Figures and figure supplements

CA1 cell activity sequences emerge after reorganization of network correlation structure during associative learning

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Figure 1. Behavior: trace eye-blink conditioning of mice. (A) Cartoon schematic of experimental system. Two-photon calcium imaging was carried out in area CA1 of the dorsal hippocampus in a head-fixed mouse which underwent trace eyeblink conditioning. A speaker (yellow, b) was used for tone stimulus delivery, while a nozzle (red, a) directed the aversive air-puff. A magnetometer (black, c) was used to monitor eyelid position in order to detect blinks. Scale bars at the bottom of the figure indicate times of stimulus delivery (yellow and red bars for tone [350 ms long] and puff [100 ms long] respectively, along with the gap in-between [250 ms]) as well as data acquisition (green bar, 15 s long) during a single trial. (B) Sample eyelid position signal traces from a mouse undergoing trace eyeblink conditioning. The color scale indicates eyelid position in arbitrary units, with high values indicating eyelid closure (blink). This mouse started reproducibly showing significant blinks before the air-puff (e.g., green arrow) mid-way through the session. (C and D) Eyelid position traces in response to pre-training tone presentation (C), and both tone and puff stimuli during trace eye-blink conditioning (D). Gray traces are from individual trials and black traces are averages across trials. Yellow and red bars at the bottom indicate times of delivery of tone and air-puff respectively. (E) Distributions of performance scores for trace (blue) and pseudo (green) conditioned mice. (F) Average performance score, which is the ratio of tone evoked significant blink (CR) rates to spontaneous blink rates, is plotted for all trace conditioned mice (blue) and pseudo-conditioned mice (green). Error bars indicate SEM. * indicates p<0.05. (G) Learning curves (gray lines) for the nine mice that learned the association to criterion. The learning trial identified for each mouse is marked with a red circle. The black curve shows average performance across mice. The vertical, dotted line indicates the mean learning trial across mice (trial 26). Each learning curve was obtained by first obtaining a binary list of significant response trials for each mouse, and then using a previously described expectation maximization algorithm to calculate the CR probability on each trial, for individual mice. The horizontal, red line indicates the probability of CRs by chance.

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Figure 2. Two-photon imaging of calcium-responses in area CA1 neurons from awake mice. (A) Schematic of the imaging preparation. o–objective lens, s–skull, cs–cover slip, hb–head bar, ag–agarose. (B) Histogram of neuron response widths in ms, calculated as the time for which a given neuron’s trial-averaged, ΔF/F trace remains above.
50% of the peak value. The red, dotted line indicates a response width of 600 ms, which is the time of interest between tone-onset and puff-onset. (C) Area CA1 cell responses show sequentially timed activity peaks after learning. Calcium response (ΔF/F) traces for six exemplar neurons from a single mouse, for sets of three trials before (panel on the left), and after (panel on the right) task learning. Neurons have been sorted as per the timing of the peak in the averaged trace. The yellow and red bars at the bottom represent the times of delivery of tone and puff respectively. The gray shading to the left covers the period of spontaneous activity prior to the onset of the tone. The red asterisks indicate the peak in each individual trace. Scale bars indicate 0.4 ΔF/F and 500 ms along the time axis. (D) Area CA1 cell activity peaks tile the entire CS-on to US-off interval. Area CA1 calcium response traces from an example dataset, sorted by the peak times of the responses. Each response trace has been averaged over all trials following the learning trial (Figure 1G), and has been normalized to the peak ΔF/F response value for each neuron. The yellow and red bars below indicate times of delivery of tone and air-puff respectively. 50% of the neurons from the field of view, with the most reliably timed responses have been shown. This is to make this plot comparable to the ones from subsequent analyses, where neurons have been similarly chosen. (E and F) Cell activity peak timings change during learning. In E, pseudo-colored ΔF/F traces for the period of interest during and after tone delivery, are plotted using data acquired during the pre-training session, where tones without air-puff were delivered. Cells have been sorted as per the timings of peaks in pre-training session data. The yellow bar at the bottom indicates time of delivery of tone (350 ms). In F, the same averaged activity traces as in E have been re-ordered according to each cell’s activity peak timing after learning has occurred, as shown in (D). Plotted in Figure 2—figure supplement 1, are panels depicting the surgical preparation, the numbers of mice from each treatment group, images of dye-loaded tissue taken at multiple depths and basic data quality control analyses. DOI: 10.7554/eLife.01982.005
Figure 2—figure supplement 1. Imaging preparation and calcium response data.
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Figure 3. After training, area CA1 cells show reliably timed, sequential calcium responses. (A) Calcium response traces for an example neuron, aligned to the time of stimulus delivery (tone and puff indicated by yellow and red bars at bottom respectively). Warmer colors in the traces indicate higher ΔF/F values. The blue rectangle on the
Figure 3. Continued

