Specific Aims

Understanding how memories experienced across large time scales are associated together is crucial to understanding episodic memories, since the ability to relate episodes across days is essential to the formation of memory. Recently it has been demonstrated in rodents that two neutral contexts experienced close in time shared a larger proportion of neural ensemble than those experienced more distant in time. Furthermore, subsequent fear conditioning in the second context increased animals' freezing level in the first context, suggesting a transfer of fear memory retrospectively [1]. These results suggest that memories that have a small temporal distance can be linked together. However, it remains unclear what factors may affect the temporal window of memory linking. Specifically, it is unknown whether the affective value of a memory influences the extent or the symmetry of the temporal window of memory linking. For instance, it is unclear whether negative valence of a memory could extend the time window within which it may be linked to a previous memory. Moreover, it is not clear whether a negative memory could link forward with a memory that happens later in time, and whether such prospective memory linking has a similar time window as retrospective memory linking (*i.e* whether the time window is symmetric regarding the temporal order of memories). Thus, the main goal of this proposal is to study how affective value and temporal order affect the time window of memory linking, with both behavioral experiments and calcium imaging in behaving animals.

It has been shown that two neutral contexts can be linked together when they are separated by 5 hours, but not when they are separated by 2 days. We have preliminary results suggesting that negative-valued context can be linked with a neutral context 2 days ago, and they have larger proportion of overlapping ensemble cells comparing to two neutral contexts. Thus, our first hypothesis is that negative emotional value extend the temporal window of memory linking retrospectively. On the other hand, we have preliminary results showing that a negative-valued context can link both backward and forward with a neutral context across 5 hours time interval, but can only link backward, but not forward, across 1 day and 2 days interval. Thus, our second hypothesis is that negative-valued memory have a longer retrospective memory linking window compared to prospective linking window. To test these hypothesis, we will carry out behavior experiments utilizing contextual fear conditioning, as well as *in vivo* calcium imaging in freely moving animals.

In addition, the analysis of neural dynamic in memory linking experiments have been limited to comparing the number of overlapping active ensemble cells across different sessions. Although such analysis provided a strong correlate of behavior results, it reduces time dimension to a binary, all-or-none representation, thus precluding the possibility of understanding the temporal structure of ensemble as well as the evolving nature of population coding within session. By applying dimension reduction analysis such as principal component analysis (PCA), we can uncover the underlying structures of neural ensembles for individual sessions and compare their similarities across linking memories versus non-linking memories. Our hypothesis is that the linked memories have higher similarities in ensemble structures compared to non-linked memories. To test this hypothesis, we can apply PCA analysis to calcium traces recorded at different behavior sessions. The resulting principal components can be thought of as subset of cells that exhibit highly correlated firing. We can then calculate a correlation of the components across different sessions, and compare the correlation between linking contexts with those between non-linking contexts. We predict that the correlation of structured ensemble components are higher for linking contexts comparing to non-linking contexts.

Aim 1: Study the effect of emotional value on the extent and symmetry of the temporal window of memory linking. Test the hypothesis that negative emotional value extend retrospective memory linking time window, and that retrospective memory linking time window is longer than those in prospective memory linking.

Aim 2: Study the neuronal correlate of memory linking. Test the hypothesis that linked memories have larger neural ensemble overlaps, and that linked memories also has more similarities in ensemble structures.

A. Significance

Understanding how temporally distinct memories can be related together is essential to understanding episodic memory. It is generally believed that hippocampus make an important contribution to episodic memory in rodents. Traditionally, studies have focused on how familiar environments are coded in hippocampus. Most notably, we have extensive knowledge of how space is coded in distinct familiar settings, as well as how time is coded in learned task contingencies. On the other hand, it is found that different population of hippocampal neurons are engaged in memories at different times, forming a neuronal ensemble of the memory, and artificially stimulating a specific ensemble can create a "false" memory. Moreover, it has been shown that neural ensembles of same environments overlap in a time-dependent manner, in that ensembles of two memories happened closer in time share more neurons than those happened further apart in time, and the shared neurons could potentially maintain a stable representation of the environment. However, it was unclear whether overlaps of ensembles encoding different contexts have a similar time-dependent property, and whether such difference in overlaps have any behavior correlates.

