Understanding Fear and Beyond in Neuronal Networks with Tensor and Graph Methods: An Interdisciplinary End-to-End Data Science Approach

Jordan Steinhauser
Psychology Department
University of California, Riverside
Riverside, CA, USA
jordan.steinhauser@email.ucr.edu

Evangelos E. Papalexakis
Computer Science and
Engineering Department
University of California, Riverside
Riverside, CA, USA
epapalex@cs.ucr.edu

Edward Korzus
Psychology Department
University of California, Riverside
Riverside, CA, USA
edward.korzus@ucr.edu

ABSTRACT

Understanding fear and where it is generated, modulated, and interpreted is paramount when trying to develop our knowledge of anxiety and stressor type disorders and how they develop. We use calcium imaging as a method to record real-time neuronal network activity from a precise region of interest in freely behaving mice. However, a continual challenge with calcium imaging methods is (1) the background noise and artifacts within the video recordings, (2) the large amounts of data that need to be processed and analyzed for underlying structure, and (3) the inability to label our data to provide an interpretable output. To address the problem of video quality and processing, we employ several algorithms to denoise, demix, and extract valid data. Using tensor decomposition and community analysis in independent analyses, we found three non-orthogonal (tensor decomposition) and orthogonal (community analysis) populations of neurons that encoded information for different aspects of the behavioral paradigms, including a habituation population, a discrimination population responsive to the novel safe and threat environment presentations, and a familiar control environment exposure population. Overall, these results are informative of the neuronal activity modulation occurring within the recorded region of interest and provide valuable insight into the different populations of neurons that are selectively co-active during different trials and environment presentations.

CCS CONCEPTS

• Applied Computing → Life and medical sciences; • Information systems → Information systems applications; Data mining; Clustering; • Computing methodologies → Machine learning; Machine learning approaches; Factorization methods

KEYWORDS

Tensor decomposition, Community analysis, Calcium imaging, Brain networks

1 Introduction

Understanding how to investigate and quantify necessary neuronal networks underlying cognition and behavior is paramount to advancing the fields of neuroscience and psychology. Numerous data mining techniques have been broadly applied to several neuroscience datasets, mainly non-invasive, low spatial-resolution fMRI data. However, few data mining techniques have been applied to neuronal circuit-level resolution analysis to determine the modulatory dynamics necessary to produce specific and appropriate behavioral outputs.

Previous work has investigated brain activity via fMRI signals with tensor methods [1] and was successful in developing an algorithm to extract common components from a group of subjects. This prior work used known, or ideal, fMRI responses to inform the conclusions; however, our experimental design is exploratory, and the data collected cannot be labeled, as its contribution to the respective brain circuit is unknown. Other work used similar calcium imaging methodology and tensor decomposition to discover clusters in the data representing key epochs of the experimental trial [2]. Our experimental design differed from this work by recording neuronal activity during a cognitive task that lacks discrete action-reaction time periods, and results in learning across several days.

In this paper, we use motivation from prior work in the learning and memory field to guide our research techniques of large-scale neuronal recording in rodent models in order to address our hypotheses. We predict that unsupervised data mining techniques will uncover latent variables in the dataset that represent distinct epochs and environments within the behavioral paradigm the animals experience.

Our contributions are summarized as follows:

- **Data collection pipeline:** We gathered calcium imaging brain data from multiple subjects that represents neuronal activity during particular environmental exposures across several recording sessions and formulated an analysis pipeline with existing work to denoise, demix, and extract neuronal activity signals. The resulting data is a multi-dimensional time series where each neuron has a sperate time-series.
- **Tensor analysis:** We found tensor decomposition to be successful in discovering latent clusters of neurons

that are highly engaged during specific trials and environments.

- **Community analysis:** We create a neuron-by-neuron adjacency matrix from the time-series, where neurons are connected via their temporal activity patterns, and found community analysis was also suitable for uncovering neuron populations that provided the same overall trial and environment representations as tensor decomposition.

