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. Such results suggest a linking of two temporally distinct memories through overlapping neuronal ensembles, and the phenomenon is termed memory linking. One of the hypothesis that could explain memory linking is the excitability hypothesis, which states that the neurons encoding an earlier memory have a transient increase in excitability, making them more likely to be active during the encoding of a later memory, thus resulting in an increase of ensembles overlap between the two memories. Such increase in the ensembles overlap, in turn, may drive memory linking.

However, the ethological implication of memory linking remains unclear. It can be speculated that memory linking may play a role in causal inference, in that linking a traumatic experience with an earlier memory may help the animal learn the causal relationship between memories and avoid future traumatic events. Thus, retrospective memory linking, where the animal link a traumatic experience with a previous neutral contextual memory, should be more important than prospective memory linking, where the animal link a traumatic experience with a neutral memory that happens later in time. Specifically, the temporal window of memory linking, which is the maximum time interval within which two memories could be linked together, should be longer for retrospective memory linking than for prospective memory linking, since it helps the animal to gather more information about the potential cause of the traumatic event. However, the excitability hypothesis would predict the opposite — Since it has been shown that negative valence increase neuron excitability, it is expected that neurons engaged in a negative memory sustain an elevated level of excitability longer than those in a neutral memory. Thus, for a negative memory, the excitability model would predict a longer temporal window for prospective memory linking comparing to retrospective memory linking. We have preliminary results suggesting a longer temporal window for retrospective memory linking. Thus, the first goal of the presented proposal is to study the temporal window regarding the direction of memory linking, and we hypothesize that retrospective memory linking has a longer temporal window than prospective memory linking.

The second goal of our proposal is to study how ensemble dynamic of two contextual memories contribute to the emergence of the increase in overlap between the two ensembles, which in turn drives memory linking. We have preliminary results suggesting that the increase in ensemble overlap between the two contexts emerges only during retrieval testing, but not during encoding. Thus, an intuitive hypothesis is that the representation of one of the contexts changed after encoding, so that it is more similar to the other context. To test this hypothesis, we will investigate the reactivation rate for each context, which is the amount of overlap between the ensembles of encoding and retrieval of the same context. We will also investigate the overlap between the ensembles of one context during retrieval and the other context during encoding, which indicate whether one of the context become more similar to the other after encoding. In addition, we will investigate whether different ensembles during encoding have an equal contribution to the overlap between the two ensembles during retrieval. To test this, for each ensemble during encoding, we will calculate a rate of neurons that is also active in both contexts during retrieval. We can then compare the rates between different ensembles during encoding to see whether they contribute equally to the overlap between the two ensembles in retrieval. Lastly, the excitability hypothesis predict a correlation between the activity level of a neuron and the likelihood of the neuron being active again in future sessions. To test this, for any two recording sessions, we will classify the neurons in the earlier session into two populations according to whether they are also active during the later session. We can then compare the mean normalized activity level of the two population to see whether the activity level of a neuron could predict whether they are active in future sessions. Taken together, these tests will give us more insights in how the neurons in different ensembles and their temporal dynamic contribute to the emergence of memory linking.

Lastly, calcium imaging in behaving animals using miniature microscope is an essential tool for proposed studies. However, one of the challenge facing this approach is the analysis of imaging data. The constrained

non–negative matrix factorization (CNMF) algorithm performed well on extracting calcium traces from raw video. However, a lack of user–friendly interface, batch–processing capability as well as visualization tools of the results limits its popularity among the community. Thus, the third goal of the presented proposal is to develop an analysis pipeline that provide an interface to the CNMF algorithm that enable the user to easily batch-process raw imaging data and visualize the results to quickly assess the quality of extraction. Such a tool will be very useful to the scientific community that employ calcium imaging with miniature microscope during their research.

Aim 1: Study the temporal window of prospective and retrospective memory linking. Test the hypothesis that temporal window for retrospective memory linking is longer comparing to prospective memory linking.

Aim 2: Study how ensemble dynamic contribute to memory linking. Test whether ensemble of one context become more similar to the other linked context after encoding. Test whether different ensembles during encoding have equal contribution to the overlap between ensembles of linked context during retrieval. Test whether activity level of neurons in one session could predict whether they are active in future sessions.

