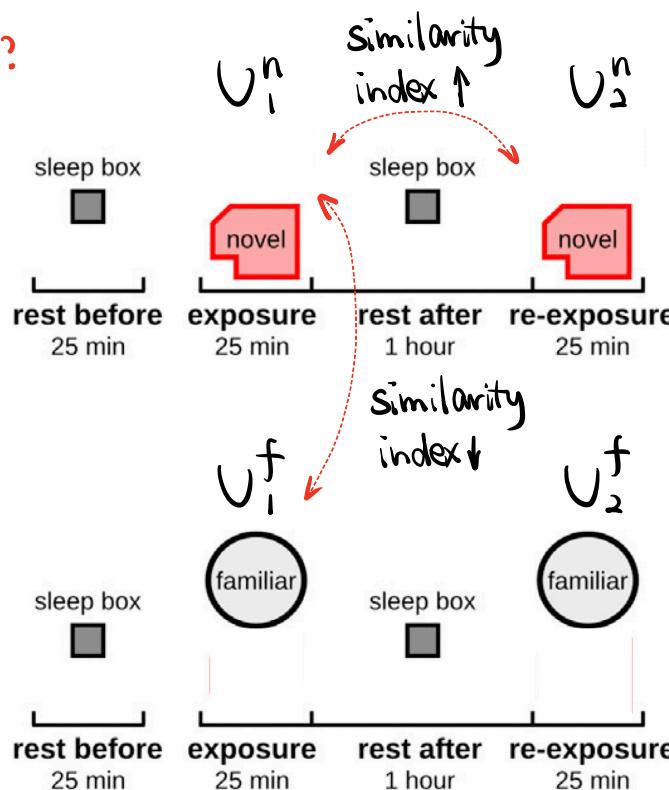
### 2.2 assembly pattern (weight vector) 的环境特异性

#### Environment specificity

To assess the environment specificity of assembly patterns, we compared each pattern detected during the first exposure to an enclosure with the set of patterns detected during re-exposure to the same enclosure and with the set of patterns detected during exposure to another enclosure of a different recording block. The similarity of two assembly patterns was quantified by a *similarity index* equal to the absolute value of the inner-product of their weight vectors (Almeida-Filho et al., 2014). A pattern's *environment-specificity index* was then defined as its maximum similarity index with any of the patterns detected during re-exposure minus its maximum similarity index with any of the patterns detected in the other enclosure.

why not minimum?

recording  
block 1

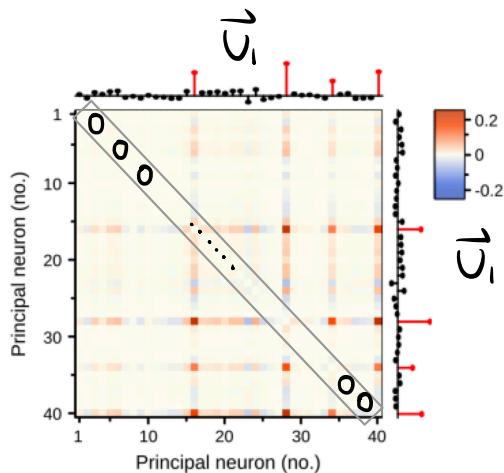


recording  
block 2

### 3. 在 first exposure session 识别出的 assembly pattern 的时变强度

#### Step 1 :

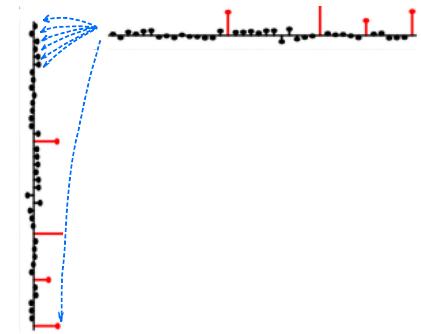
For each assembly pattern, a projector matrix is constructed by taking the outer product matrix of its weight vector and setting the diagonal to zero (to ensure only co-activations of neurons can contribute to the expression of an assembly pattern)



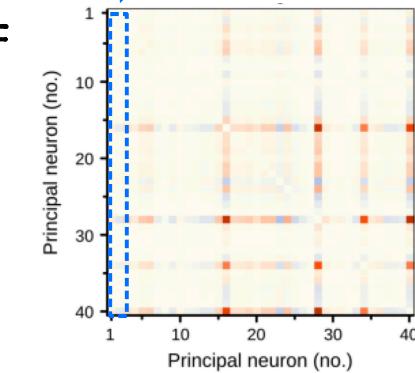
projector matrix

$\vec{U}_1$  表示各 cell 在该 assembly pattern 中的重要程度 (firing 程度?)