2 Data Collection Methodology

The following is a brief description of the behavioral paradigm used to address our hypotheses. We employ a contextual differential fear learning (cFDL) task (Figure 1) where mice must learn to discriminate between a threatening and a safe context [3,4]. Specifically, mice are exposed to three environments every day for a total of 11 days: a control environment that is familiar because it is similar to their home cage, a novel safe environment and a novel threatening environment, both of which are similar yet distinct. First, the mice are habituated to all three environments for three days. Then they undergo fear conditioning where they learn to fear the threatening environment. Finally, they are exposed to all three environments for eight days during the discrimination phase of the behavioral paradigm, where they learn to discriminate between the two novel, distinct environments by the end of the behavioral experiment.

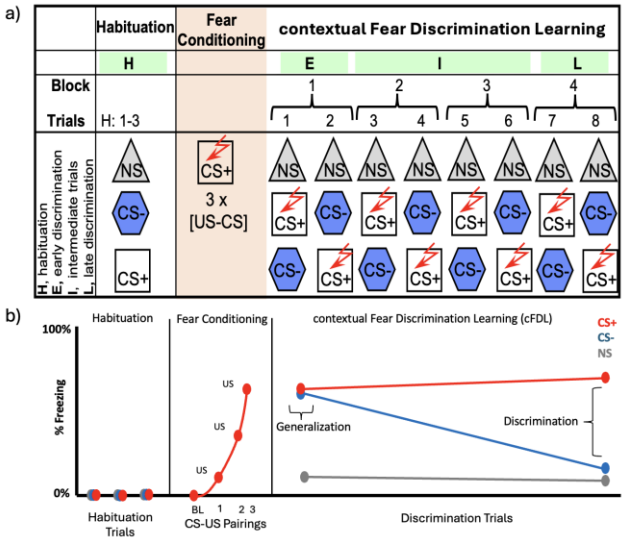

Figure 1: Schematic of behavioral paradigm. (a) Depicts schedule of behavioral paradigm with three environmental exposures (trials) every day, except on the Fear Conditioning day. The first three days are Habituation. Day 4 is Fear Conditioning where mice are conditioned to associate a tone (CS) with an aversive stimulus (footshock, US). The subsequent eight days are the contextual Fear Discrimination Learning phase of the paradigm where mice first generalize the novel safe (blue, CS-) and novel threatening (red, CS+) environments. However, by the end of the experiment, mice will learn to discriminate between the two similar, yet distinct, environments. (b) A hypothetical model of data presentation that we expect to observe during the behavioral paradigm. Preliminary data supports the hypothetical model.

During this behavioral task, we record neuronal activity from hundreds of medial prefrontal cortex (mPFC) neurons using a technique called "calcium imaging" which entails chronically implanting a microendoscopic grin lens into mPFC and using a miniaturized head mounted microscope (referred to as a "miniscope") to record the calcium fluorescence emitted from each neuron in the field of view (Figure 2) [5,6]. The resulting data are 30 frame per second videos that are approximately 200 seconds in duration and display 800 neurons on average, that fluoresce when the neuron is active.

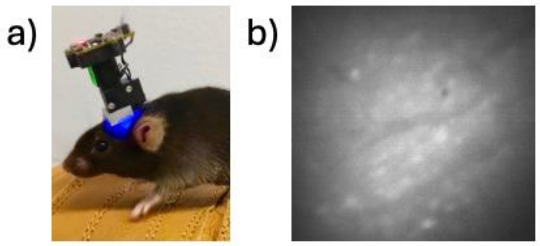

Figure 2: Calcium Imaging Data Collection. (a) Image of a mouse with a head-mounted miniaturized microscope. (b) Example field of view of *in vivo* calcium imaging. Dark lines represent blood vessels that serve as landmarks when aligning video recordings across sessions. Bright spots represent active neurons. Neuron activity is tracked via fluorescence across the 200 second trial.