Recently, it has been found in rodent hippocampus that the neuronal ensembles of two distinct contextual memories separated by 5 hours time interval has more overlapping cells than those separated by 2 days or 7 days. Interestingly, subsequent fear conditioning in the later context induce elevated freezing level in the former context when the two contexts are separated by 5 hours, indicating a transfer of fear memory from the second context to the first. Such results suggest a linking of two temporally distinct memories through overlapping neuronal ensembles. Following these findings, two models have been proposed to explain the phenomenon of memory linking: On cellular and circuit level, It has been hypothesized that memory linking happens through excitability mechanism, where the ensemble neurons of first memory sustain an elevated excitability during the memory linking time window, and thus are more likely to be recruited during the encoding of the second memory, facilitating the linking of the two memory; At the same time, from a conceptual and computational aspect, temporal context model suggests that memory linking is a peculiar case of a more general temporal context framework, which argues that features of memories are associated with an ever-drifting temporal context, and all recollection of episodic memories, as well as formation of semantic memories, happen through the retrieval of the associated temporal context.

However, two important and inter-related questions remain unclear for the memory linking phenomenon: **a) whether and how the temporal order of the experiences affect the temporal window of memory linking. b) whether and how negative emotional value of the experiences affect the temporal window of memory linking.** Regarding the first question, the excitability hypothesis would predict no effect of temporal order, *i.e.* a memory would link to a past memory equally well as to a future memory, whereas temporal context model would predict a stronger prospective memory linking, *i.e.* a memory would share more overlapping ensemble and have stronger association with the other memory that happens later in time. Regarding the second question, the excitability hypothesis would predict an effect of negative emotional value on prospective memory linking, so that a memory that has a more negative value, comparing to a neutral memory, would have a stronger association with memories in the future, but not in the past. Meanwhile, the temporal context model would fail to provide a prediction regarding the second question since emotional valence has not been integrated into the model.

However, from a ethological perspective, the predictions regarding the two questions would be different from those predicted by the two existing models. Specifically: a) relating a memory to past experiences is more beneficial than relating a memory to future experiences, since only past experiences may have a causal role which is important to learn. b) relating a highly traumatic memory to other experiences (especially past experiences) is more beneficial than relating a neutral memory to others, so that the animal may learn to avoid the same situation in the future. An important example of such perspective is conditioned taste aversion, where the animals learned to associate the negative experience (sickness) with a past experience (consumption of food), and such association depends on the valence of the negative valence (how much sickness was induced).

Thus, the main goal of this proposal is to study the effect of temporal order and emotional value on memory linking. The study would help us understand the mechanism and behavioral significance of memory linking, and may extend our knowledge on episodic memory in general.

In addition, the analysis of neural recording data in memory linking experiments have been limited to com-

paring overlapping ensemble cells that are active during recording sessions. Such analysis provided a simple estimation of similarities between ensembles and successfully supported behavior data. However, it reduces the time dimension of each recording session to a binary, “active-or-not” representation, precluding any analysis on the structure of the ensemble within session. This limitation is mainly due to the task-free and one-trial-learning nature of memory linking, where there is no task variables to align the recording data to, nor is there enough time for place cells to be formed and detected. Dimension reduction approaches, or more specifically principal component analysis (PCA) is a very useful tool in such circumstances, since it can transform higher dimension recording data to lower dimension, temporally structured components in an unsupervised manner. Thus another goal of the presented proposal is to apply PCA analysis to neural recording data during memory linking experiments. Such analysis could uncover the underlying structures of memory ensembles, and help us understand the nature of memory linking on the ensemble level.

C. Approach

Aim 1: Study the effect of emotional value on the extent and symmetry of the temporal window of memory linking.

To test the hypothesis, we will use contextual fear conditioning combined with calcium imaging in behaving mice. We choose contextual fear conditioning because it is a robust and well-established test for long-term memory, and moreover a strong memory can be formed within one learning session. We choose miniature calcium imaging due to its capability to record neuronal activities in behaving mice and to track same field-of-view across long period of time, which is essential for the purpose of memory linking studies. We will focus on recording in dorsal CA1 region since it is believed to make a major contribution to contextual memory.

