**Supplementary information for**

# Parallel processing of past and future memories through reactivation and synaptic plasticity mechanisms during sleep

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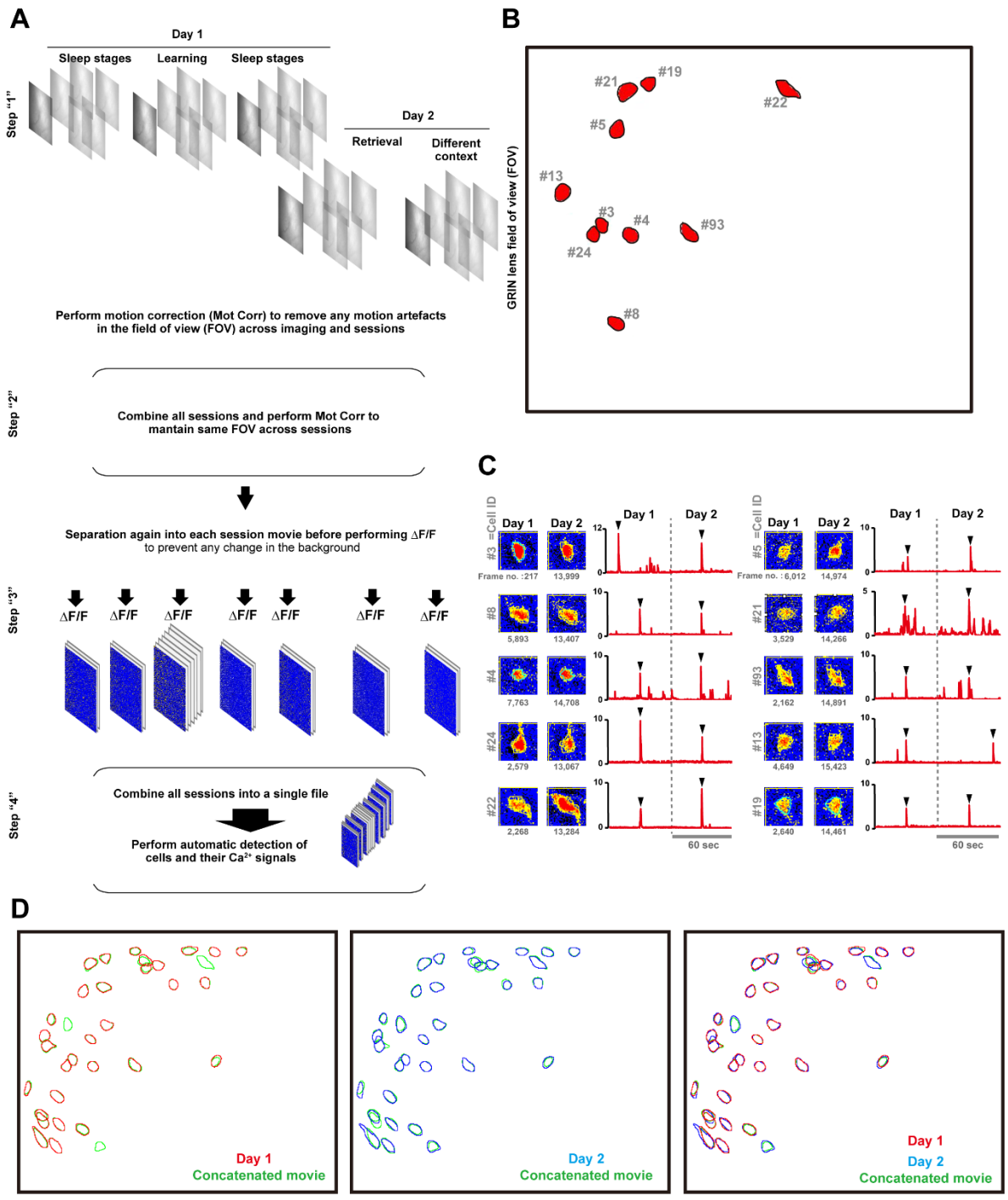

A
Day 1
Sleep stages
Learning
Sleep stages
Day 2
Retrieval
Different context
Step "1"
Perform motion correction (Mot Corr) to remove any motion artefacts in the field of view (FOV) across imaging and sessions
Step "2"
Combine all sessions and perform Mot Corr to mantain same FOV across sessions
Separation again into each session movie before performing ΔF/F to prevent any change in the background
Step "3"
ΔF/F
Step "4"
Combine all sessions into a single file
Perform automatic detection of cells and their Ca2+ signals
B
GRIN lens field of view (FOV)
#21
#19
#22
#5
#13
#3
#4
#93
#24
#8
C
=Cell ID
Day 1
Day 2
Frame no. :217
13,999
Frame no. : 6,012
14,974
5,893
13,407
3,529
14,266
7,763
14,708
2,162
14,891
2,579
13,067
4,649
15,423
2,268
13,284
2,640
14,461
60 sec
D
Day 1
Concatenated movie
Day 2
Concatenated movie
Day 1
Day 2
Concatenated movie

Suppl. Fig. 1

**Suppl. Fig.1 Extracting calcium transients from engram cells and nonengram cells across sessions maintaining the same field of view (FOV).**

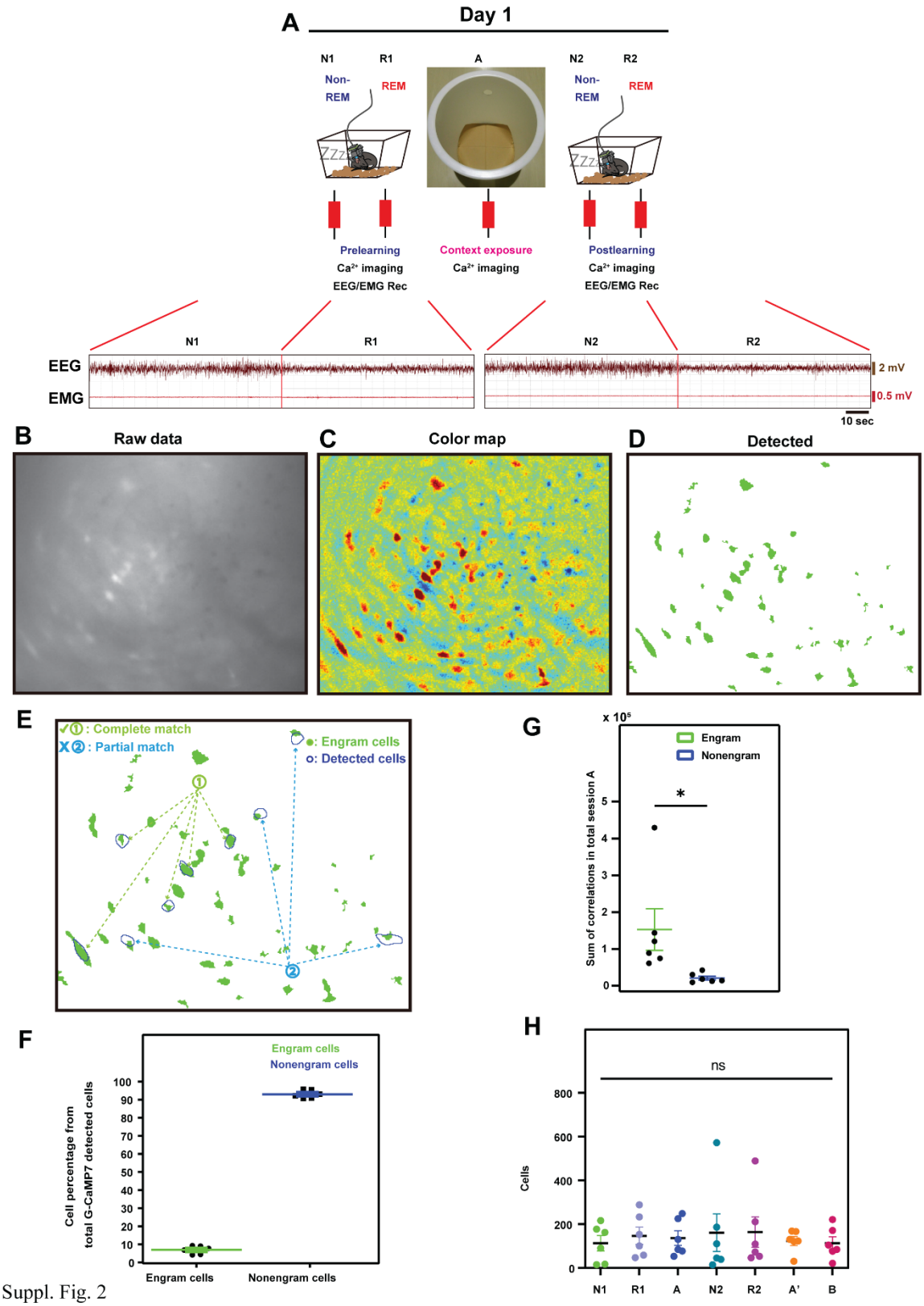
(A) Schematic diagram showing the preprocessing of a Ca2+ imaging video of the whole recorded behavioral paradigm.

(B) Spatial positions of representative cells in the FOV that were active on both days of recording.

(C) ROI of the selected cells in panel (B), and their representative Ca2+ traces on day 1 and day 2. Black arrowheads point to the time selected for evaluating cell morphology. The number of frames is indicated under each ROI.

(D) Footprints of matched cells detected by the HOTARU system from the concatenated movie and videos of single days (Days 1 & 2). Cells detected from the concatenated video are shown in green (left, center, right), cells detected from the day 1 video are shown in red (left, right), and cells detected from the day 2 video are shown in blue (center, right).

The Experiment was independently repeated six times.


