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 later context increased animals' freezing level in the previous context, suggesting a transfer of fear memory retrospectively [1]. Such phenomenon is coined as temporal memory linking. However, it remains unclear what factors may affect the temporal window within which two memories can be linked together. Specifically, it is unknown whether the affective valence 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, (*i.e* linking to a memory prospectively). Furthermore, it's unclear 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 temporal window of memory linking, with both behavioral experiments and calcium imaging in behaving animals.

Two models have been developed to explain the memory linking phenomenon from two perspective: the excitability hypothesis from cellular level and temporal context model from computational level. For a negative memory, both model would predict a stronger prospective memory linking than retrospective memory linking. At the same time, neither model would readily expect a change of retrospective memory linking window as a function of emotional valence. However, from an ethological point of view, retrospective memory linking is more important than prospective memory linking, since past memories may have a causal contribution to future events, but not the other way around. Moreover, memory linking of more negative events should extend further back in time, since that helps animals to gather more information to avoid future traumatic events. Thus, we hypothesis that negative emotional valence extend retrospective memory linking time window, and that retrospective memory linking time window is longer than those in prospective memory linking. 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.

Besides behavior, the other defining feature of memory linking is an increase of overlaps between the ensembles of two linked memories. Thus, our second goal is to study the neural correlates of memory linking during the manipulation of emotional valence and temporal order of contexts. We hypothesize that if the temporal window of memory linking changed, and transfer of fear is observed between two contexts, the overlaps between the ensembles encoding the two contexts should also increase. In addition, by applying dimension reduction analysis such as principal component analysis (PCA), we can uncover the underlying temporal structures of neural ensembles for individual sessions. Thus we also hypothesize that the similarities between principal components of two ensembles are higher if the corresponding contexts are linked together. In other words, the similarities between principal components of two memory ensembles should be consistent with the overlaps between the two ensembles.

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 carry out experiments utilizing contextual fear conditioning. 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.

The experimental design is shown in Figure 1. Specifically, animals will be divided into four groups according to two factors: the shock intensity and the temporal order of the contexts. Two of the four groups receive "low shock", while the other two groups receive "high shock"; At the same time, two of the four groups are assigned to "prospective" experiments, while the other two groups are assigned to "retrospective" experiments. Thus, overall the four groups form a 2 by 2 matrix, where groups on the same rows receive same amplitude of shock, and groups on the same column experience contexts in the same order. This design allow us to easily compare between groups, and uncover both the effects and interactions of emotional valence and temporal order.

In all groups, context A, B and C are distinct contexts that differ in lighting conditions, arena shapes, floor textures and scents. For "retrospective" experiments, animals are first put into context A to explore for 5 minutes. Then after a variable time intervals, the animals are put back into context B for 5 minutes, where they receive a delayed shock at fourth minute with an amplitude of either 0.75 mA or 1.5 mA, depending on whether the animals are in "low shock" or "high shock" group. The variable time intervals are achieved by further dividing animals into sub-groups according to different time intervals, and between-group comparison can be carried out. 2 days after these, the testing sequence is carried out, where the animals are put back to context A, B and C in that order, with 2d interval in between. Animals' freezing levels are assessed from behavior video recordings using
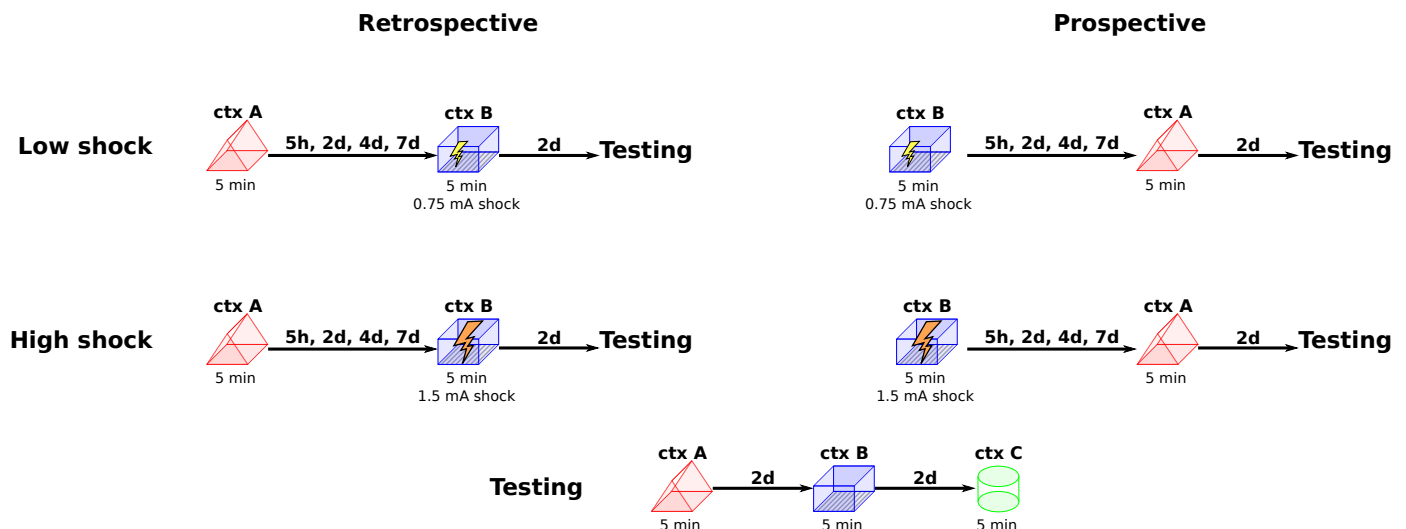

Figure 1: behavior experimental design

standard software. For "prospective" experiments, the contexts, time intervals, shock intensity assignments and testing sequence remain identical to those in "retrospective" experiments. The difference is that in "prospective" experiments, the animals are first put into context B, where they receive a delayed shock, and then put into the neutral context A to explore.

According to preliminary results, we expect to see that in "low shock" and "retrospective" groups, animals are able to link context A and B together when they are separated by either 5 hours or 2 days, but not when they are separated by 4 days or 7 days. Specifically, within "low shock" and "retrospective" group, we expect to see higher freezing in context A for 5 hours and 2 days sub-group, but not for 4 days or 7 days sub-group. Similarly, we expect in "low shock" and "prospective" groups, the animals are only able to link together context A and B when they are separated by 5 hours, but not when they are separated by either 2 days, 4 days or 7 days. Such result would suggest that retrospective memory linking has longer temporal window than prospective memory linking. On the other hand, we expect to see in "high shock" and "retrospective" group, the animals may be able to link context A and B across either 5 hours, 2 days or 4 days, but not 7 days. Such result would suggest that more negative emotional value of a memory can extend the memory linking time window retrospectively.

Aim 2: Study the neuronal correlate of memory linking.

To study the neural correlate of memory linking, we would carry out miniature calcium imaging in behaving animals. 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 experimental design is identical to Aim 1 as shown in Figure 1. Neuronal activities would be recorded during all experiment sessions to allow collection of rich dataset.

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.

The main comparison is the ensemble overlap between context A and B, B and C, as well as A and C during retrieval/testing. We expect to see a higher overlap between context A and B when the two context are linked together and animals exhibit elevated freezing level in both of them, while the overlap between A and B are not expected to be significantly higher than those between B and C or A and C when the two contexts are not linked together.

In addition, a PCA analysis can be carried out to reveal temporal structures of each ensemble. Specifically, 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.