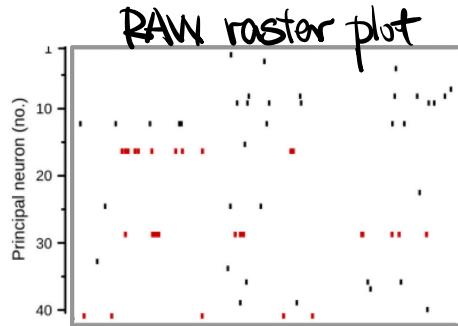
$$\text{projector matrix} = \vec{U}_1 \cdot \vec{U}_1^T$$



第一列表示 cell 1 与其它(不包括自己)各 cells 的“共同贡献” “共同权重” or “co-firing 程度”



#### Step 2 :

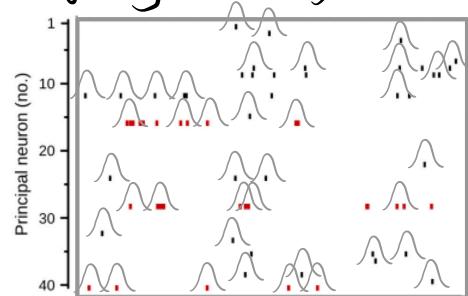


raw raster plot

20kHz

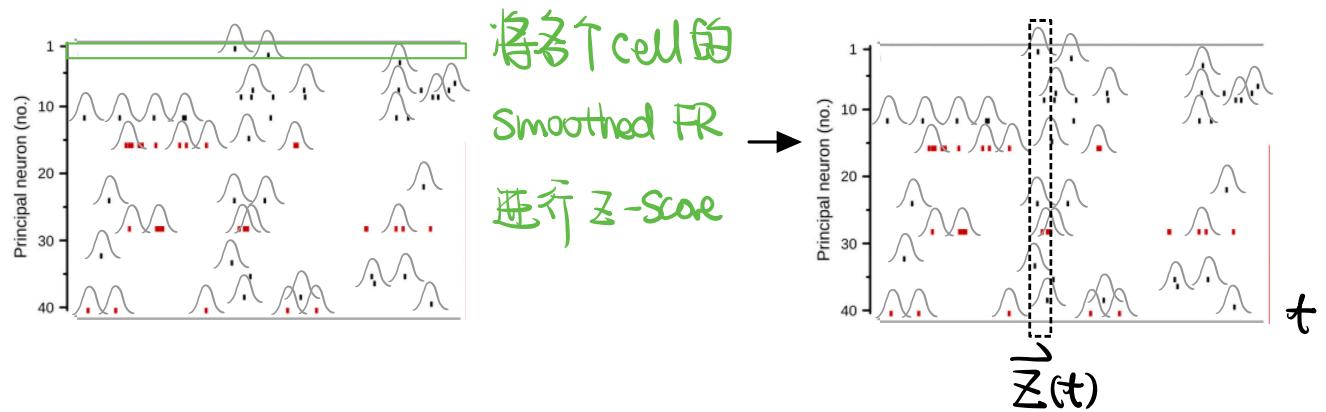
conv with a Gaussian kernel to get smoothed →  
instantaneous firing rates of each cell