A
Day 1
N1
R1
A
N2
R2
Non-REM
REM
Non-REM
REM
Prelearning
Ca2+ imaging
EEG/EMG Rec
Context exposure
Ca2+ imaging
Postlearning
Ca2+ imaging
EEG/EMG Rec
EEG
EMG
2 mV
0.5 mV
10 sec
B
Raw data
C
Color map
D
Detected
E
✓①: Complete match
X②: Partial match
●: Engram cells
○: Detected cells
F
Engram cells
Nonengram cells
Cell percentage from total G-CaMP7 detected cells
Engram cells
Nonengram cells
G
x 10⁵
Engram
Nonengram
Sum of correlations in total session A
H
ns
Cells
N1
R1
A
N2
R2
A'
B

Suppl. Fig. 2

**Suppl. Fig.2 Electroencephalogram (EEG) and electromyogram (EMG) traces and identification of double-positive KikGR and G-CaMP7 cells**

(A) Representative EEG and EMG traces during calcium imaging sleep recording to differentiate between nonrapid eye movement (NREM) and rapid eye movement (REM) sleep stages, in prelearning (N1 and R1) and postlearning (N2 and R2) sleep.

(B-D) Representative snapshot image of KikGR+ expression raw data (B), colored map (C), and automatic detection of KikGR+ (D) (see Methods).

(E) Criteria for selecting KikGR+ cells. Cells showing a complete match between the KikGR-expressing cell from the snapshot (green filled contours) and the HOTARU system (blue contours; category 1; i.e., cells double-positive for c-fos tet tagging and G-CaMP7 fluorescence) were considered engram cells. Cells that showed only a partial match between the blue and green contours (category 2) were excluded from further analysis and considered as nonengram cells.

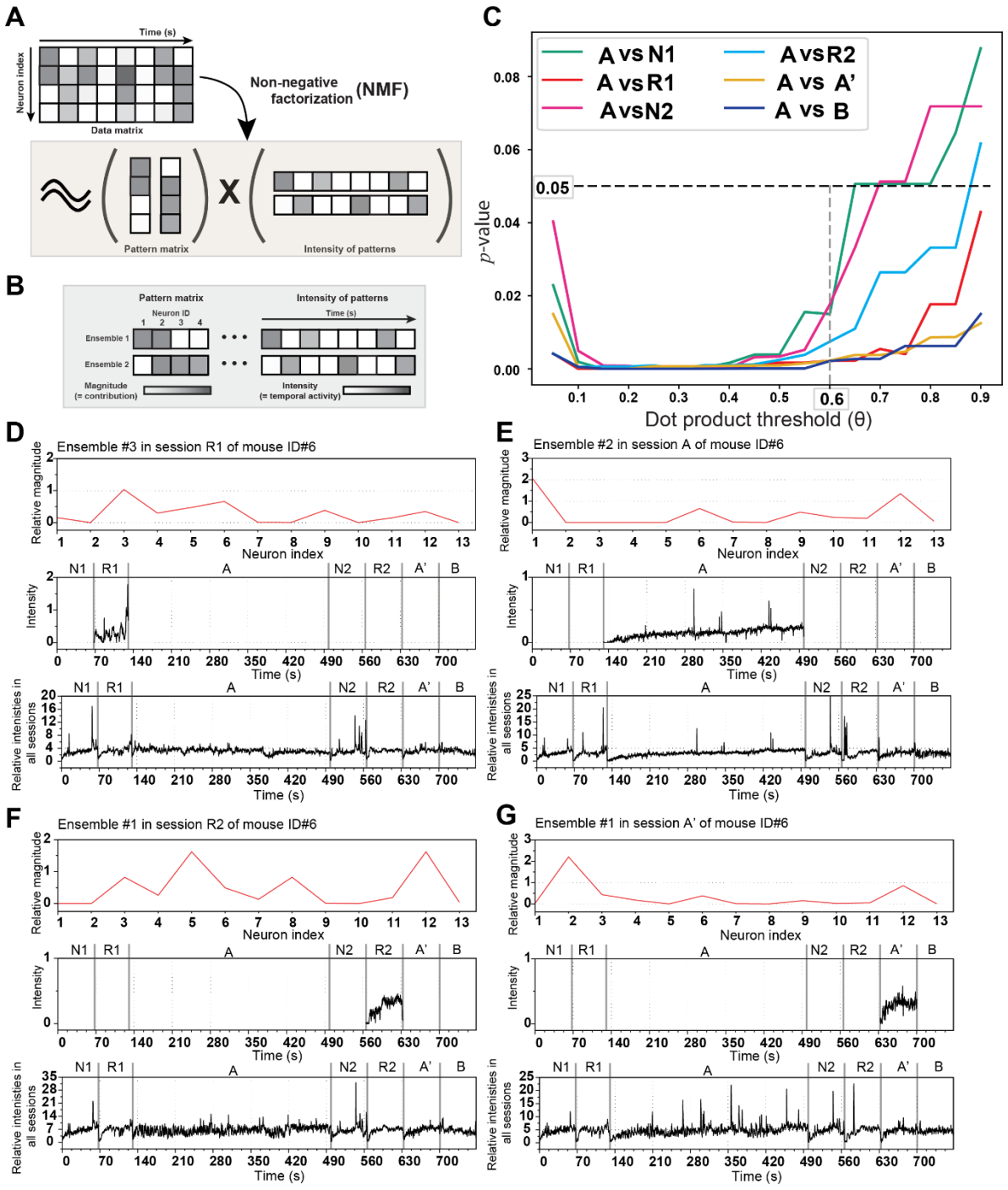
(F) Percentage of engram cells ($KikGR^+$ & $G\text{-}CaMP7^+$) to nonengram cells (total detected $G\text{-}CaMP7^+$).

(G) Sum of correlations of engram and nonengram cells during total exposure of session A.

(H) Total number of cells detected in each session.

*$p < 0.05$, n.s., nonsignificant, n=6 mice. Data represent the mean ± s.e.m. Statistical comparisons were conducted using a two-tailed Wilcoxon signed rank test (G) and repeated measure one-way ANOVA (H). The Experiment was independently repeated six times.

Source data are provided as a Source Data file. Detailed statistics are shown in Supplementary Data 1.


A
Time (s)
Neuron index
Data matrix
Non-negative factorization (NMF)
Pattern matrix
Intensity of patterns
B
Pattern matrix
Intensity of patterns
Neuron ID
Time (s)
Ensemble 1
Ensemble 2
Magnitude (= contribution)
Intensity (= temporal activity)
C
A vs N1
A vs R1
A vs N2
A vs R2
A vs A'
A vs B
p-value
0.05
0.6
Dot product threshold (θ)
D
Ensemble #3 in session R1 of mouse ID#6
Relative magnitude
Neuron index
Intensity
Time (s)
Relative intensities in all sessions
N1 R1 A N2 R2 A' B
E
Ensemble #2 in session A of mouse ID#6
F
Ensemble #1 in session R2 of mouse ID#6
G
Ensemble #1 in session A' of mouse ID#6

Suppl. Fig. 3

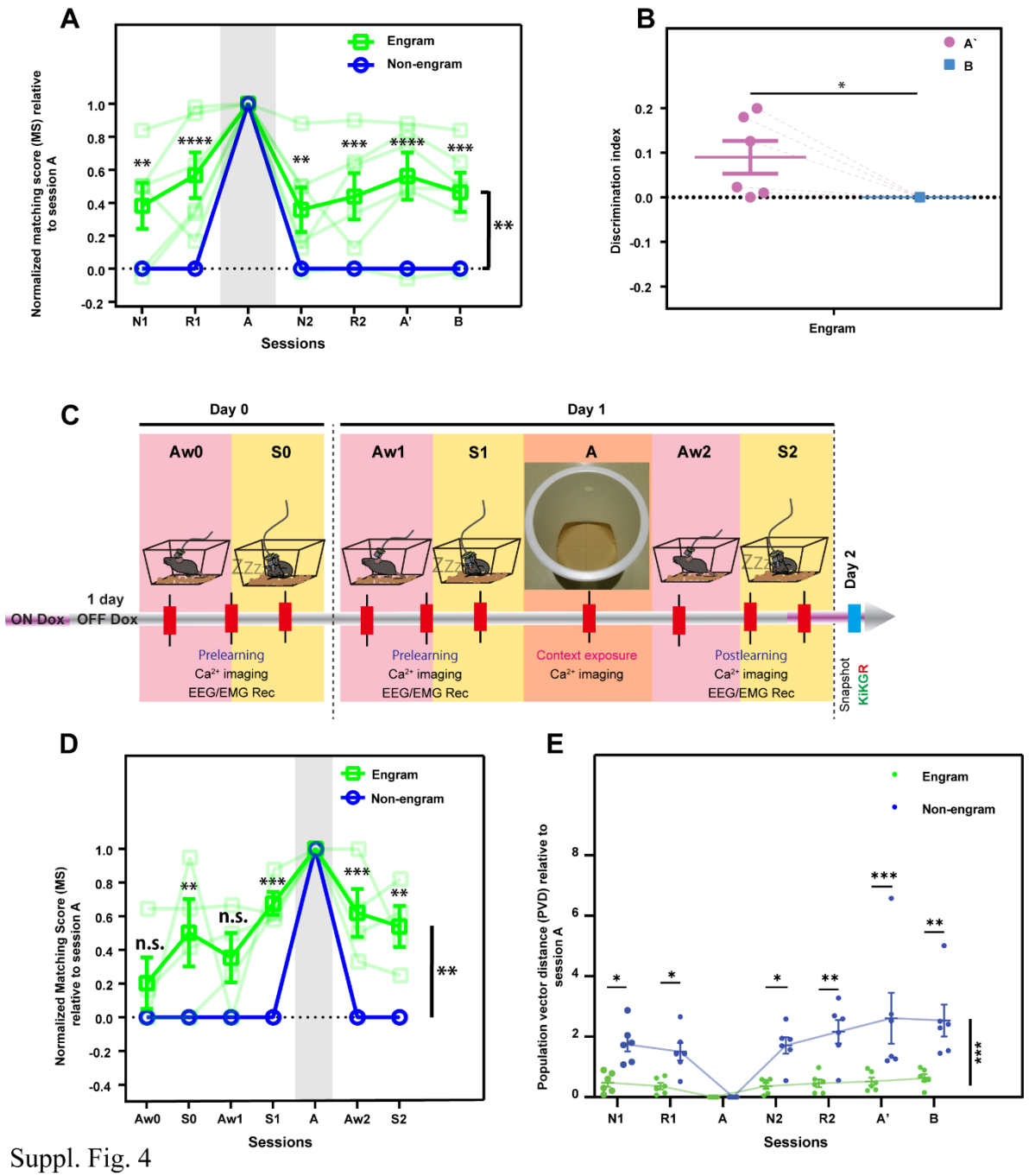
**Suppl. Fig. 3 Ensembles detected by NMF analysis across sessions.**

(A-B) Illustrative diagram of the non-negative matrix factorization (NMF) analysis (A), and the relative magnitude of each neuron participating in that ensemble and the activity of that ensemble across time (B).