Aim 3: Develop analysis pipeline for calcium imaging with miniature microscope.

A. Significance

It is generally believed that hippocampus make an important contribution to episodic memories in rodents. Traditionally, studies on hippocampal neuronal coding have been focused on how information are encoded in the **activity pattern** of neurons. Usually, such studies involve measuring neuronal activities during repeated retrieval of same memory, and measure how the activities of neurons respond consistently to a behavior variable across retrieval sessions. For instance, "place cells" have been found to encode the location of the animal in a familiar environment [1], and "time cells" have been found to encode elapsed time during well–learned, time–dependent tasks [2]. On the other hand, another trend of studies have focused on how information might be encoded in the **identity** of neurons. For example, the idea of "neural ensemble" states that different population of neurons are engaged in the encoding of different memories, and memory retrieval happens through the reactivation of the corresponding neural population. The population of neurons that encode a memory is thus termed the ensemble of the said memory. Indeed, it is found that artificially stimulating an ensemble is sufficient to drive the retrieval of the corresponding memory and elicit behavior response [3]. The two distinct but not mutually exclusive types of hippocampal coding — through activity pattern and through neuron identity — can be brought together by a conceptual framework coined as "memory space" [4]. Briefly, it is hypothesized in the "memory space" concept that the activities of cells encode details of a memory episode, such as stimulus, location and time, whereas the common cells that are shared across episodes, or the overlapping neuron population between ensembles, may serve as "nodes" that bridge together different memory episodes. In other words, the neuronal activities encode information within episodes, while the identity of ensembles encode relationship between episodes. Consistent with this concept, it is found that the ensembles of the **same** familiar environment "drift" across time, so that different but overlapping ensembles were activated during the retrieval of memory of the same environment at different times. More importantly, it is found that the overlap between ensembles depend on time, in that ensembles of episodes that happened closer in time share more neurons in common. Moreover, it is found that the subset of neurons that were active across all retrieval episodes sustain a stable spatial map of the environment so that the location of the animals can be reliably decoded with only the activities from this subset of cells. Taken together, these results suggest that overlapping neurons between ensembles encode relationship between memory episodes, in that they encode both the information that is common across episodes (the same spatial environment) and the temporal relationship of episodes (the temporal distances between them), which is consistent with the prediction of "memory space" concept [5]. However, two important aspects of the "memory space" concept remained untested: a) whether the ensembles overlap also encode temporal distance between memories of **different** contexts; b) whether the overlapping ensemble can actually drive the "bridging" of different memory episodes. Understanding how distinct memories can be related together is essential to understanding episodic memory, since the ability to associate different episodes across long periods of times is essential to forming memories.

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, and the phenomenon is termed memory linking [6]. 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 [7]; 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 [8].

However, various aspects of memory linking remain under-studied. Most notably, it is unclear what factors

affect the temporal window of memory linking, defined as the maximum time interval within which two memories could be linked together. Besides, the effect of temporal order on memory linking remains unclear - it has been demonstrated that memory linking can happen retrospectively, in that the fear associated with a later memory can transfer back to a neutral memory that happened earlier. It is unknown, however, whether memory linking could happen prospectively, where the fear associated with a memory can transfer forward to a neutral memory that happens later in time. Moreover, if prospective memory linking exists, it is interesting to see whether it has a same temporal window as retrospective memory linking. Taken together, 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 valence of the experiences affect the retrospective temporal window of memory linking.** Regarding the first question, the excitability hypothesis would predict longer memory linking window for prospective memory linking, since negative emotional valence increase the excitability of neurons, making them sustain an elevated excitability for longer period of time comparing to those engaged in neutral memories, thus extend the time window where a negative memory could be linked to a neutral memory in the future, but not in the past. Similarly, the temporal context model would predict a stronger prospective memory linking as well. Regarding the second question, the excitability hypothesis would not predict an effect of negative emotional valence on retrospective memory linking, since the excitability of neurons engaged in a neutral memory should not be affect by emotional valence of a memory happens in the future. 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 valence 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 to behavior, it is important to see whether there is neural correlates of memory linking in hippocampus. Traditionally, the analysis of neural recording data in memory linking experiments have been limited to comparing 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.