smoothed instantaneous firing rates of each cell



? 完成

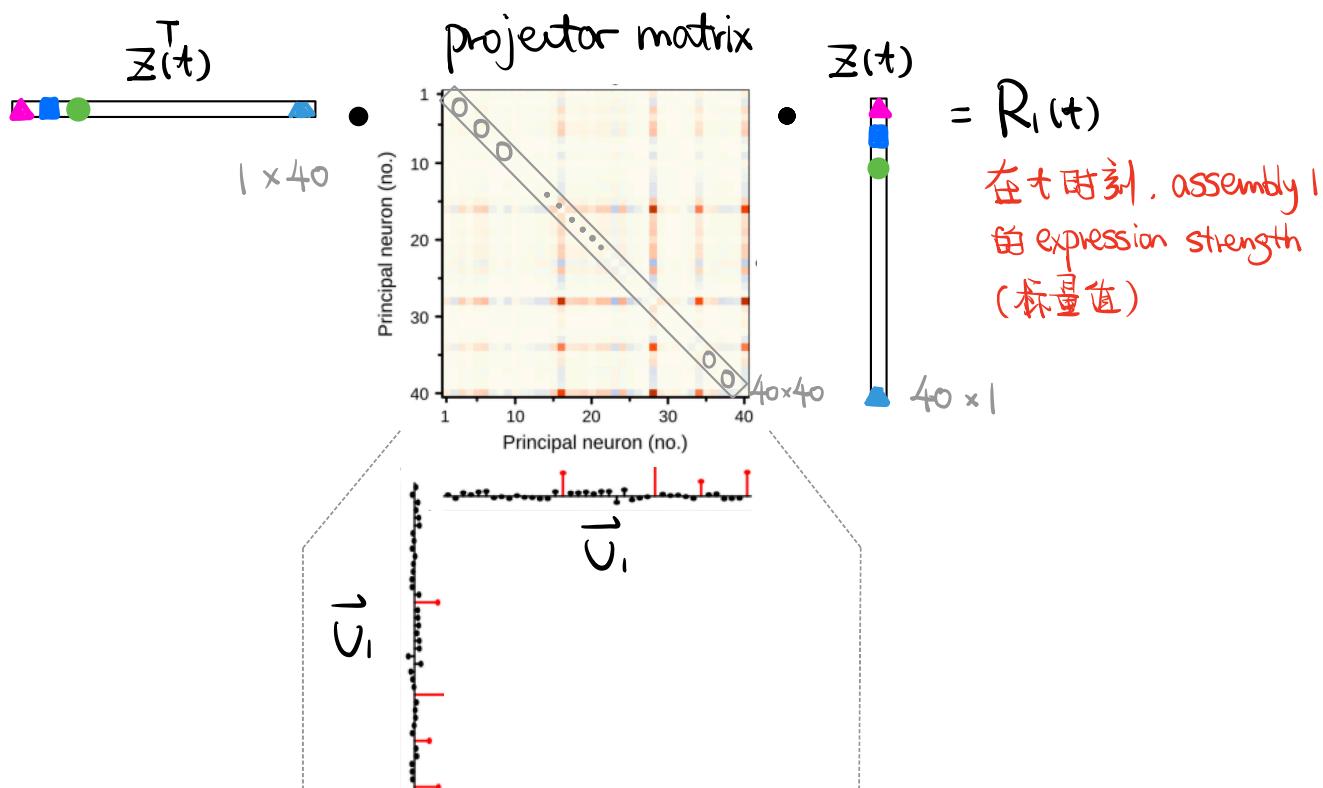
As smoothing function, a Gaussian kernel was chosen with standard deviation  $w/\sqrt{12}$ , so that the kernel had the same standard deviation as fixed time bins of  $w$  ms (Kruskal et al., 2007). The value  $w$  determines the width of the “integration window” in which spikes are still considered to be coincident. We set  $w$  to 25 ms to match the bin-size used to identify the patterns.