‘trial number’ axis indicates the trial averaging window comprising all trials after the learning trial. (B) The same data as in A, except with each trial’s ΔF/F trace given a random time offset. (C) Averaged calcium response curves obtained from aligned, as well as random time-offset traces. The averaged curve from time-aligned traces (blue curve) has a prominent peak, which is absent in the random time offsets case (red curve). This indicates that the neuron fires reliably at a fixed time relative to stimulus delivery. The area under the shaded region (peak ± 1 frame) was used to calculate the reliability score. (D) Area CA1 cells from trace learners show significantly higher activity-timing reliability scores. Average reliability scores for all neurons in the entire dataset for learners of the trace-conditioning task (blue), pseudo-conditioned mice (green) spontaneous activity data (red) and data from non-learners (cyan; * indicates p<0.01). (E) Change in reliability score with learning for neurons from trace conditioned (blue), pseudo-conditioned (green) and spontaneous activity data (red) respectively. Reliability of firing at the final peak response time (PT) gradually increased over the training session. Reliability scores were computed in five-trial bins. The shaded regions represent SEM. (F) Change in reliability scores between early and late blocks of training trials. The increase over the training session for trace-learners was significantly higher than for spontaneous activity data or for pseudo-conditioned mice (* indicates p<0.01). This increase was calculated by subtracting reliability scores of the first half of the session from those of the second half. (G and H) Distributions of single-cell peak response times (PT) at different stages of learning: pre-training (G) and early in training (H). The distribution shifts from one showing distinct peaks at the time of tone delivery in the pre-training stage (G), to one showing a peak at the time of the air-puff during early training (H). Only the PTs of cells with high reliability scores were included in these plots. The red and yellow bars at the bottom indicate times of delivery of tone and air puff respectively. (I) Distribution of single cell peak response times (PT) after the learning trial in trace conditioned mice. For trials after the learning trial, PT is uniformly distributed across all times in the trace interval between tone and puff. Only the PTs of cells with significant reliability scores were included in this plot. (J) Average, time-decoder performance score, pooled across all trace conditioned mice (blue), pseudo-conditioned mice (green), spontaneous background activity (red) and non-learners (cyan). Dotted line indicates chance level scores, error bars indicate SEM (* indicates p<0.01).

Figure 3—figure supplement 1, presents further characterization of the reliability score increase. A schematic explaining the time-decoder algorithm is depicted in Figure 3—figure supplement 2.

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Figure 3—figure supplement 1. Activity peak times change during learning.

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Figure 3—figure supplement 2. Time-decoder algorithm.
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- Trial Set for Time Prediction Scoring
- Trial Set for Averaged, Population Response Vectors

- Vector of Dot Products
- Calculate Dot Products

Frame 1 Frame 2 Frame 3 ...
Figure 4. Area CA1 cell noise-correlations increase transiently during training. (A) Average neuron-pair noise correlations plotted as a function of training trials for task learner mice (blue curves), pseudo-conditioned mice (green curves) and non-learners (cyan curves). Solid lines represent average noise correlations between neurons that share similar time tuning (same PT), whereas dashed lines indicate average noise correlations between random neuron pairs. Pair-wise noise correlations have been determined from spontaneous activity traces over five trial windows. Shaded regions indicate SEM. (B) Summary statistics comparing average noise correlations across early (trials 1–10), middle (trials 21–30) and late (trials 36–45) stages of training, between task learner mice (blue bars) and pseudo-conditioned mice (green bars). Solid bars represent average noise correlations between neurons that share similar time tuning (same PT) whereas hatched bars represent average noise correlations between random neuron pairs. Error bars represent SEM. ( * indicates within condition [same time-tuning v/s random cell-pairs], # indicates across conditions [trace Figure 4. Continued on next page)
learners v/s pseudo-conditioned) and @ indicates comparisons across stages of learning (early v/s middle v/s late), p<0.01; n.s. indicates not significant. Figure 4—figure supplement 1A depicts the point in the session at which behavioral CR rates, CA1 cell timing reliability and spontaneous activity correlations reach their peaks, on an individual mouse basis. It also presents further characterization of the changes in NC during learning.