The presented proposal will adopt a similar experimental design. Specifically, animals will be divided into two groups: “neutral” and “negative”, which represent the affective value of the shocking context during encoding. Both groups will explore context A, B and C for 10 minutes. The time point at which the animals experience A, B and C is spaced out in such a way that the temporal distances between each of them and the shocking context S is 7 days, 2 days and 5 hours respectively. During the exploration of the shocking context S, a 2 seconds long, 0.1 mA delayed shock will be delivered at fifth minute to the animals in “negative” group, but not “neutral” group. Both groups explore the shocking context S for 10 minutes. Then 2 days later, animals in the “neutral” group will be put back in context S, where a 2 seconds long, 0.1 mA immediate shock is delivered after 10 seconds of exploration. Finally, another 2 days later (that is, 4 days interval for “negative group”), each group of animals are further divided into 5 sub-groups, where their freezing levels are assessed in parallel for context A, B, C, S, as well as N, which is a novel context. Neuronal activities in dorsal CA1 are recorded with miniature endoscope throughout the whole experiment.

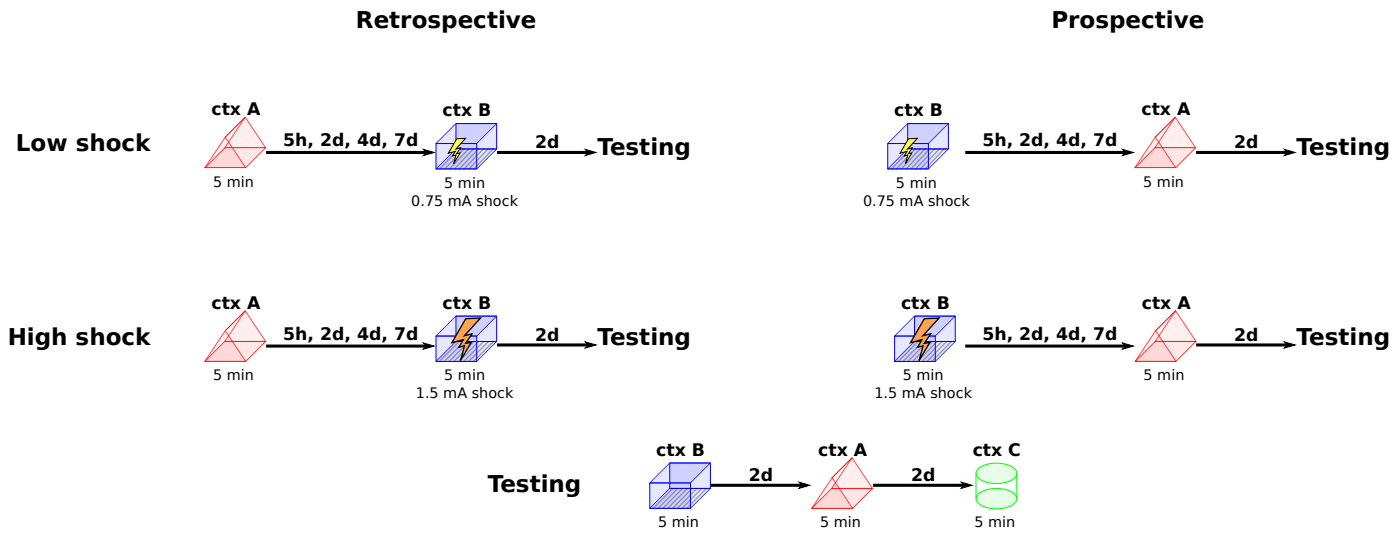

Figure 1: behavior experimental design

We expect to see significantly higher freezing levels in context B, C, and S when comparing to A or N in "negative" group, while only freezing levels in C and S are significantly higher when comparing to either A, N or C in "neutral" group. Furthermore, we expect in "negative" group that the overlap of neural ensembles between B and S, as well as between C and S, are significantly higher than the overlaps between A and S or N and S, which is supposedly at "chance" level. Whereas in "neutral" group, the overlaps between A and S, B and S, as well as N and S should all be low and at "chance" level, while only the overlaps between C and S are significantly higher than others. Taken together, these results would suggest that the paring of context S with shock during encoding extend the time window of memory linking to at least 2 days back, while the memory linking time window for a neutral context is only longer than 5 hours but shorter than 2 days.

Aim 2: Test the hypothesis that prospective memory linking has a different temporal window comparing to retrospective memory linking.