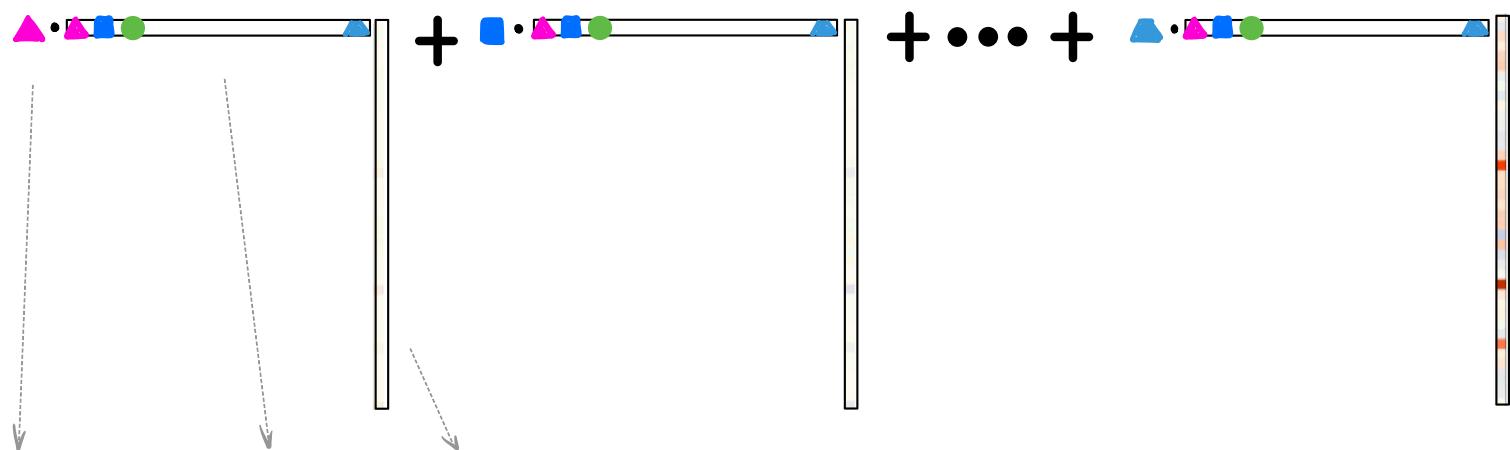
$\vec{z}(t)$  : 在  $t$  时刻 各 cells 的 Z-Score firing rate.  
这  $t$  firing rate 经过了 smooth 和 各  $t$  cell 自身的 Z-Score

### Step 3:

assembly pattern ( $v_i$ ) expression strength over time  $R_i(t)$



上述公式可以详细分解为：



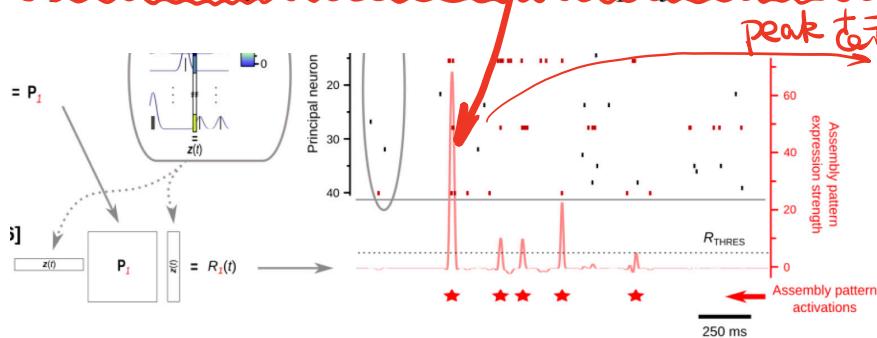
在  $t$  时刻,  
cell 1 与各 cells  
的 FR 乘积, 表示  
在这个时刻,  
cell 1 与各 cells  
的 co-firing 程度

在  $t$  时刻,  
cell 1 与其它 (不包括自己)  
各 cells 的“共同发射”  
“co-firing 程度”

两者。相关度。越相关, 当前时刻 cell 1  
越正在发挥“在 assembly pattern 1 中的作用.”

所以, 第一项表示 cell 1 在  $t$  时刻发挥“在 assembly pattern 1 中的作用”的程度  
所有项的和即表示所有 cells 发挥“在 assembly pattern 1 中的作用”的程度  
即, assembly pattern ( $V_1$ ) expression strength at time  $t$ .

resulting expression strength time-courses showed a low baseline with sparse, transient peaks (see Figure 1C). These peaks correspond to co-activations (within 25 ms) of at least two neurons with high weight in that pattern, whereby the magnitude of these peaks is governed by both (1) the weights of the active neurons in that pattern and (2) the number of spikes discharged by each neuron within the window. Note that the scale of the expression strength can be interpreted as a “projected z-score”. ?