2.1 Data Processing

The first step in the data processing pipeline is using a Normalization Correction (NormCorr) program. Each raw video of calcium imaging recorded during a behavioral trial in freely behaving animals is motion corrected by NormCorr, a non-rigid, piecewise motion correction algorithm [7]. This algorithm stabilizes the frames of the video across time and corrects for physiological inner frame transformations of the imaged brain tissue. Next step is to use a Constrained Nonnegative Matrix Factorization (CNMF-E), a matrix factorization technique that imposes a non-negativity constraint, which ultimately reduces the dimensionality of the data while ensuring all elements of the matrices are non-negative, in order to extract underlying patterns from calcium imaging data [8]. Last is Cell Registration (CellReg), which allows for the accurate tracking and registration of hundreds of cells across countless recording sessions [9]. This program has several parameters that are adjustable to best represent individual subjects' calcium imaging recordings and outputs cell identification information that provides large amounts of crucial calcium imaging information that can then be mined using a variety of knowledge discovery techniques (Figure 3).

2.2 Data Description

Our data is multidimensional time series data. Each neurons' spatial location, calcium fluorescence, and determined spiking activity is recorded for each trial and tracked across several trials. In particular, the calcium fluorescence generates a trace with the change in fluorescent intensity on the y-axis (Δf/f) across the total length of the trial (200 seconds) (Figure 3). This trace and spike data is generated for each trial (33 trials), resulting in a calcium activity data that is best represented as a three-dimensional array with trial length on the x-axis, individual neurons' activity on the y-axis, and each trail along the z-axis. An important variable within the threatening environment exposures is the presentation of the aversive stimulus (footshock) at 180 seconds into the trial. For example, Neuron 1 (bottom blue trace in Figure 3) can be categorized as an aversive stimulus responding neuron because it only becomes active after the footshock is delivered.

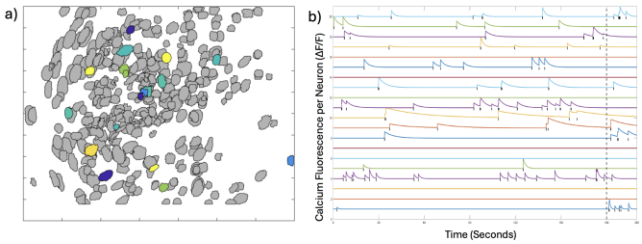

Figure 3: Data processing representation. (a) Preprocessing algorithms provide aggregated characteristics of individual neurons across all trials in the recorded field of view, including spatial location. (b) Calcium fluorescence for 20 exemplar neurons that are active sporadically throughout the 200 second trial. Trial time is represented on the x-axis and change in calcium fluorescence for each neuron (20 example neuron traces shown) is represented on the y-axis. Some neurons are not active during this environment presentation and therefore depict no change in fluorescence (e.g. flat line). Black dashed line denotes presentation of aversive stimulus.

3 Tensor Decomposition

Our data can be represented as a third-order tensor, which is a three-dimensional array. Tensor decomposition is an unsupervised data mining technique to reduce the data from high dimensionality to low dimensionality and discover latent variables, or components, within the data [10]. In particular, the components encode information about the pattern of activity of neurons that are coactive during specific temporal points during the stimulus presentation and during the trials. We decomposed the tensor into three low-dimensional factors that reconstruct the original tensor when combined. The Neuron Factor provides information regarding how much each individual neuron participates in that specific component. The Temporal Factor informs how variable the activity of the clustered neurons attributed to that component are within the designated time bin. The Trial Factor importantly shows how the neurons clustered within that component participated throughout the trials of the behavioral paradigm. The third-order tensor is an element of the set of real numbers within the three modes, represented as,

$$\mathcal{X} \in \mathbb{R}^{I \times J \times K} \quad (1)$$

Canonical polyadic (CP) decomposition, also known as PARAFAC or CANDECOMP, was used because the model is suitable for exploratory data mining and due to the results being easily interpretable [11]. CP decomposition for our three-mode tensor is the sum of the three-way vector outer products of the neuron (n), time (t), and trial (a) factors,

$$\mathcal{X} \approx \sum_{r=1}^{R} n_r \circ t_r \circ a_r \quad (2)$$

where R is the number of components that adequately model the data.

Specifically, we used CP nonnegative matrix factorization with multiplicative updates to estimate the best rank-R CP model of $\mathcal{X}$ with nonnegative constraints on the three factors [12]. To determine the number of components that best model the data, we used the so-called "Core Consistency Diagnostic" or CORCONDIA (Figure 4) [13]. This diagnostic runs a specified number of iterations for a variety of components to find the model that optimally represents the complexity of the data without overfitting the data. CORCONDIA will ideally provide the maximum number of components that explain the variability in the model with minimal decrease of the fit of the model to the data [14]. We found that most of the subjects in our experimental groups were best represented with three components; therefore, we specified the number of components as three for all subjects' data.

