

## 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 [1]. 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.

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 similarity across linking memories versus non-linking memories.

**Aim 1: Test the hypothesis that negative valued memories have extended retrospective linking window comparing to neutral memories.** 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. To test this hypothesis, we will use contextual fear conditioning along with *in vivo* calcium imaging in freely moving animals.

**Aim 2: Test the hypothesis that prospective memory linking has a different temporal window comparing to retrospective memory linking.** It is hypothesized that memory linking might be mediated through a sustained increase in neuronal excitability after the first memory. If such hypothesis is true, we should expect similar temporal window of memory linking regardless of which of the two memories is later associated with fear, as long as the temporal distance of the two memories stay the same. However, we have preliminary results showing that associating the first context with foot-shock did not induce increase of freezing level in the second context at 5 hours time interval, suggesting that prospective memory linking is not observed at time scales that induced retrospective memory linking.

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

## A. Significance

### A.1. Instructions.

Optional subtitle

Explain the importance of the problem or critical barrier to progress in the field that the proposed project addresses.

Explain how the proposed project will improve scientific knowledge, technical capability, and/or clinical practice in one or more broad fields.

Describe how the concepts, methods, technologies, treatments, services, or preventative interventions that drive this field will be changed if the proposed aims are achieved.

### A.2. Subheading.

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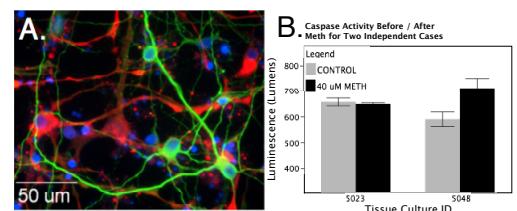
### A.3. Another subheading:

optional subtitle

**Table 1:** Example Table

City	N <sup>a</sup>	%Silly
San Diego	289	41%
Seattle	262	32%
Galveston	261	15%
St Louis	269	7%
New York	271	4%
Baltimore	231	2%
<i>Total</i>	1,586	21%

<sup>a</sup>All participants clowns.



**Figure 1:** Example wrapped figure. (A) Impressive microscopy image. (B) Impressive data.

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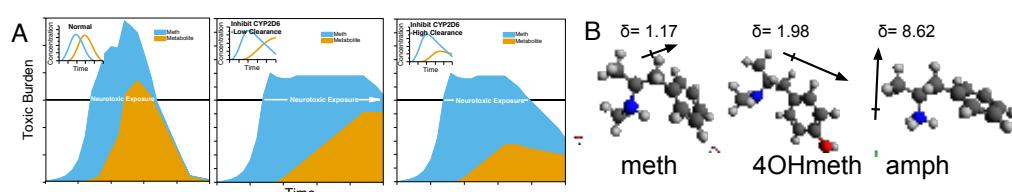
### **B. Innovation**

### **B.1. Instructions.**

Explain how the application challenges and seeks to shift current research or clinical practice paradigms.

Describe any novel theoretical concepts, approaches or methodologies, instrumentation or interventions to be developed or used, and any advantage over existing methodologies, instrumentation, or interventions.

Explain any refinements, improvements, or new applications of theoretical concepts, approaches or methodologies, instrumentation, or interventions.



## **C. Approach**

### **C.1. Instructions.**

Describe the overall strategy, methodology, and analyses to be used to accomplish the specific aims of the project. Unless addressed separately in Item 15 (Resource Sharing Plan), include how the data will be collected, analyzed, and interpreted as well as any resource sharing plans as appropriate.

Discuss potential problems, alternative strategies, and benchmarks for success anticipated to achieve the aims.

If the project is in the early stages of development, describe any strategy to establish feasibility, and address the management of any high risk aspects of the proposed work.

Point out any procedures, situations, or materials that may be hazardous to personnel and precautions to be exercised. A full discussion on the use of Select Agents should appear in Item 11, below.

As applicable, also include the following information as part of the Research Strategy, keeping within the three sections listed above: Significance, Innovation, and Approach.

### **C.2. Preliminary Studies for New Applications**

Preliminary Studies for New Applications: For new applications, include information on Preliminary Studies. Discuss the PD/PI's preliminary studies, data, and or experience pertinent to this application. Except for Exploratory/Developmental Grants (R21/R33), Small Research Grants (R03), and Academic Research Enhancement Award (AREA) Grants (R15), preliminary data can be an essential part of a research grant application and help to establish the likelihood of success of the proposed project. Early Stage Investigators should include preliminary data (however, for R01 applications, reviewers will be instructed to place less emphasis on the preliminary data in application from Early Stage Investigators than on the preliminary data in applications from more established investigators).

## 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. Tuszyński, 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.