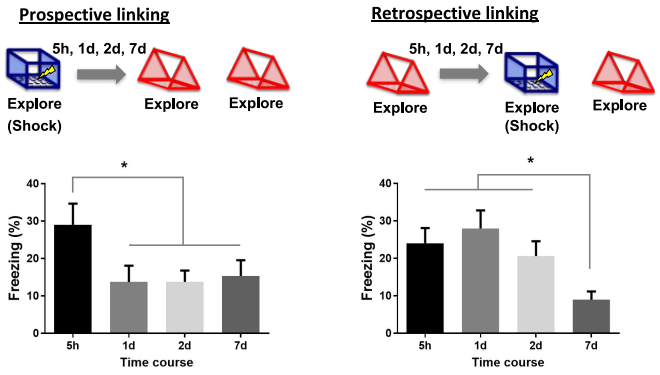

Figure 1: Prospective memory linking window is longer than retrospective linking window for a negative memory.

B. Preliminary

Prospective memory linking window is longer than retrospective linking window for a negative memory

In the preliminary study shown in Figure 1, animals are put into two distinct contexts separated by various time intervals. In the "prospective linking" group, animals received a delayed shock in the first context, and then explore and get tested in the second context. In the "retrospective linking" group, animals explored first context, and then get a delayed shock in the second context, and then put back to the first context for testing. Elevated freezing level in the testing context, where no shock ever occurred for both groups, indicate a transfer of fear and a linking of the two contexts. For both groups, the exploration and testing session last 10 minutes, a shock of 0.75 mA was delivered at fifth minute. The various time intervals are 5 hours, 1 day, 2 days and 7 days for both groups.

In prospective linking group, we observed a significant higher freezing level in testing context with 5 hours interval, but not with either 1 day, 2 days or 7 days interval. This suggest that the fear memories were able to transfer forward to a neutral context 5 hours in the future. On the other hand, in retrospective linking group, we observed higher freezing level with either 5 hours, 1 day or 2 days interval, but not with 7 days interval. This suggest the fear memory was able to transfer backward up to 2 days. Taken together, these results suggest an extended temporal window of retrospective memory linking comparing to prospective memory linking.

Negative emotional valence extend temporal window of memory linking

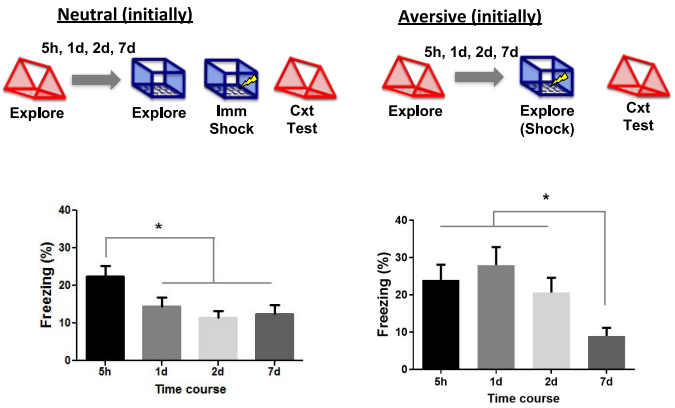

Figure 2: Negative valence increased temporal window of memory linking.

In the preliminary study shown in Figure 2, animals are first put into a neutral context for exploration. After

various time interval, animals are put into a second context. For animals in the aversive group, they received a delayed shock at fifth minute of the exploration, which associate a negative emotional value to the second context. For animals in the neutral group, the exploration of the second context was uninterrupted, and they received a immediate shock in the second context 2 days after the initial exploration, thus the emotional valence of the second context was initially neutral for this group. Both groups were put back to the first context to test for freezing. An elevated freezing level in the first context indicate a transfer of fear.

For the neutral group, we observed significant higher freezing level in the first context with 5 hours interval, but not with either 1 day, 2 days or 7 days interval, suggesting that the two contexts were only linked across 5 hours when the second context was initially neutral during encoding. On the other hand, we observed significant higher freezing level with either 5 hours, 1 day or 2 days interval, but not with 7 days interval, suggesting the two memories were able to link across 2 days when the second memory was initially negative during encoding. Taken together, these results suggest that negative emotional valence was able to extend the time window of memory linking.