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Figure 4. Continued

Figure 4—figure supplement 1. Peaks of CR rate curve, CA1 activity timing reliability and NC; characterization of NC changes during learning.

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Figure 5. Correlated neurons are spatially clustered. (A) Neurons within clusters show within-group, correlated activity. Spontaneous, $\Delta F/F$ calcium activity traces for neurons from an example dataset. Neurons are sorted as per clusters identified by meta k-means clustering. Cluster boundaries are indicated by red, dotted-lines. The color scale represents $\Delta F/F$ amplitude. A short, 6 s stretch of the traces outlined by the yellow box have been re-plotted on the right for clearer visibility. The white asterisk indicates a bout of correlated activity in a cluster. (B) Pair-wise noise correlation matrix for neurons sorted as per cluster identity, for the dataset in (A). Color scale represents the pairwise correlation coefficient during spontaneous activity (noise correlation). Boxes indicate within-cluster spontaneous activity correlations. (C) Masks of neuron ROIs for the dataset shown in A and B, color coded as per cluster identity. (D) Comparison of distributions of within cluster (gray) and across cluster (black) correlation-coefficients. The distributions are significantly different ($p<10^{-4}$). (E) The same field of view, with neuron ROIs color-coded by timing of their activity peak in post-training trials (top) and by correlation cluster number (bottom). The number of cells in the top panel is smaller as only 50% of the cells with the highest reliability scores were found to be time-tuned. (F) Correlated cell-clusters are spatially organized. Inter-cell distances or the distances between the centroids of pairs of neurons, when both belong to the same noise-correlation cluster (Intra-Cluster) or when they belong to different clusters (Inter-Cluster). Error bars indicate SEM. (*) indicates $p<10^{-4}$. Figure 5—figure supplement 1 shows that the spontaneous activity correlations between pairs of cells fall off with increasing distance between the cells being considered.

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Figure 5—figure supplement 1. Spontaneous activity correlations fall with increasing inter-cell distance.

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Figure 6. Correlated neuron-groups are spatially re-distributed during trace conditioning. (A and B) Sample trace-conditioned (A) and pseudo-conditioned (B) mouse neuron-clusters, identified in early (left), middle (center) and late (right) trials, for pre-training (top) as well as training (bottom) sessions. Neurons have been color-coded as per the noise-correlation cluster they belong to, and cluster numbers have been sorted for maximum cluster member overlap across trial-groups. (C) Spatial organization of correlated cell-clusters changes during learning. Summarized statistical testing of stability of clusters over the training session. A cluster similarity score (SS) was computed to measure the similarity between clusters from different parts of the session. Mean similarity scores for clusters of neurons from trace conditioned mice are shown in blue, pseudo-conditioned mice in green, and non-learners in cyan. Pre-training session scores are displayed as hatched bars and training session scores, solid bars. Error-bars denote SEM. The black, dotted line denotes chance level overlap score, and the red, dotted line indicates mean overlap score with perfectly overlapping clusters (* indicates p<0.01).

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Figure 7. Summary and model schematics. (A) Schematic summarizing findings: early in the session (top), neuron activity peaks are tuned to stimuli. Spontaneous activity is largely un-correlated. By the middle of the session (center), time-tuned activity during the stimulus period begins to emerge, after spontaneous activity correlations have risen. At the end of the session (bottom), stimulus-period activity is time-tuned, but spontaneous correlations have fallen back towards baseline levels. (B) Cartoon model of possible network changes occurring at the synaptic level: early in training (top), CA3 to CA1 synapses (gray circles) are at baseline strengths. As training progresses, specific synapses are strengthened (larger gray circles), towards the middle of the session (middle). At this point, neurons that share time tuning also begin to display increased noise-correlations as they receive more common input. Late in the training session (bottom), after repeated re-organization of the network, synapses undergo homeostatic normalization (gray circles reduced in size), thus causing total common input and spontaneous activity correlations to drop. (C) Schematic diagram depicting network changes at the level of groups of cells. CA1 cell clusters (horizontal row at bottom of each panel; clusters are color-coded) have high correlations in spontaneous activity, receiving shared input (direction indicated with black arrows) from groups of CA3 cells that are also correlated in their spontaneous activity. These correlation-groups of cells are spatially clustered. As learning progresses, the clusters of correlated CA3 cells change, resulting in changes in the groups of correlated CA1 cells. These new groups are also spatially organized, but differ significantly from pre-training groups.

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