(C) Calculating *p*-values in each session at several dot product thresholds (θ) from 0.1 till 0.9. Different colored lines correspond to the difference of p values between the matching scores between engram and non-engram ensembles of each designated session. Using paired t-test between engram and non-engram cells.

(D-G) Representative ensembles detected by the NMF analysis showing the neurons participating in that ensemble (top) and their relative intensity in different sessions; R1 (D), A (E), R2 (F), and A` (G) (middle), and their relative intensities across all the sessions (bottom).

The Experiment was independently repeated six times.


A
Engram
Non-engram
Normalized matching score (MS) relative to session A
Sessions
N1
R1
A
N2
R2
A'
B
B
A`
B
Discrimination index
Engram
C
Day 0
Day 1
Aw0
S0
Aw1
S1
A
Aw2
S2
1 day
ON Dox
OFF Dox
Prelearning
Ca2+ imaging
EEG/EMG Rec
Context exposure
Ca2+ imaging
Postlearning
Ca2+ imaging
EEG/EMG Rec
Day 2
Snapshot
KIKGR
D
Engram
Non-engram
Normalized Matching Score (MS) relative to session A
n.s.
n.s.
Aw0
S0
Aw1
S1
A
Aw2
S2
Sessions
E
Engram
Non-engram
Population vector distance (PVD) relative to session A
N1
R1
A
N2
R2
A'
B
Sessions

Suppl. Fig. 4

**Supp. Fig. 4 Sleep but not awake activity is more correlated to next learning event.**

(A) MS analysis in reference to session A (same as Fig.1D but with using same number of neurons to run the analysis). n = 6 mice.

(B) Discrimination index between sessions A' and B for engram cells, n = 6 mice. ($Discrimination\ ratio = \frac{MS\ (A\prime)-MS\ (B)}{MS\ (A\prime)+MS\ (B)}$).

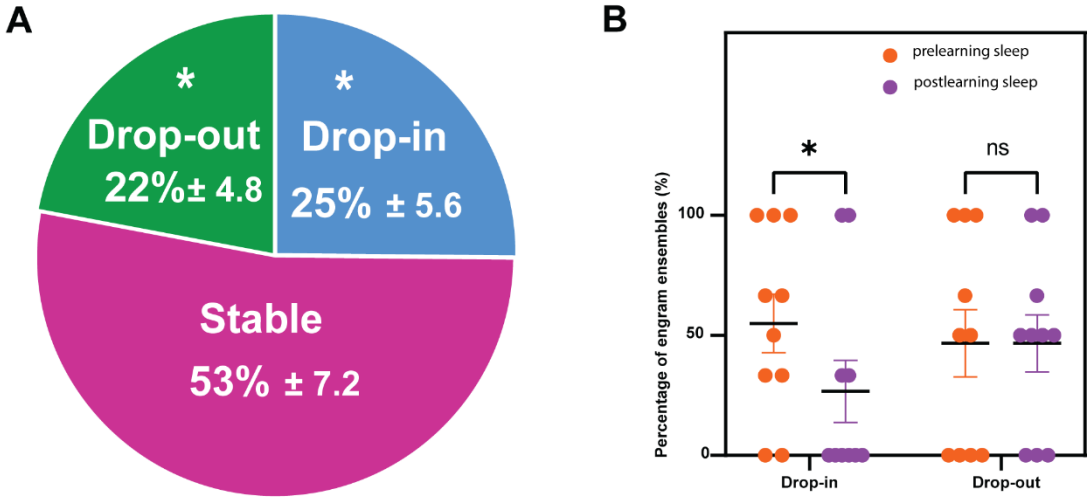
(C) Experimental design for calcium imaging and awake, sleep behavior paradigm. Awake (Aw0) and sleep (S0) recorded session at 1 day before learning (Day 0), and then awake (Aw1) and sleep (S1) in prelearning sleep sessions, then learning (A), and followed by postlearning awake (Aw2) and sleep sessions (S2) on day 1. On day 2, a snapshot for KikGR is taken.

(D) Normalized MS analysis in reference to session A for both engram (green) and nonengram (blue) cells across sessions. n = 4 mice.

(E) Population vector distance (PVD) analysis in reference to session A for both engram (green) and nonengram (blue) cells across sessions. n = 6 mice.

**p < 0.01, ***p < 0.001, ****p < 0.0001, n.s., nonsignificant. Data represent the mean ± s.e.m. Statistical comparisons were made using two-way repeated-measures ANOVA with Bonferroni's multiple comparisons test (A), paired t test one-tailed (B), two-way repeated-measures ANOVA with Bonferroni's multiple comparisons test (D), two-way repeated-measures ANOVA with Holm Sidak's multiple comparisons test (E). The Experiment was

independently repeated four times. Source data are provided as a Source Data file. Detailed statistics are shown in Supplementary Data 1.


A
*
Drop-out
22%± 4.8
*
Drop-in
25% ± 5.6
Stable
53% ± 7.2
B
prelearning sleep
postlearning sleep
*
ns
Percentage of engram ensembles (%)
100
50
0
Drop-in
Drop-out

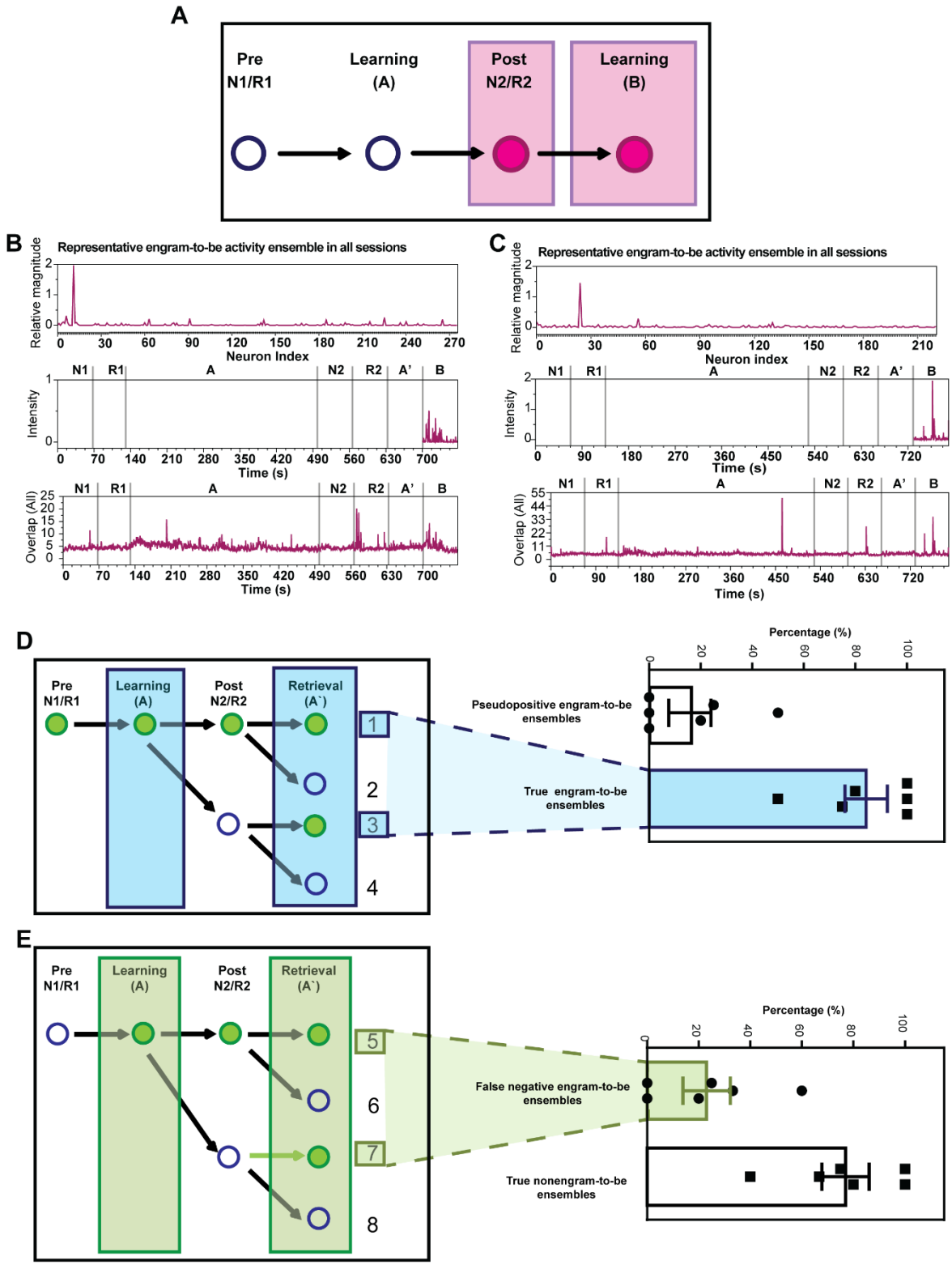
Suppl. Fig. 5

**Suppl. Fig. 5 Engram ensembles dynamics across memory processing stages**

(A) Percentage of drop-out, drop-in and stable engram ensembles across sessions, n = 10 mice.