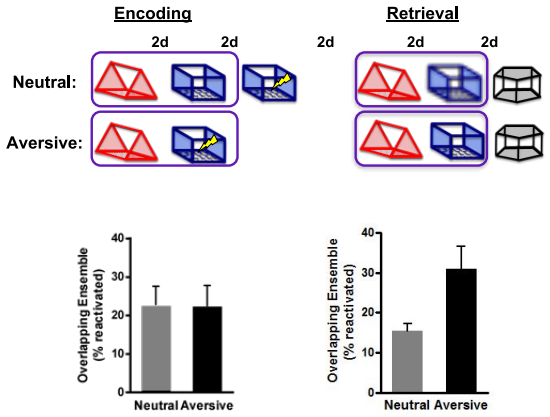

Figure 3: Negative valence increased the ensemble overlap of two memories across 2 days

In another preliminary study shown in Figure 3, calcium imaging was carried out during all the behavior sessions. The experiment design is similar to the previous behavior study, except the time interval between the initial exploration of the two contexts was fixed at 2 days, since there was a significant behavior effect of negative valence at 2 days interval. Consistent with the behavior results, we found a significant increase in neural overlaps of the two ensembles during the retrieval of the two contexts in the aversive group. Interestingly, between the two groups, there is no significant difference between the neural overlaps of the two ensembles during encoding of the two contexts, suggesting that the changes in the overlaps of the representation of the two contexts, possibly memory linking as well, happened during offline periods between the initial encoding and the testing of the memories.

Minian: a python analysis pipeline for calcium imaging data

One of the challenge facing miniature microscope in behaving animals is the analysis of calcium imaging data. Previously, a constrained non–negative matrix factorization algorithm has been developed to extract calcium traces of different neurons from raw video. However, a lack of user-friendly interface and visualization tools for result inspection limit its popularity among community. Moreover, a method of cross-registering neurons across sessions has not been integrated into the analysis pipeline. To address these issues and facilitate the analysis of imaging data, we have developed a pipeline based on jupyter notebook, which is a document format that combines codes and texts. The adoption of jupyter notebook enables us to take and share text notes, edit and execute codes, as well as dynamically visualize results all in an integrated document, so that we can easily share reproducible analysis to the community (Figure 4). Furthermore, a simple cross-registration method based on euclidean distance of centroids of neurons has been integrated into the pipeline. Application of this method to our preliminary data shows that the method could identify same neurons across sessions with satisfactory accuracy.