Similar to Aim 1, we use contextual fear conditioning combined with calcium imaging to test the hypothesis.

The presented proposal use a similar setup. Specifically, animals are divided into two groups, "prospective" and "retrospective". The animals in "retrospective" group will explore context A, B and C before the shocking context S, while those in "prospective" group will explore A, B and C after the shocking context S. The time points at which the animals explore A, B and C are spaced out such that the temporal distance between the shocking context S and context A, B or C are 2 days, 1 day and 5 hours respectively. In both groups, animals explore context A, B, C, S for 10 minutes, where during exploration of context S, a 2 seconds, 0.1 mA shock will be delivered at the fifth minute. 2 days after the exploration are finished for the last context, each group will be further divided into 5 sub-groups, where their freezing levels are assessed in parallel in context A, B, C, S, as well as N, which is a novel context. Neuronal activities in dorsal CA1 are recorded with miniature endoscope throughout the whole experiment.

We expect to see elevated freezing level for context B, C and S comparing to A and N in "retrospective" group, whereas in "prospective" group freezing levels are only higher in C and S, but not A, B or N. Furthermore, we expect the overlap in ensembles between B and S as well as between C and S are higher in "retrospective" group, whereas in "prospective" group the overlap are only higher between C and S but not between B and S, when comparing to the "chance" level overlap between N and S. Taken together, these results would suggest that "prospective" memory linking has a shorter temporal window than "retrospective" temporal linking.

Aim 3: Test the hypothesis that linked memories have higher similarity of ensemble structures.

The raw videos from calcium imaging recording could be processed with an open-source analysis toolkit CaImAn implementing a constrained non-negative matrix factorization algorithm. After the process, a spatial matrix representing the spatial footprint of each putative neurons, as well as a temporal matrix representing the calcium traces of each putative neurons will be extracted from the raw data. A custom-written script is used to visually assess the accuracy of the extraction as well as manually refine the results. After this, neurons from different recording sessions are cross-registered based on the euclidean distances between the centroids of their spatial footprint, and a unique master index can be assigned to each neuron in the whole experiment.

For each recording session, given a matrix representing the calcium traces of N neurons along T time-steps (usually frames), a PCA analysis can be applied to extract R principal components, where each components contain a "neuron vector" $\vec{n}$ of length N, and a "temporal vector" $\vec{t}$ of length T. Thus the dimension of the data is reduced from $N \times T$ to $R \times (N + T)$. The PCA is carried out in a way so that: a) the "neuron vector" of each principle component represent a group of neurons that has a highly correlated firing pattern, and the "temporal vector" represent that averaged pattern treating the whole group as single neuron. b) a dot product can be computed with each "neuron vector" and "temporal vector", and the sum of R such dot products should closely reproduce the original $N \times T$ data. c) the R components should explain most of the variance in the original data, thus the value of R can be determined by thresholding the proportion of variance explained.

Once the principal components of each recording sessions are extracted, we can calculate a cross-correlation of the "neuron vector"s between any two session. We can then compare such correlation matrices between linked context and unlinked contexts. We expect to see higher correlations between linked contexts, suggesting that the temporally correlated structures within each ensemble are more likely to be preserved across linked contexts than

across unlinked contexts.

The presented approach has two caveats that might require further refining: Firstly, a method to assess the quality of cross-registration is lacking. For this issue, an algorithm developed by Yaniv lab might be more suitable since it can also output the confidence of cross-registration. However, as long as the current approach does not produce systematic bias towards linked contexts, that is, as long as the field-of-view of recordings remain relatively stable, there is no reason to expect a significant artifact from presented methods. Secondly, the application of PCA analysis presume that the neuronal ensembles are structured such that subsets of cells fire together. It may fail to detect other temporal structures, such as sequence of firing. For this, other dimension reduction algorithms might address the issue.

References

[1] D. J. Cai, D. Aharoni, T. Shuman, J. Shobe, J. Biane, W. Song, B. Wei, M. Veshkini, M. La-Vu, J. Lou, S. E. Flores, I. Kim, Y. Sano, M. Zhou, K. Baumgaertel, A. Lavi, M. Kamata, M. Tuszynski, M. Mayford, P. Golshani, and A. J. Silva. A shared neural ensemble links distinct contextual memories encoded close in time. *Nature*, 534(7605):115–118, June 2016.