(B) Percentage prelearning and postlearning sleep ensembles from drop-in and drop-out engram ensembles. n = 10 mice.

*$p < 0.05$, n.s., nonsignificant. Data represent the mean ± s.e.m. Statistical comparisons were made using two-way repeated-measures ANOVA with Holm Sidak's multiple comparisons test (A), paired t test two-tailed (B). The Experiment was independently repeated ten times. Source data are provided as a Source Data file. Detailed statistics are shown in Supplementary Data 1.


A
Pre
N1/R1
Learning
(A)
Post
N2/R2
Learning
(B)
B
Representative engram-to-be activity ensemble in all sessions
Relative magnitude
Neuron Index
Intensity
Time (s)
Overlap (All)
N1
R1
A
N2
R2
A'
B
C
Representative engram-to-be activity ensemble in all sessions
Relative magnitude
Neuron index
Intensity
Time (s)
Overlap (All)
D
Pre
N1/R1
Learning
(A)
Post
N2/R2
Retrieval
(A`)
1
2
3
4
Percentage (%)
Pseudopositive engram-to-be ensembles
True engram-to-be ensembles
E
Pre
N1/R1
Learning
(A)
Post
N2/R2
Retrieval
(A`)
5
6
7
8
Percentage (%)
False negative engram-to-be ensembles
True nonengram-to-be ensembles

Suppl. Fig. 6

**Suppl. Fig. 6 Engram ensemble activity across sessions**

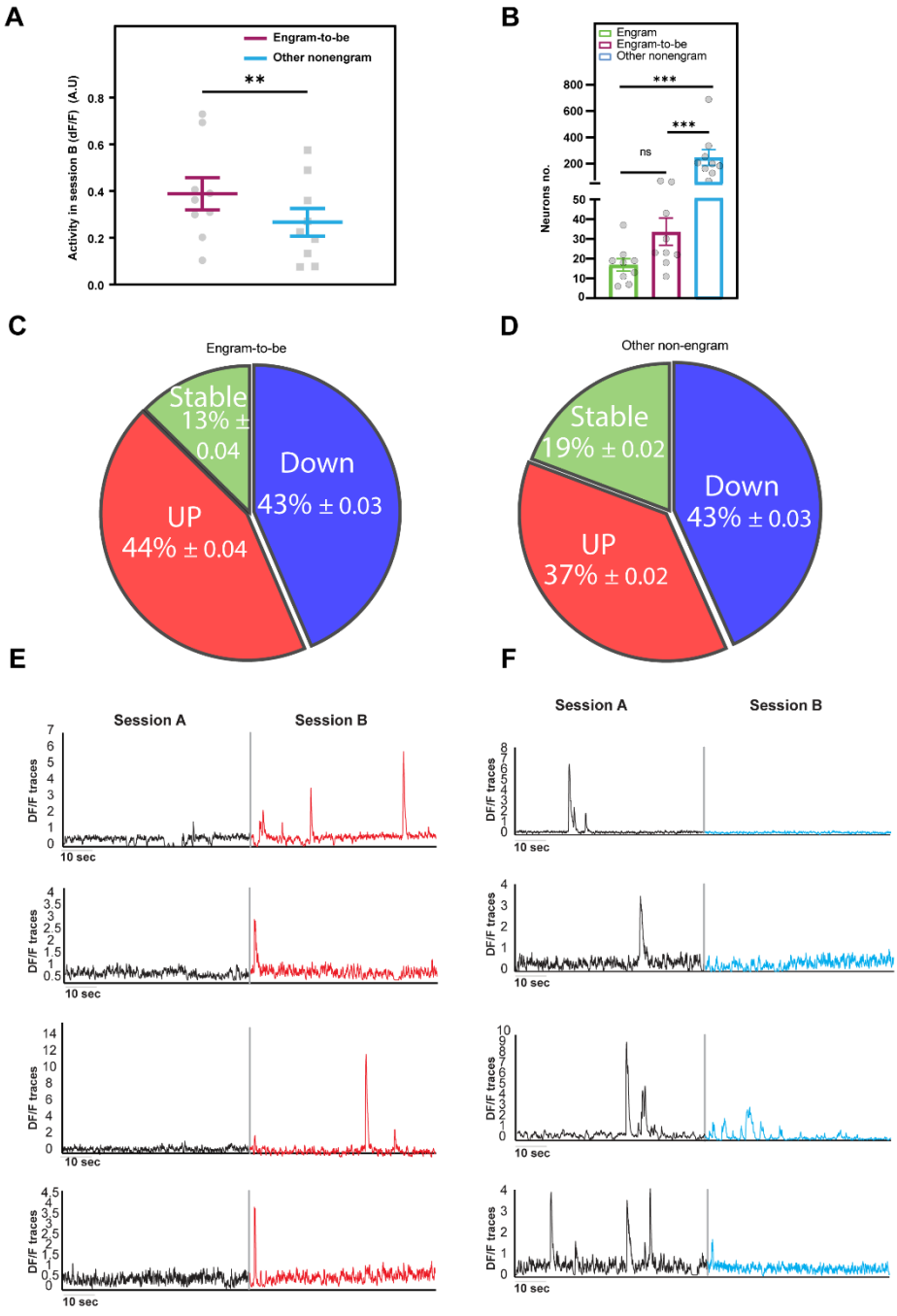
(A) Schematic diagram showing engram-to-be ensemble activities across sessions.

(B and C) Representative engram-to-be ensembles detected by NMF analysis showing the neurons participating in that ensemble (top), their relative intensity in session B (middle), and their relative intensities across all sessions (bottom).

(D) Left, schematic diagram showing all possible engram-to-be ensembles according to the criteria, in a similar manner to Figure 2. Right, percentages of true engram-to-be ensembles that were preactivated and retrieved (patterns 1 and 3), and pseudopositive engram-to-be ensembles that were preactivated and not retrieved (patterns 2 and 4). Ensemble percentages for each category were calculated as follows: (True= (1+3)/ (1+2+3+4)), (Pseudo-positive= (2+4)/ (1+2+3+4)), n = 6 mice.

(E) Left, schematic diagram showing all possible ensembles assigned as non-engram-to-be ensembles. Right, percentage of false-negative engram-to-be ensembles (true engram-to-be ensembles) that were not preactivated but activated during learning and retrieval (patterns 5 and 7), and true nonengram-to-be ensembles not preactivated and not retrieved (patterns 6 and 8). False negative= (5+7)/ (1+3+5+7), True negative= (6+8)/ (2+4+6+8), n = 6 mice.

Data represent the mean ± s.e.m. The Experiment was independently repeated six times.


A
Engram-to-be
Other nonengram
**
Activity in session B (dF/F) (A.U)
0.0
0.2
0.4
0.6
0.8
B
Engram
Engram-to-be
Other nonengram
***
***
ns
Neurons no.
0
10
20
30
40
50
200
400
600
800
C
Engram-to-be
Stable
13% ± 0.04
Down
43% ± 0.03
UP
44% ± 0.04
D
Other non-engram
Stable
19% ± 0.02
Down
43% ± 0.03
UP
37% ± 0.02
E
Session A
Session B
DF/F traces
10 sec
F
Session A
Session B
DF/F traces
10 sec

Suppl. Fig. 7

**Suppl. Fig. 7 The engram-to-be population showing higher activity in session B than in session A**

(A) Basal activity of engram-to-be and other nonengram in session B. n = 9 mice.

(B) The number of neurons in engram (green), engram-to-be (magenta), and other nonengram cells (cyan). Statistical comparisons were made using one-way ANOVA, n = 9 mice.

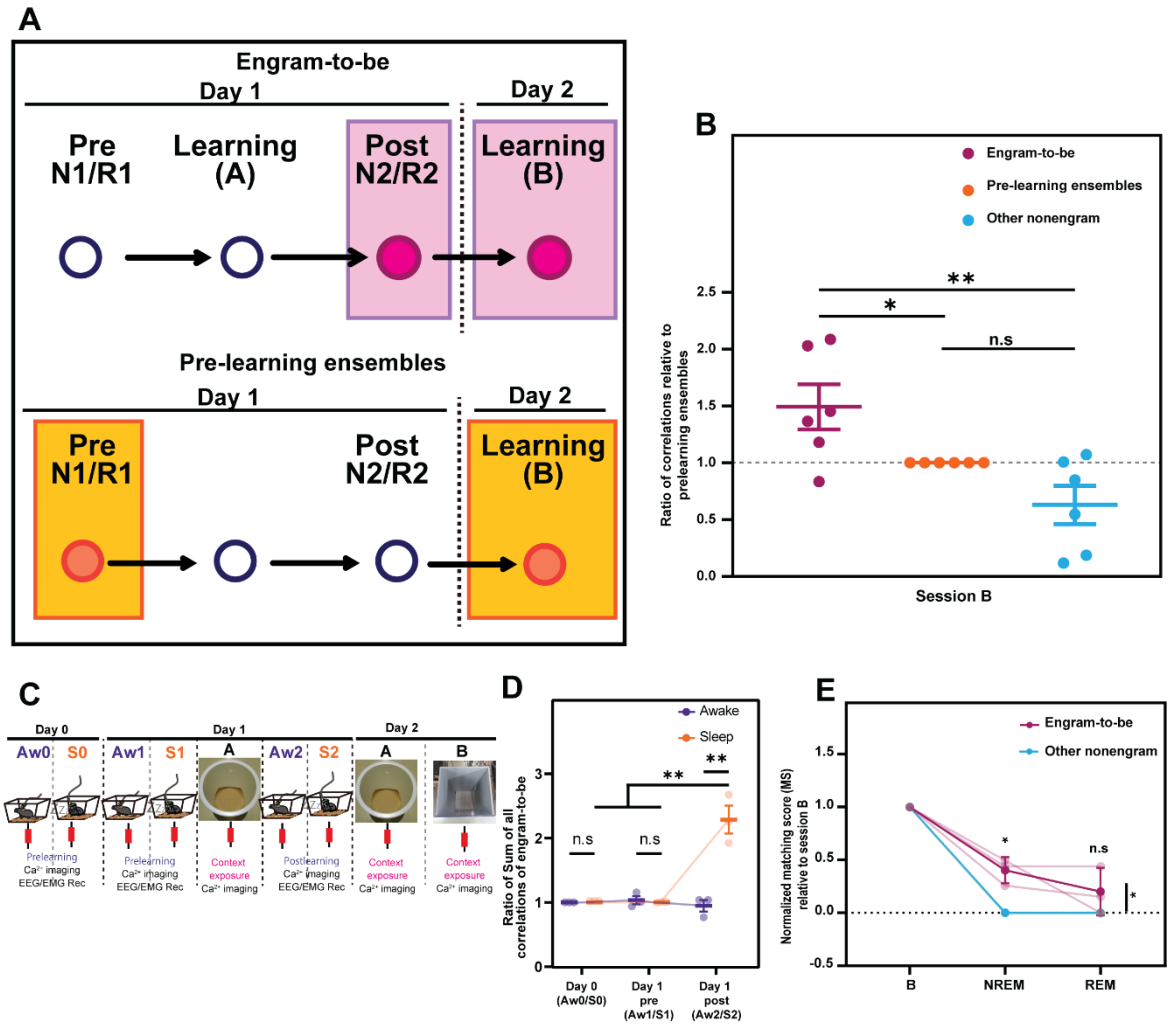
(C) Percentage of engram-to-be cells showing a significant increase in their activity in session B compared with session A as the up population, cells showing a significant decrease as the down population, and cells that did not show a significant change as the stable population, shown in red, blue, and green, respectively.