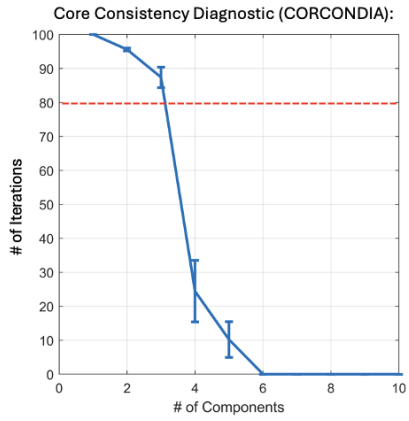

Figure 4: Core Consistency Diagnostic (CORCONDIA) Plot. Diagnostic used to determine the appropriate number of components that optimally represent the data by judging the core consistency value (y-axis) for n number of components. Red dashed line represents ideal threshold of 80% consistency.

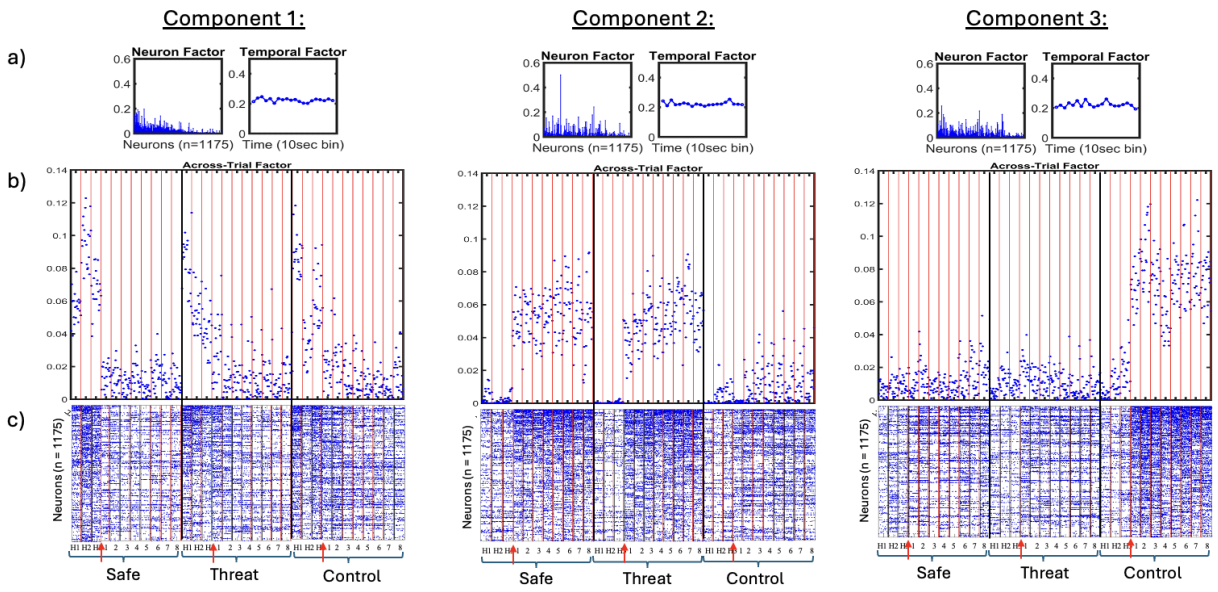

Figure 5: Tensor decomposition results (single subject data). (a) Neuron Factor and Temporal Factor subplots for Components 1-3. (b) Trial Factor subplot organized into three sections for each component. First third represents all 11 trials when the subject experienced the safe environment, starting from Day 1 Habituation through Day 8 of cFDL. The second third is similar to the first and represents when the subject experienced the threatening environment. The last third is similar to the first and represents when the subject experienced the familiar control environment. (c) Spike raster plot reorganized with neurons' calcium spiking activity based on the Neuron Factor value of the respective component (the more the neuron contributes to component, the higher the neuron factor value).