至少有两个在  $V_1$  中具有大权重的 neurons 进行了 co-firing (in 25ms).  
peak 的幅度与 active neurons 的权重和 spikes 数量 (in 25ms) 有关

**assembly pattern 被认为激活的条件:** Assembly pattern activations were defined as peaks in the expression strength exceeding  $R_{\text{THRES}} = 5$ . During the exposure session in which the patterns were detected, this threshold resulted in a mean assembly pattern activation rate of  $0.95 \pm 0.02$  Hz ( $n = 521$  assembly patterns). Note that each detected assembly pattern thus corresponded to many activations.

# 4.

## 4.1 assembly pattern's spatial firing rate map (以 assembly 为 对象

### Assembly pattern activation maps

The time stamps of these assembly pattern activations during active exploration in the first exposure session were used to generate *assembly maps* (e.g., **Figures 1C and 4B**). For this, the horizontal plane of the recording room was divided into spatial bins of approximately  $2 \times 2$  cm. Activation count maps (number of assembly pattern activations per spatial bin) and an occupancy map (time spent by the animal in each spatial bin) were generated, and all maps were spatially smoothed by convolution with a Gaussian kernel with standard deviation of one bin width. Smoothed activation count maps were then normalized by dividing them by the smoothed occupancy map to generate the spatial assembly maps.

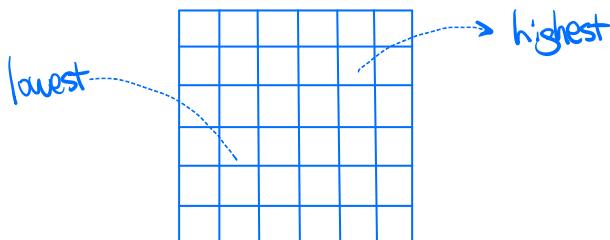
→ time stamps 作为中间量来连接空间坐标，还可以用来计算 spike count.

## 4.2

The coherence and sparsity of an assembly map (see **Table S1**) were calculated from the unsmoothed maps.

? Coherence, which reflects the similarity of the assembly pattern activation rate in adjacent bins, was calculated as Fisher's  $r$  to  $z$  transform of the Pearson correlation (across all visited bins) between the rate in a bin and the average rate of its eight nearest neighbours (Muller and Kubie, 1989). Sparsity, which reflects how tightly concentrated in space the activity of an assembly pattern is, was calculated as  $(\sum P_i R_i)^2 / \sum P_i R_i^2$ , where  $P_i$  is the probability of the animal occupying bin  $i$  and  $R_i$  is the assembly pattern activation rate in bin  $i$  (Skaggs et al., 1996). A pattern's assembly field (see **Table S1**) was defined as those bins with an assembly pattern activation rate above its "field threshold", which was calculated as the rate of the bin with the highest rate minus 40 % of the difference in rate between the bins with the highest and lowest rate.

$$\text{field threshold} = \text{highest} - 40\% (\text{highest} - \text{lowest})$$