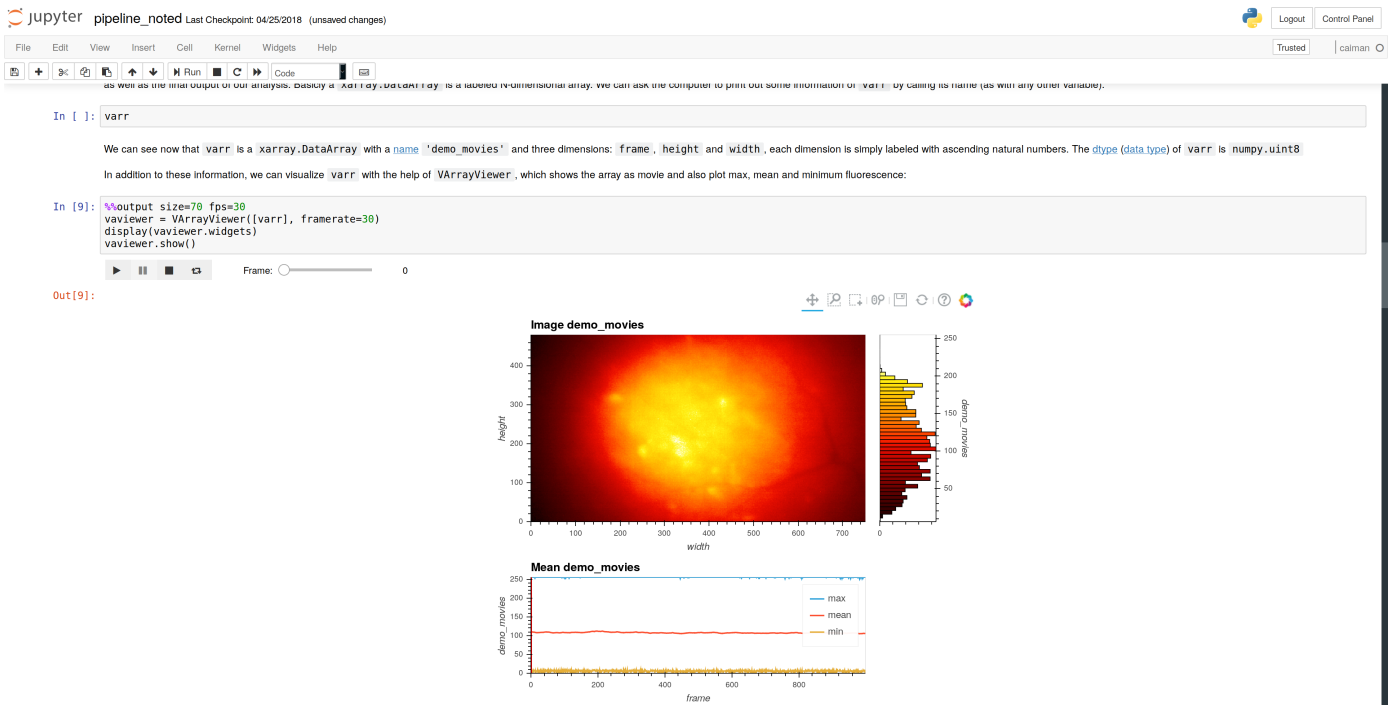

Figure 4: integration of notes, codes and visualization of results in a single document.

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 5. 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

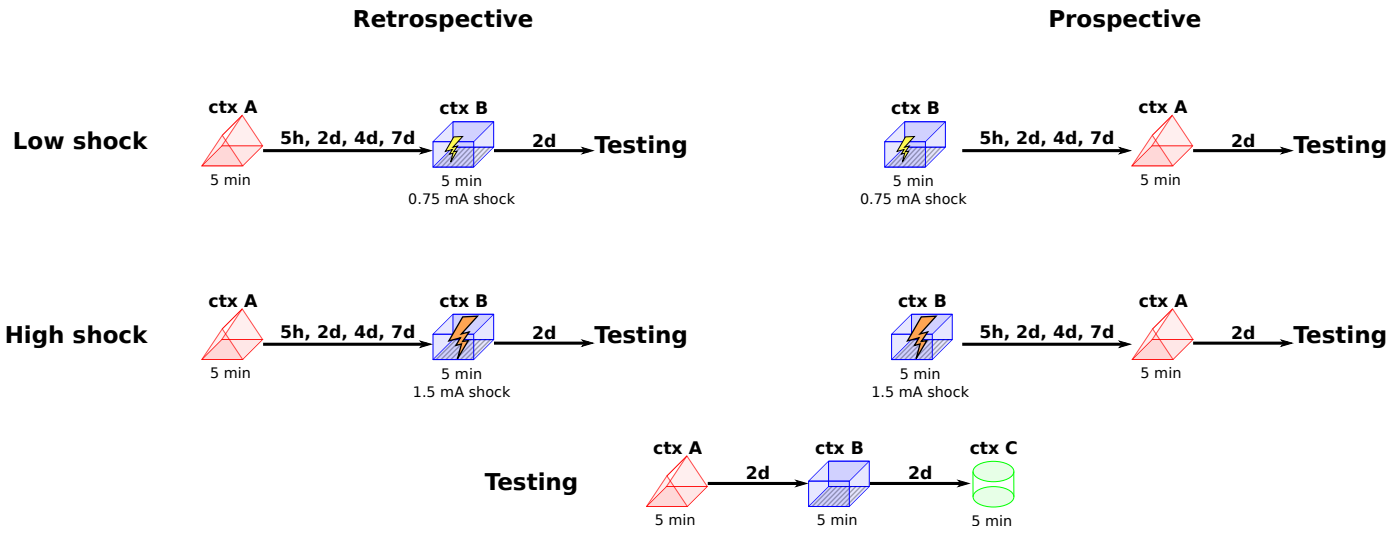

Figure 5: behavior experimental design

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 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 how ensemble dynamic contribute to memory linking.