3.1 Tensor Decomposition Results

Tensor decomposition revealed groups of neurons that similarly engage during distinct time periods of the behavioral paradigm, and only when the subject is in a specific environment (Figure 5). Component 1 clustered neurons that were highly engaged when exposed to all three environments (novel safe, novel threatening, and familiar control) during the habituation phase of the behavioral paradigm. Component 2 clustered neurons that were highly engaged during cFDL with processing information during the novel safe and novel threatening environment exposures throughout the discrimination trials and not during the habituation phase, nor when experiencing the familiar control environment. Component 3 clustered neurons that were highly engaged during the familiar control environment exposures throughout the discrimination phase of the behavioral paradigm and not during the habituation phase.

These results show that tensor decomposition is a valuable method to identify factors within our data that cluster groups of neurons highly coactive and engaged in similar activity patterns.

4 Community Analysis

Graphs are useful models for representing and interpreting complex networks, such as brain networks, by using nodes to signify neurons and edges to signify connections between neurons [15]. Graphs are also extremely versatile and offer a variety of modifications to be applied to the algorithm in order to adjust the characteristics and outputs of the graph. Each neuron detected using calcium imaging is designated as a node. Calcium imaging is unable to provide synaptic resolution information to determine if a neuron is directly connected to another neuron via a synapse; therefore, we connect neurons based on their temporal firing patterns. If a neuron spikes around the same time as another neuron, they are connected with an edge. Neurons that fire within the same time window as another neuron have the highest weight and neurons that do not fire relatively close in time with another neuron are not connected with an edge. Additionally, we can add directionality to the neuronal network graph by only connecting nodes with a directed edge if the neuron spikes in the same time window or forward in time. With node and edge information, we generated adjacency matrices for each subject for each trial of the behavioral paradigm, where the adjacency matrices represent all neurons and their pairwise connections to other neurons within a trial. All adjacency matrices for each trial are summed into a cumulative adjacency matrix, representing the entire network of neurons during the behavioral paradigm. The summed edge weights maintain temporal information as a greater edge weight denotes frequently correlated firing between neurons within the same time window across multiple trials.

Next, we employed community analysis, an unsupervised technique to find optimized structure of nonoverlapping groups of