(D) Percentage of other nonengram cells showing a significant increase in their activity in session B compared with session A as the up population, cells showing a significant decrease as the down population, and cells that did not show a significant change as the stable population, shown in red, blue, and green, respectively.

(E) Representative traces of engram-to-be cells from the up population showing a significant increase in their activity in session B compared with session A.

(F) Representative traces of other nonengram cells from the down population showing a significant decrease in their activity in session B compared with session A.

**$p < 0.01$, ***$p < 0.001$, n.s., nonsignificant. Data represent the mean ± s.e.m. Statistical comparisons were made using paired t test two-tailed (A), one way ANOVA with Tukey's multiple comparisons test (B). The Experiment was independently repeated six times. Source data are provided as a Source Data file. Detailed statistics are shown in Supplementary Data


A
Engram-to-be
Day 1
Day 2
Pre
N1/R1
Learning
(A)
Post
N2/R2
Learning
(B)
Pre-learning ensembles
Day 1
Day 2
Pre
N1/R1
Post
N2/R2
Learning
(B)
B
Engram-to-be
Pre-learning ensembles
Other nonengram
Ratio of correlations relative to prelearning ensembles
Session B
n.s
C
Day 0
Day 1
Day 2
Aw0
S0
Aw1
S1
A
Aw2
S2
A
B
Prelearning
Postlearning
Context exposure
D
Awake
Sleep
Ratio of Sum of all correlations of engram-to-be
n.s
Day 0 (Aw0/S0)
Day 1 pre (Aw1/S1)
Day 1 post (Aw2/S2)
E
Engram-to-be
Other nonengram
Normalized matching score (MS) relative to session B
n.s
B
NREM
REM

Suppl. Fig. 8

**Suppl. Fig. 8 Engram-to-be cells correlated activity in pre- and post-session B sleep.**

(A) Schematic diagram showing engram-to-be (top) and prelearning (bottom) ensemble activities across sessions.

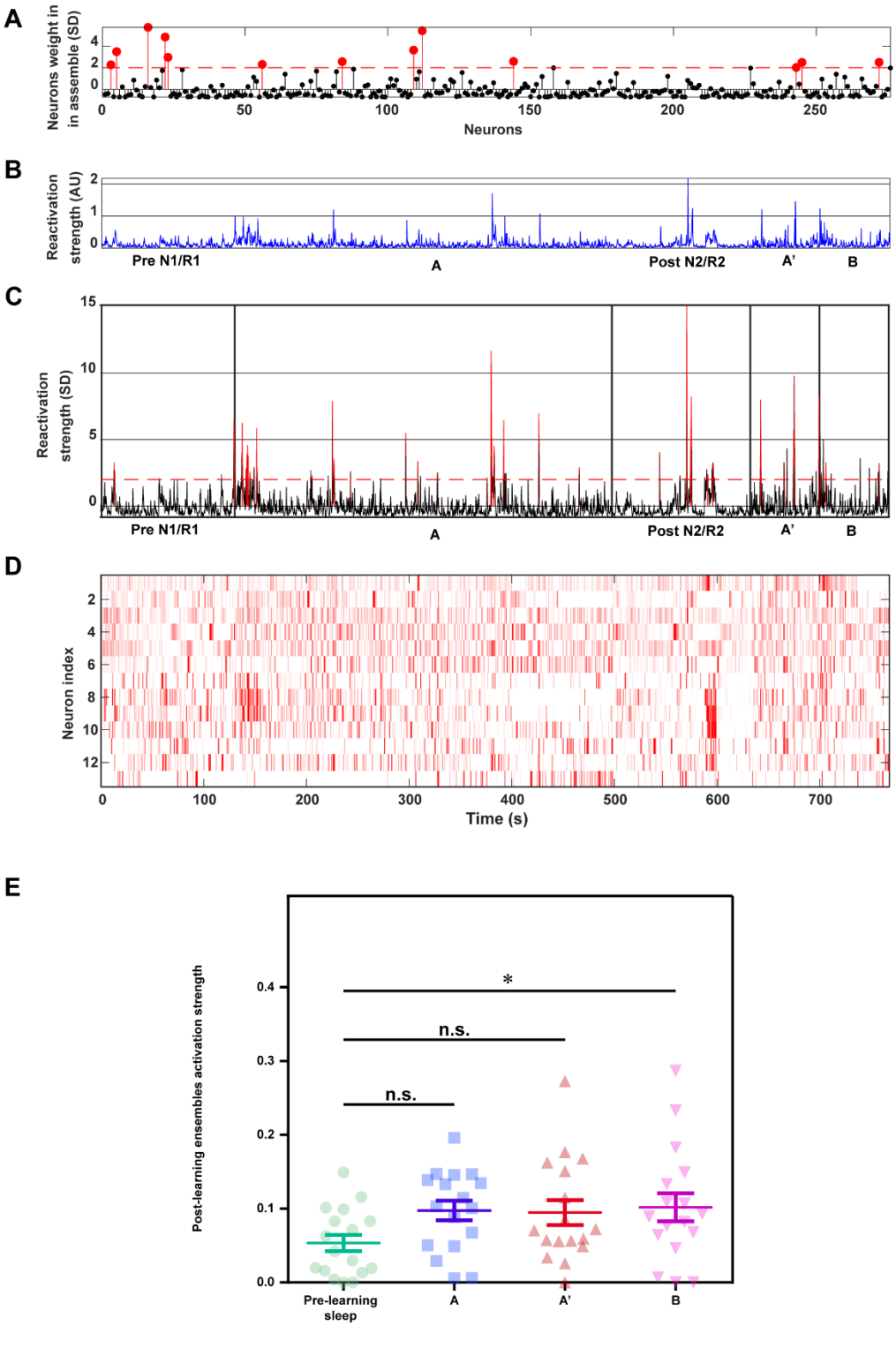
(B) Ratio of sum of all correlations (Mtot(t)) for engram-to-be and prelearning in session B. n = 6 mice.

(C) Experimental schedule for calcium imaging during awake, sleep behavior paradigm.

(D) Ratio of sum of all correlations (Mtot(t)) for engram-to-be in Day 0 (Aw0 and S0), Day1 pre (Aw1 and S1) and Day2 post (Aw2 and S2) compared to their respective Day 0, n = 3 mice.

(E) Matching score (MS) analysis in reference to session B. n = 3 mice.

*p < 0.05, **p <0.01, n.s., nonsignificant. Data represents the mean ± s.e.m. Statistical comparisons were made using using one-way repeated-measures ANOVA with Holm-Sidak's multiple comparisons test (B), repeated measure Two-way ANOVA with Sidak's multiple comparisons test (D; between groups) and Tukey's multiple comparisons test (D; within group), repeated measure Two-way ANOVA with Bonferroni's multiple comparisons test (E). The Experiment was independently repeated three times. Source data are provided as a Source Data file. Detailed statistics are shown in Supplementary Data 1.


A
Neurons weight in in assemble (SD)
Neurons
B
Reactivation strength (AU)
Pre N1/R1
A
Post N2/R2
A'
B
C
Reactivation strength (SD)
Pre N1/R1
A
Post N2/R2
A'
B
D
Neuron index
Time (s)
E
Post-learning ensembles activation strength
*
n.s.
n.s.
Pre-learning sleep
A
A'
B

Suppl. Fig. 9

**Suppl. Fig. 9 PCA-ICA analysis shows higher activation strength of postlearning sleep ensembles during session B.**

(A) Weight of neurons participating in a representative ensemble, red dots are significant participating neurons (>2SD), red dashed linerepresents threshold (2SD).