To study how ensemble dynamic contribute to memory linking, we will carry out calcium imaging studies shown in Figure 6. The design was similar to the behavior experiments in previous aim. Briefly, the animals are placed in context A to explore, then after a variable time interval, the animals are placed in context B, where they receive a delayed shock at second minute. Then two days later, the animals are put back in context A, context B, and a novel context C in that order for testing. Calcium imaging in behaving animals are carried out in all sessions. Thus the neural dynamics during all sessions can be collected and analyzed.

For simplicity, we use uppercase letters combined with numbers to denote the population of neurons that were active during a recording session: we use $A1$ and $A2$ to denote neurons that are active during the encoding and retrieval session of context A, respectively. We use $B1_{pre}$ and $B1_{post}$ to denote cells that active before and after the delivery of the shock during the encoding session of context B. In addition, we use $B2$ to denote active neurons in the retrieval session of context B, and lastly we use C to denote the active neurons encoding context C. Additionally, we use set operation conventions to denote the specific population of neurons that we are interested in. Specifically, we use set intersection "$\cap$"to denote the population of neurons that are active in both sessions. For example, $A1 \cap A2$ denote the cells that are active in both the encoding and the retrieval session of context A. Moreover, we use set difference "$\setminus$"to denote the population of neurons that are active in the first session but

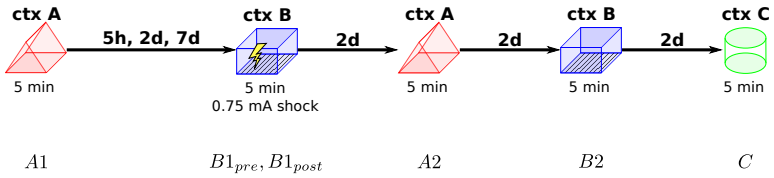

Figure 6: imaging experiment design

not the second session. For example, $A1 \setminus A2$ denote the cells that are active only during the encoding session of context A, but not reactivated during the retrieval session of context A. Finally, we use "$N\{\ldots\}$" to denote the number of cells in the population that we are interested in. For example, $N\{A1\}$ denote the total count of cells that are active during the encoding of context A.

To test whether representation of one context become more similar to the other after encoding, we first look at the reactivation rate of the neurons initially encoding a context during the retrieval of that context. Specifically, we calculate reactivation rate as the following:

$$R_{A1} = \frac{N\{A1 \cap A2\}}{N\{A1\}}$$
$$R_{B1pre} = \frac{N\{B1_{pre} \cap B2\}}{N\{B1_{pre}\}}$$
$$R_{B1post} = \frac{N\{B1_{post} \cap B2\}}{N\{B1_{post}\}}$$

where R_{A1}, R_{B1pre}, R_{B1post} denote the reactivation rate of the ensembles for context A, pre-shock phase for context B, and post-shock phase for context B, respectively. In addition, we also want to look at whether one context become more similar to the other after encoding. To test this, we can calculate a reactivation rate of the neurons initially encoding one context during the retrieval of the other context. Specifically, we calculate the rate as the following:

$$R_{BA} = \frac{N\{A1 \cap B2\}}{N\{A1\}}$$
$$R_{ABpre} = \frac{N\{B1_{pre} \cap A2\}}{N\{B1_{pre}\}}$$
$$R_{ABpost} = \frac{N\{B1_{post} \cap A2\}}{N\{B1_{post}\}}$$

Conceptually, R_{BA} indicate how close is the representation of context B during retrieval to the initial representation of context A. Similarly, R_{ABpre} and R_{ABpost}indicate how close is the representation of context A during retrieval to the initial pre-shock or post-shock representation of context B, respectively. If there is a drift of the representation of one context towards the other after encoding, we should see a decrease of reactivation rate of that context between encoding and retrieval, as well as a higher overlap between the retrieval of that context and the encoding of the other context.