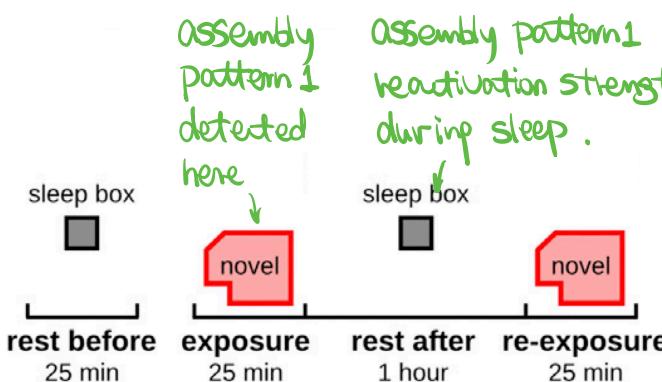


assembly pattern's spatial firing rate map

## 4.3

### Reactivation and reinstatement strength ( reactivation & reinstatement 的区别 )

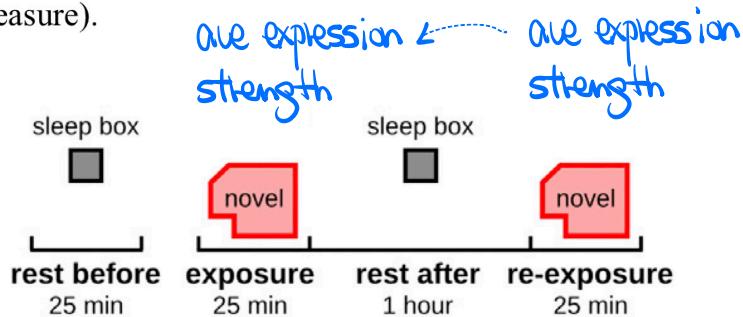
To compare offline reactivation across assembly patterns, regardless of their difference in baseline strength (e.g., see **Figure S1F**), the *reactivation strength* of an assembly pattern identified during the exposure session was defined as its average expression strength during (the rest after) minus its average expression strength during (the rest before). Note that a null score for reactivation strength indicates that a pattern is not reactivated (i.e., equal strength during rest after as during rest before), while the more positive the stronger the reactivation of that pattern.



assembly pattern 1  
reactivation strength  
during sleep.

如果 one expression strength  
of assembly pattern 1 during  
sleep 等于 rest before  
session (baseline), then,  
assembly 1 在 sleep session  
中被重新激活。

The *reinforcement strength* was similarly defined as its average expression strength during re-exposure minus its average expression strength during the first exposure. Note that a null score for reinforcement strength indicates that a pattern is “perfectly” reinstated (i.e., no loss in strength from initial exposure to re-exposure), while the more negative the worse the reinstatement of that pattern (see the legend of **Figure S3E** for further discussion of this measure).



**Reactivation:** 在 sleep box 中，cell assembly 是否再次活跃

**reinstatement:** 在 re-exposure 中，cell assembly 是否再次活跃

## 4.4

### Gradually strengthened and early stabilized assembly patterns

Assembly patterns identified during the first exposure to a novel enclosure were divided into two sets based on the time-course of their expression strength. For each pattern, a linear trend model (including intercept) was fitted to its expression strength during the exposure session calculated in 1 sec bins. Patterns with a significant positive slope were defined as *gradually strengthened* and those without significant positive slope as *early stabilized*. Note that these two sets of patterns were defined solely based on their strengthening dynamics during the initial exposure session, and that we then show that both sets later differ in their sensitivity to SWR disruption. Further note that this division of patterns into two sets was only used for the analyses corresponding to **Figure 4**; all other analyses were always based on all detected assembly patterns. In **Figure 4A**, for visualization-purposes only, the individual expression strength time-courses were smoothed with a Gaussian kernel ( $SD = 5$  sec) before calculating the presented average and SEM time-courses. One gradually strengthened assembly pattern detected in a novel enclosure had both an unusual high reactivation and reinstatement strength.

## 4.5

### Neuron-pair and single-neuron measures

For each pair of principal neurons, the *co-firing coefficient* (see **Figure 1D**) was calculated as the Pearson correlation coefficient between the binned (25 ms time bins) spike counts of the two neurons forming that pair (McNamara et al., 2014). To calculate the *place-field similarity* (PFS; see **Figures 1E, S1C** and **S3G**) scores between two neurons in a given exploratory session, spatial rate maps were first generated for both neurons in a way similar to the assembly maps, with the assembly pattern activations replaced by the neuron’s spike train. The PFS was then defined as the Pearson correlation coefficient from the direct comparison of the spatial bins between the spatial rate maps of both neurons (McNamara et al., 2014).

For the single-neuron analyses (see **Figures S1D** and **S3H**), a given neuron’s place field *similarity score* between two exploratory sessions was calculated as the Pearson correlation coefficient from the direct bin-wise comparison between that neuron’s spatial rate maps from both sessions, whereby only spatial bins were included that were visited by the animal in both sessions (Kentros et al., 1998). For all neuron-pair and single-neuron analyses, only principal neurons with at least 100 spikes discharged during active exploration in each session considered were included. In addition, for the neuron-pair analyses, only pairs of neurons recorded from different tetrodes were included.