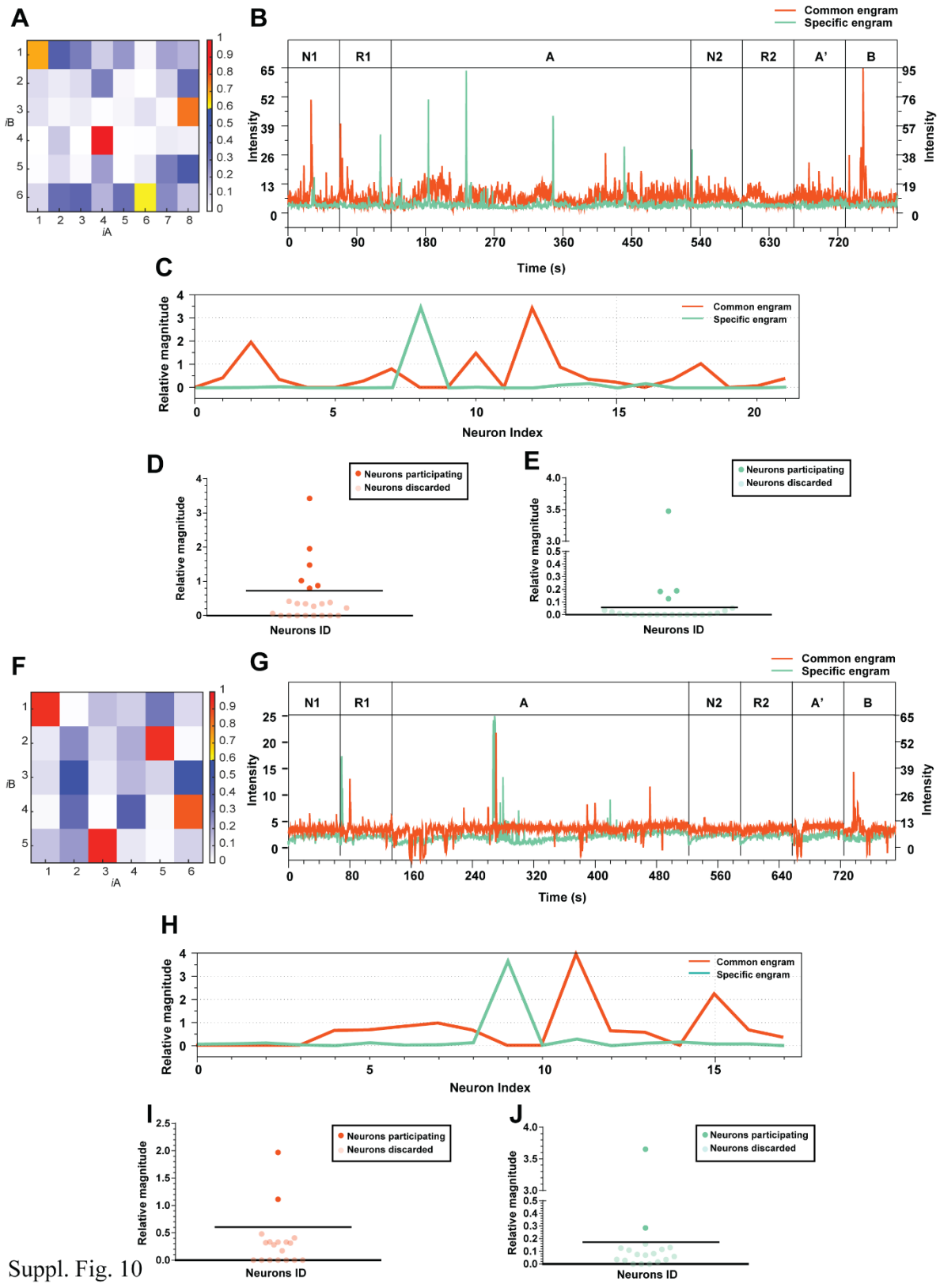
(B) Reactivation strength (AU) of a given representative ensemble in sessions prelearning sleep, A, A', postlearning sleep and B.

(C) Reactivation strength (SD) across the whole sessions, where red dashed line indicates threshold for activity (2SD) in sessions prelearning sleep, A, A', postlearning sleep and B.

(D) Activities of the 13 neurons participating in the ensemble (z-score) across time.

(E) Average of activation strength of all detected in ensembles in postlearning sleep in other sessions., N = 6 mice, n = 17 patterns.

*$p < 0.05$, n.s., nonsignificant. Data represent the mean ± s.e.m. Statistical comparisons were made using one-way RM ANOVA with Dunnett's multiple comparisons test (E). The Experiment was independently repeated six times. Source data are provided as a Source Data file. Detailed statistics are shown in Supplementary Data 1.


A
B
Common engram
Specific engram
N1
R1
A
N2
R2
A'
B
Intensity
Time (s)
C
Relative magnitude
Neuron Index
D
E
Neurons participating
Neurons discarded
Neurons ID
F
G
H
I
J

Suppl. Fig. 10

**Suppl. Fig. 10 Common and specific engram ensemble activity in all sessions.**

(A) Dot product analysis (*i*) of NMF-extracted ensembles in engram cells in sessions A and B.

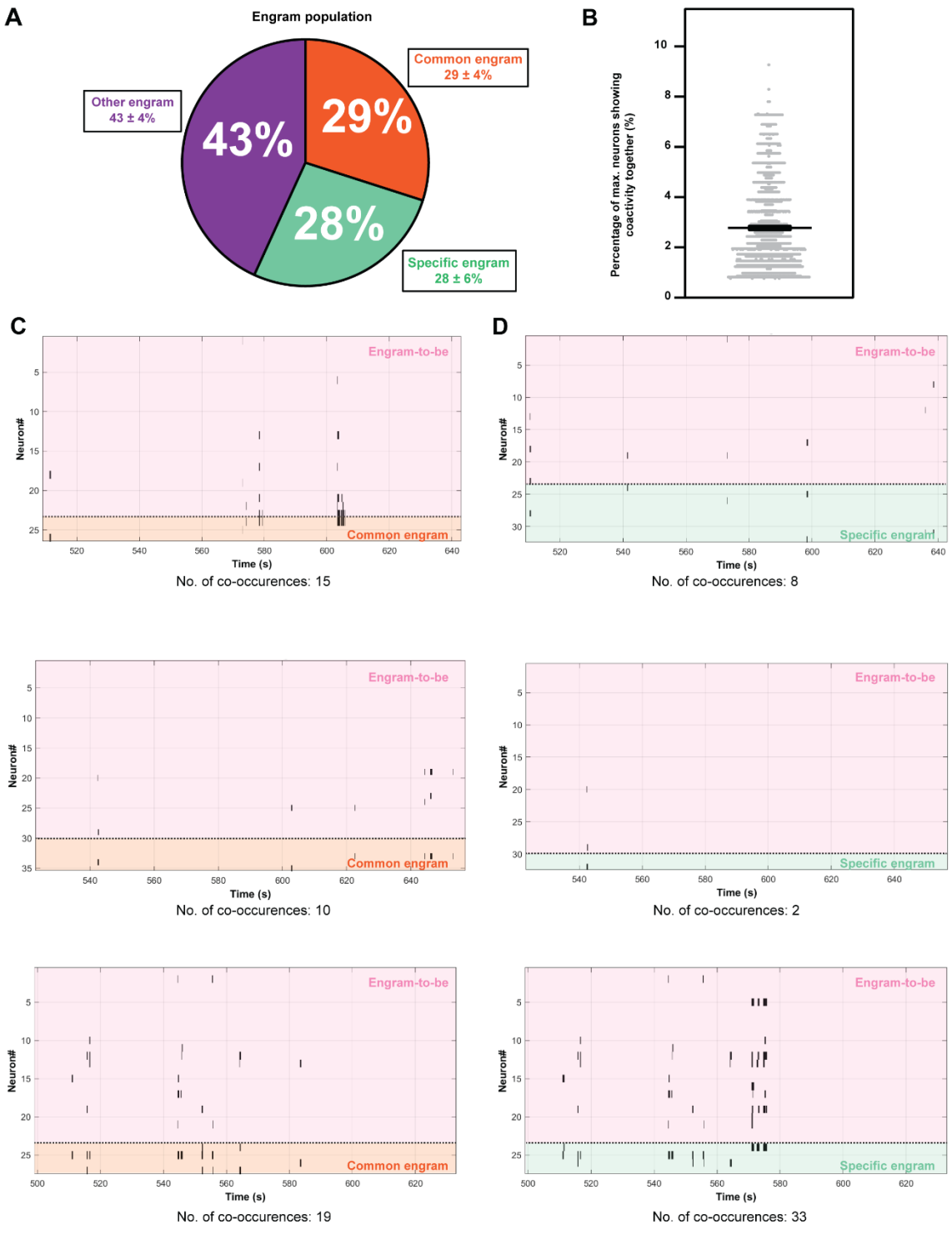
(B) Representative ensemble of common engrams (orange) and specific engrams (light green) across all sessions. The intensity of common engram ensembles is represented on the primary y-axis, and the intensity of specific engram ensembles is represented on the secondary y-axis.

(C) Relative magnitude of all neurons participating in common and specific engram ensembles, shown in orange and light green, respectively.

(D & E) Graph showing actively participating neurons in common (orange) and specific (light green) engram ensembles created by showing those neurons having activity above double the median of the ensemble activity. The line represents double the median.

(F-J) Same as (A-D) from a different subject.

Data represent the mean ± s.e.m. The Experiment was independently repeated five times.


A
Engram population
Common engram
29 ± 4%
Other engram
43 ± 4%
Specific engram
28 ± 6%
43%
29%
28%
B
Percentage of max. neurons showing coactivity together (%)
C
D
Engram-to-be
Common engram
Specific engram
Neuron#
Time (s)
No. of co-occurences: 15
No. of co-occurences: 8
No. of co-occurences: 10
No. of co-occurences: 2
No. of co-occurences: 19
No. of co-occurences: 33

Suppl. Fig. 11

**Suppl. Fig. 11 Engram-to-be co-occurrences with common and specific engram populations during postlearning sleep (N2 & R2) sessions.**

(A) Classification of engram population into common (29%), specific (28%) or other (43%) engram, n = 5 mice.

