Specific Aims

Understanding how memories experienced across large time scale are associated together is crucial to understanding episodic memories. Recently it has been demonstrated in rodents that two neutral contexts experienced closer in time shared a larger proportion of neural ensemble. Furthermore, subsequent fear conditioning in the second context increased animals' freezing level in the first context, suggesting a transfer of fear memory retrospectively [**?**]. These results showed 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 influence the time window within which it may be linked to a previous memory. Moreover, it is not clear whether memory linking can happen prospectively, where the associated fear of previous context may be transferred to a later context. Furthermore, if there is prospective memory linking, it is interesting to see whether the temporal window of prospective memory linking is similar to those in retrospective linking, *i.e* whether memory linking 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 5 hours apart, but not when they are 2 days apart. We have preliminary results suggesting that negative-valued context can be linked with a neutral context two 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 5h 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 successfully provided strong support for 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 behavioral effect of emotional value and temporal order on memory linking.

Aim 1a: Test the hypothesis that negative emotional value extend retrospective memory linking window.

Aim 1b: Test the hypothesis that negative valued memory has a longer retrospective memory linking window compared to prospective memory linking.

Aim 2: Test the hypothesis that overlap in neural ensembles correlate with the behavior effect of emotional value and temporal order on memory linking.

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