To study how different ensembles during encoding may contribute to the overlap during the retrieval, we can calculate the proportion of cells during encoding that also serve as the overlapping cells during retrieval. Specifically:

$$C_X = \frac{N\{X \cap (A2 \cap B2) \setminus (A2 \cap B2 \cap C)\}}{N\{X\}}$$

where X is one of the three ensembles during encoding, that is: $A1$, $B1_{pre}$, or $B1_{post}$. The term $(A2 \cap B2) \setminus (A2 \cap B2 \cap C)$ give us the population of neurons that are active specifically in both the retrieval of context A and B, but not active during all three retrieval sessions of context A, B and C. The intersection between this population and the ensembles of one of the encoding sessions will then estimate the contribution of neurons from one of the encoding sessions to this specific population that may drive memory linking. Thus, C_{A1}, C_{B1pre} and C_{B1post} will indicate the contributions to the overlap during retrieval from the ensembles encoding context A, pre-shock phase of context B, and post-shock phase of context B respectively. We can then compare these three rate to see whether different ensembles during encoding contribute equally to the population of neurons that may drive memory linking.

Lastly, in line with the excitability hypothesis, we expect the activity level of a neuron should affect the likelihood of that neuron being reactivated during future sessions. Specifically, for each neuron, the activity level of that

neuron can be summarized by the area-under-the-curve of the calcium trace of that neuron. By taking a z-score of such activity level across all neurons in a recording session, we can obtained a centered activity level for each neuron that's also normalized to the overall activity level of all neurons in that session. We can denote such centered and normalized activity level in session X as $A_X\{\ldots\}$, where $\ldots$ indicate a specific population we are interested in. Then, for any given two recording session X and Y, where X happens before Y, four quantities can be calculated and compared:

$$\begin{aligned} Activity_X^{common} &= A_X\{X \cap Y\} \\ Activity_X^{alone} &= A_X\{X \setminus (X \cap Y)\} \\ Activity_Y^{common} &= A_Y\{X \cap Y\} \\ Activity_Y^{alone} &= A_Y\{Y \setminus (X \cap Y)\} \end{aligned}$$

$Activity_X^{common}$ indicate the activity level in X of those cells that were reactivated in Y, whereas $Activity_X^{alone}$ indicate the activity levels in X of the cells that were only activated during X, but not Y. We can then compare the mean of $Activity_X^{common}$ and $Activity_X^{alone}$. According to excitability hypothesis, we expect to see higher $Activity_X^{common}$ than $Activity_X^{alone}$, since the activity level in X should affect whether they are reactivated in later sessions. On the other hand, we expect no difference between $Activity_Y^{common}$ and $Activity_Y^{alone}$, since the activity level of cells in the later session should not affect whether they are co-activated in both sessions.

Aim 3: Develop analysis pipeline for calcium imaging with miniature microscope.

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 5. 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] T. Hartley, C. Lever, N. Burgess, and J. O'Keefe. Space in the brain: how the hippocampal formation supports spatial cognition. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 369(1635), February 2014. PMID: 24366125.

[2] H. Eichenbaum. Time cells in the hippocampus: a new dimension for mapping memories. *Nature Reviews Neuroscience*, October 2014. PMID: 25269553.

[3] S. Ramirez, X. Liu, P. P.-A. Lin, J. Suh, M. Pignatelli, R. L. Redondo, T. J. Ryan, and S. Tonegawa. Creating a false memory in the hippocampus. *Science*, 341(July):387–392, July 2013.

[4] H. Eichenbaum, P. Dudchenko, E. Wood, M. Shapiro, and H. Tanila. The hippocampus, memory, and place cells: Is it spatial memory or a memory space? *Neuron*, 23:209–226, 1999. PMID: 10399928.

[5] A. Rubin, N. Geva, L. Sheintuch, and Y. Ziv. Hippocampal ensemble dynamics timestamp events in long-term memory. *eLife*, 4:e12247, December 2015.

[6] 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.

[7] G. Kastellakis, A. J. Silva, and P. Poirazi. Linking Memories across Time via Neuronal and Dendritic Overlaps in Model Neurons with Active Dendrites. *Cell Reports*, 17(6):1491–1504, November 2016.

[8] M. W. Howard, M. S. Fotedar, A. V. Datey, and M. E. Hasselmo. The Temporal Context Model in spatial navigation and relational learning: Toward a common explanation of medial temporal lobe function across domains. *Psychological review*, 112(1):75–116, January 2005. PMID: 15631589.