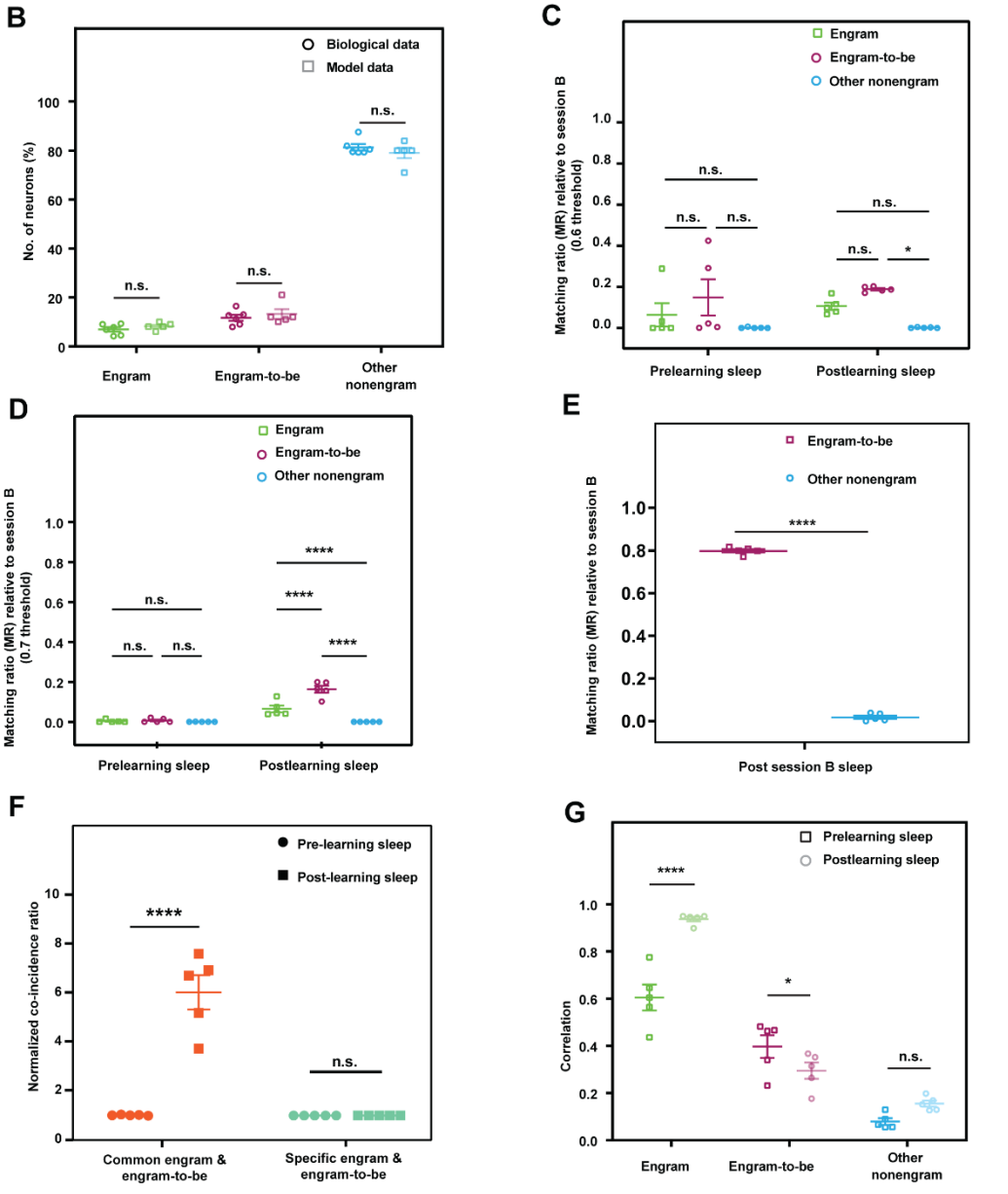
(B) Scatter plot showing the percentage of maximum number of neurons to show coactivity in a single time unit.

(C-D) Representative images of number of co-occurrences between engram-to-be with common engram (left) and engram-to-be with specific engram (right) during postlearning sleep sessions (NREM and REM). Each tick at certain time point in both 2 populations is counted as a single co-occurrence (top, middle, and bottom plots are representative examples from different subjects).

Data represent the mean ± s.e.m. The Experiment was independently repeated five times.

A

| | | Response to pattern A | Response to pattern B |
|---|---|---|---|
| Engram | Common engram | Active | Active |
| | Specific engram | Active | Inactive |
| Non engram | Engram-to-be | Inactive | Active |
| | Other nonengram | Inactive | Inactive |




Suppl. Fig. 12

**Suppl. Fig. 12 The neural mathematical model follows the biological data.**

(A) Classification of cell criteria in the neural model data in reference to the biological data.

(B) Number of neurons in each cell category in both the biological data (dark colored) and neural model data (light colored). Biological data (n = 6 mice) and model data (n = 5 simulations).

(C) The matching ratio relative to session B between engram, engram-to-be and other nonengram cells (0.6 threshold). n = 5 simulations.

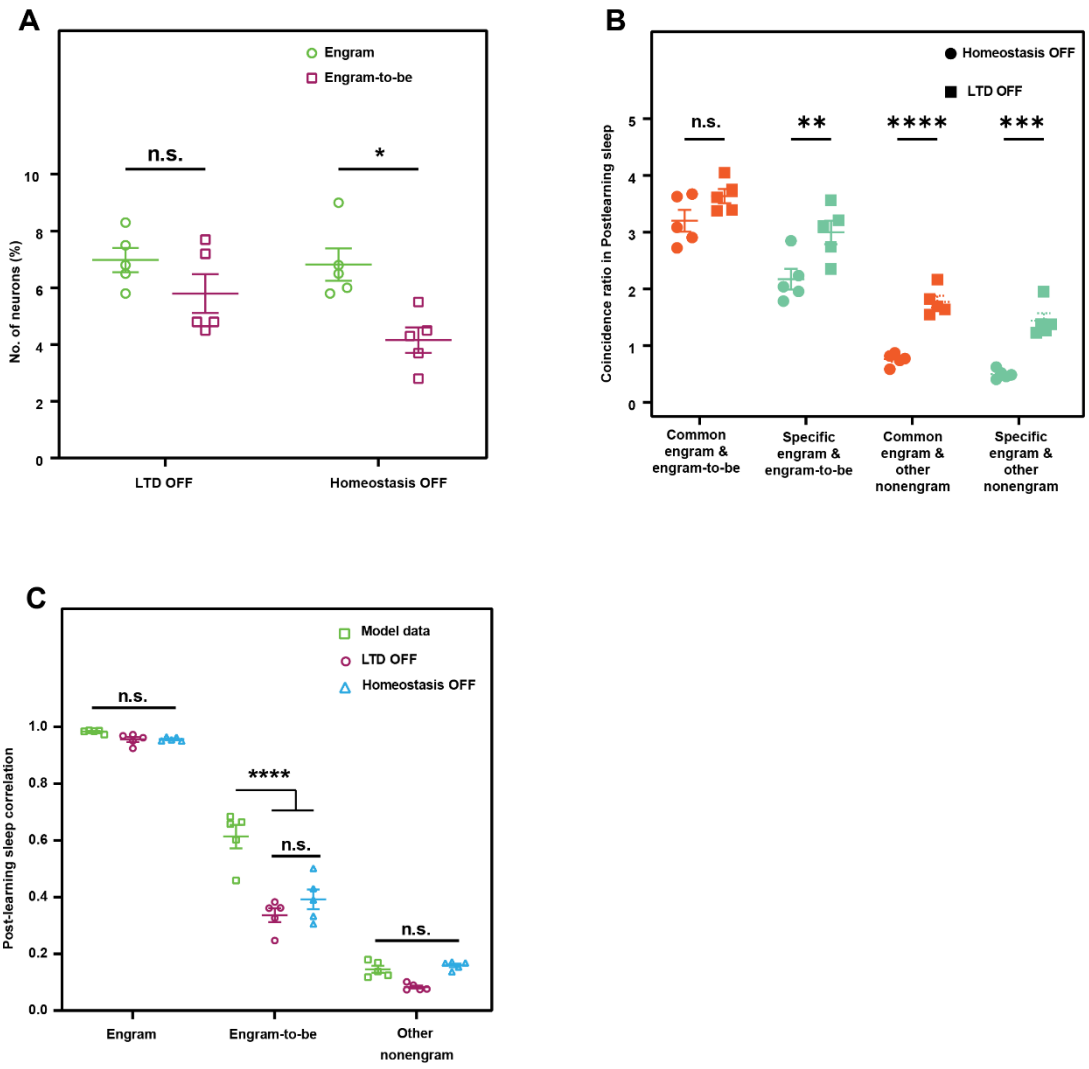
(D) The matching ratio relative to session B between engram, engram-to-be and other nonengram cells (0.7 threshold). n = 5 simulations.

(E) The matching ratio in post session B sleep between engram-to-be and other nonengram cells. n = 5 simulations.

(F) Normalized coincidence ratio during pre and postlearning sleep. n = 5 simulations.

(G) Pearson correlation in the sleep plasticity OFF model in pre- (light colored) and postlearning sleep (dark colored) in engram cells (green), engram-to-be cells (pink), and other nonengrams (light blue). n = 5 simulations.

*$p < 0.05$, ****$p < 0.0001$, n.s., nonsignificant. Data represent the mean ± s.e.m. Statistical comparisons were made using two-way ANOVA with Sidak's (B), Bonferroni's (C) and Tukey's (D) multiple comparisons test, unpaired t test two-tailed (E), repeated measures two-way ANOVA with Bonferroni's (F) and Sidak's (G) comparison test. The Experiment was independently repeated five times. Source data are provided as a Source Data file. Detailed statistics are shown in Supplementary Data 1.


A
Engram
Engram-to-be
n.s.
*
No. of neurons (%)
LTD OFF
Homeostasis OFF
B
Homeostasis OFF
LTD OFF
n.s.
**
****
***
Coincidence ratio in Postlearning sleep
Common engram & engram-to-be
Specific engram & engram-to-be
Common engram & other nonengram
Specific engram & other nonengram
C
Model data
LTD OFF
Homeostasis OFF
n.s.
****
n.s.
n.s.
Post-learning sleep correlation
Engram
Engram-to-be
Other nonengram

Suppl. Fig. 13

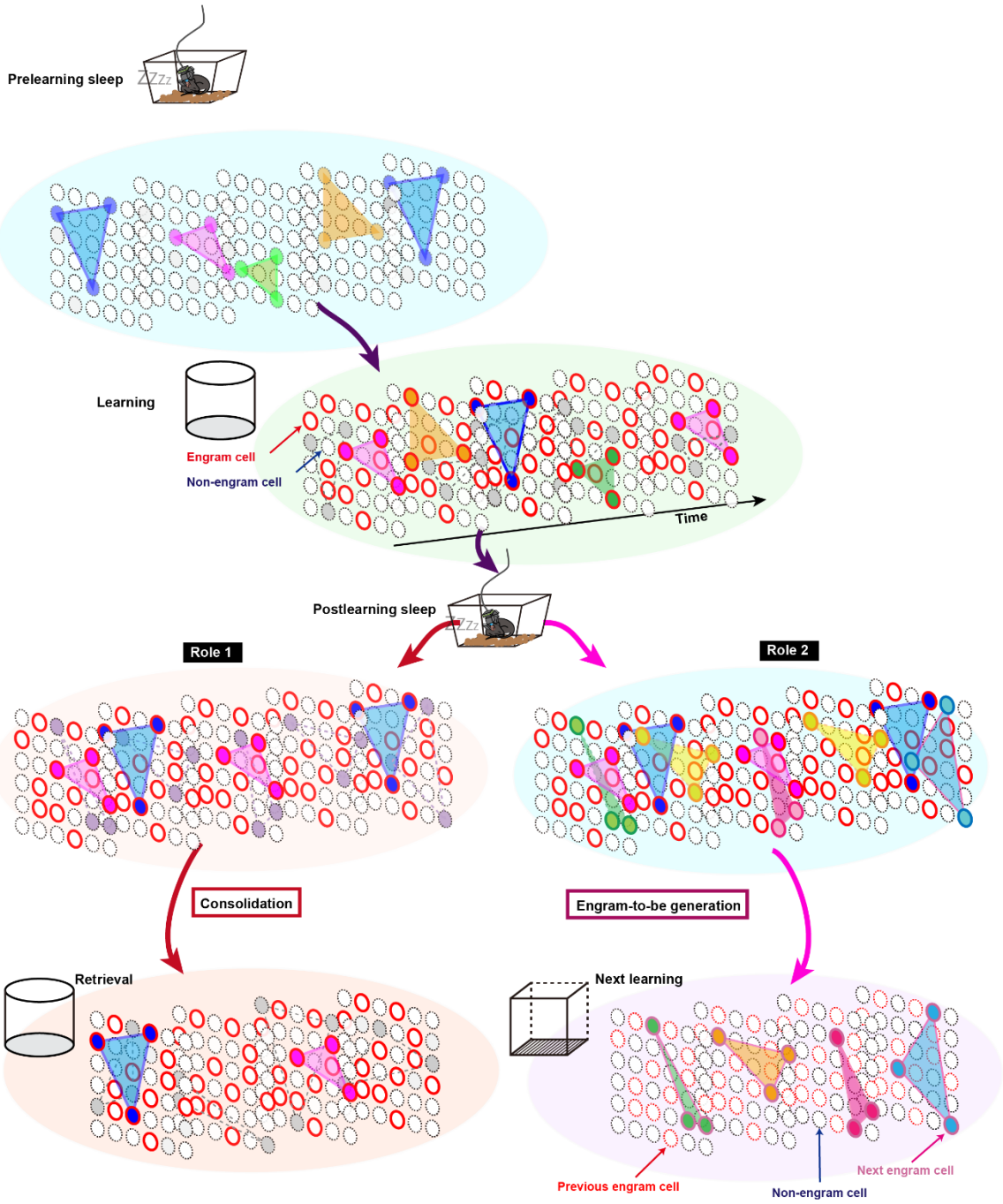
**Suppl. Fig. 13 Synaptic depression and homeostatic plasticity play different roles for engram-to-be ensembles emergence and activity.**

(A) Number of neurons in engram and engram-to-be in both synaptic depression OFF (LTD OFF) and homeostatic plasticity OFF (homeostasis OFF). Statistical comparisons were made between LTD OFF and homeostasis OFF, n = 5 simulations.

(B) Normalized coincidence ratio during postlearning sleep in the LTD OFF and homeostasis OFF model, n = 5 simulations.

(C) Comparing the pairwise correlations within groups (engram, engram-to-be, and other nonengram) computed through the normal model parameters, LTD OFF and homeostasis OFF model, n = 5 simulations.

*p < 0.05, **p< 0.01, ***p<0.001, ****p < 0.0001, n.s., nonsignificant. Data represent the mean ± s.e.m. Statistical comparisons were made two-way ANOVA with Sidak's (A, C) and Bonferroni's (B) and comparison test. The Experiment was independently repeated five times. Source data are provided as a Source Data file. Detailed statistics are shown in Supplementary Data 1.


Prelearning sleep
ZZzz
Learning
Engram cell
Non-engram cell
Time
Postlearning sleep
ZZzz
Role 1
Role 2
Consolidation
Engram-to-be generation
Retrieval
Next learning
Previous engram cell
Non-engram cell
Next engram cell

Suppl. Fig. 14

**Suppl. Fig. 14 Schematic model showing ensembles activities across memory processing stages.**

Ensembles organized and activated in a synchronous manner during prelearning sleep preactivated ensembles are later recruited in upcoming engram. Cell population consisting of engram (red circles) and nonengram cells (dotted blue circles). During postlearning sleep, there are two simultaneous processes occurring; engram ensembles activated during learning are then consolidated and later reactivated during retrieval (Role 1). Synaptic plasticity mechanisms, such as synaptic scaling and synaptic depression (LTD) during sharp wave ripples, lead to emergence of new ensembles that are coactivated with previous engram cells as a mechanism for preparing future engram cells for next learning (Role 2). Cell population after a given learning experience consisting of previous engram (dotted red circles), nonengram (dotted blue circles) and next engram cells (pink circles).

| ID/ Cell type | Engram | Engram-to-be | Other non-engram | Total | Data are used for |
|---|---|---|---|---|---|
| **ID 1** | 19 | 23 | 163 | 205 | Fig. 1E, Fig. 2B, C, Fig. 3C, D, E, Fig.4 C-F, S Fig.1 B-D, S Fig.2 B-H, S Fig. 3C, S Fig.4A, B, E, S Fig. 5, S Fig. 6D, E, SFig8B, S Fig. 9E, S Fig 11, S Fig 12B |
| **ID 2** | 18 | 30 | 183 | 231 | Fig. 1E, Fig. 2B & C, Fig. 3C, D, E, Fig.4A, C-F, S Fig.2 B-H, S Fig. 3C, S Fig. 5D, E, S Fig.4A, B, E, S Fig. 5, SFig8B, S Fig. 9E, S Fig 10F-J, S Fig 11, S Fig 12B |
| **ID 3** | 22 | 22 | 199 | 243 | Fig. 1E, Fig. 2B, C, Fig. 3C, D, E, Fig. 4 C-F, S Fig.2 B-H , S Fig. 3C, S Fig.4A, B, E, S Fig. 5, S Fig. 6C-E, SFig8B, S Fig. 9E, S Fig 10A-E, S Fig 11B, S Fig 12B |
| **ID 4** | 11 | 43 | 207 | 261 | Fig.1E, Fig. 2B, C, Fig. 3C, D, E, S Fig.2 B-H , S Fig. 3C, S Fig.4A, B, E, S Fig. 5, S Fig. 6D, E, S Fig. 7E, F, SFig8B S Fig. 9E, S Fig 11B, S Fig 12B |
| **ID 5** | 6 | 11 | 70 | 87 | Fig.1E, Fig. 2B, C, Fig. 3C, D, E, S Fig.2 B-F , S Fig. 3C, S Fig.4A, B, E, S Fig. 5, S Fig. 6D, E, SFig8B, S Fig. 9E, S Fig 12B |
| **ID 6** | 13 | 23 | 253 | 289 | Fig.1D, E, Fig. 2B, C, Fig. 3C-F, Fig. 4 C-F, S Fig.2 2F-H , S Fig. 3 C-G, S Fig.4A, B, E, S Fig. 5, S Fig. 6B, D, E, SFig8B, S Fig. 9A-E |
| **ID 7** | 19 | 70 | 336 | 425 | Fig.3D-F, S Fig. 2H, S Fig.4D, S Fig. 5, S Fig.7A, B, SFig8B-E |
| **ID 8** | 37 | 314 | 688 | 1039 | Fig.3D-F, Fig.4C-F, S Fig.4D, S Fig. 5, S Fig.7A, B, SFig8B-E |
| **ID 9** | 8 | N/A | 33 | 41 | S Fig.4D, S Fig. 5 |
| **ID 10** | 7 | 18 | 129 | 154 | Fig.3D-F, S Fig.4D, S Fig. 5, S Fig.7A, B, SFig8B-E |
| **Average** | 16 | 56 | 226 | 298 | |
| **Percentage** | 7 (± 1.5) | 14 (± 2) | 79 (± 2) | 100 | |

**Supplementary Table 1: Number of engram, engram-to-be and other non-engram cells detected from animals used in this study. Data represent the mean ± s.e.m.**