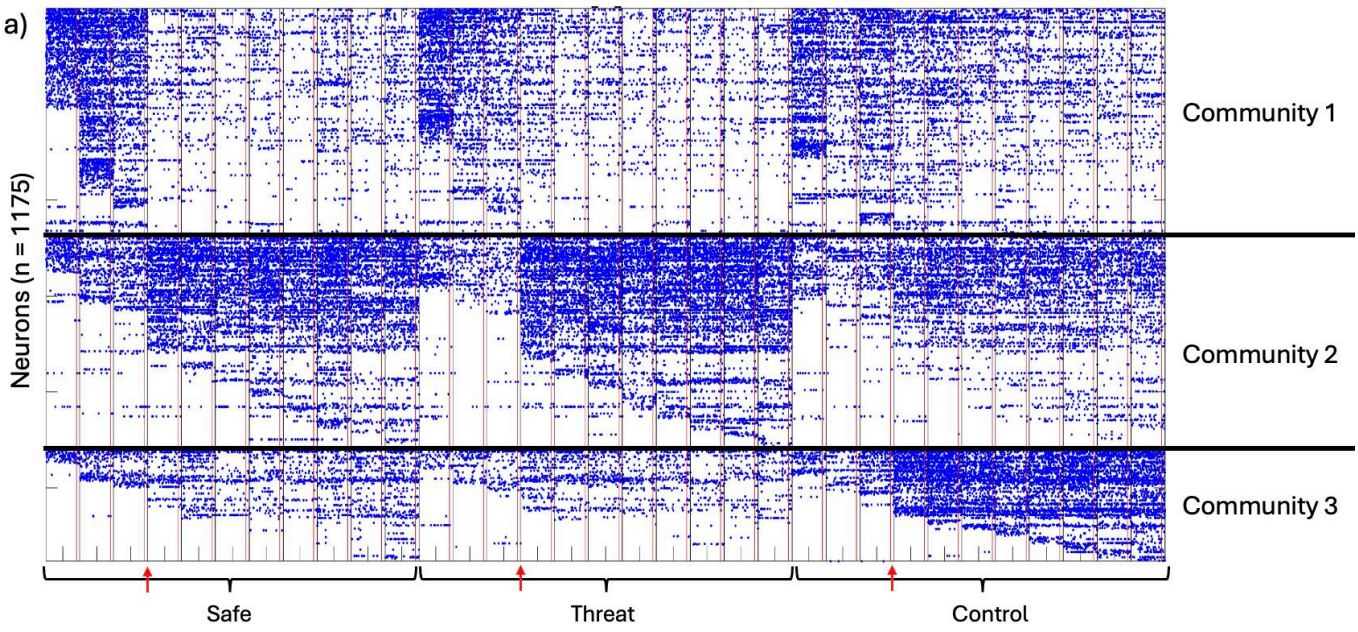

Figure 6: Community analysis results (single subject data). (a) Spike raster plot with neurons sorted and ordered based on their assignment to specific communities. The x-axis is organized into three sections with the first third representing all 11 trials when the subject experienced the safe environment, starting from Day 1 Habituation through Day 8 of cFDL, the second third similar to the first, representing when the subject experienced the threatening environment, and the last third similar to the first and representing when the subject experienced the familiar control environment.

nodes, on the cumulative adjacency matrix, in order to discover populations of neurons that are highly coactive [16]. There are several different production-quality community detection algorithms that can be utilized, each with different inputs, parameters, and outputs. We found the Louvain community detection algorithm to be best for modeling our data. The Louvain algorithm detects communities within large networks and maximizes the modularity score for each community [17].

4.1 Community Analysis Results

Community analysis detected three communities in which all neurons were segregated accordingly into (Figure 6). Community 1 consisted of neurons that exhibited similar activity when the subjects experienced the novel safe, novel threatening, and familiar control environments during the habituation phase of the behavioral paradigm. Community 2 was made up of neurons that exhibited similar activity in the novel safe and novel threatening environments during the discrimination phase of the behavioral paradigm. Community 3 contained neurons that displayed similar activity in the familiar control environment during the discrimination phase of the behavioral paradigm.

These results demonstrate the ability of graph-based community analysis to discover populations of neurons that exhibit similar spiking activity and segregate these neurons into discrete, orthogonal communities.

5 Conclusions

Ultimately, this research has critical implications for translational human research on coping with fear. Several techniques, such as fMRI recording on human participants, electroencephalogram recording on non-human primates, or calcium imaging on rodent models, can each be respectively applied to help develop our understanding of fear, ultimately leading to knowledge generation that can assist with the development of clinical solutions for fear related dysfunction, including generalized anxiety disorder and post-traumatic stress disorder [18, 19]. To investigate the large data outputs from these imaging techniques, we need to be able to apply knowledge discovery methods that can interrogate datasets, whether they are labeled or unlabeled. Additionally, this research aims to generate a path where interdisciplinary collaborations can successfully be applied through an application-driven development of data mining algorithm generation. Specifically, this work allows for the direct application of tensor and graph methods on investigating latent variables within large neuronal datasets currently being collected, ultimately providing the ability for cognitive and behavioral research to be enacted using an end-to-end data science approach. Furthermore, this framework can be applied to countless disciplines, including biology, physics, and sociology to name a few, and encourages application-driven algorithm generation [20]. Data mining techniques, such as tensor

decomposition and community analysis, will help us investigate and define neuronal activity and associated network dynamics within the brain that are unique and necessary to guide specific learning and behavior outcomes, such as learning to modulate and control fear.

Future directions include further investigations, using calcium imaging methodology and network manipulations, into the network activity within other structures implicated in the fear circuit. Additionally, other unsupervised techniques can be applied to this calcium imaging data set to interrogate how these populations of neurons interact in order to produce the appropriate behavioral output. Future work aims to determine the precise neuronal populations necessary for generating the behavioral output and to understand what perturbations to the system cause inadequate network functioning, resulting in unsuccessful threat perception and safety learning.

ACKNOWLEDGEMENTS

The authors would like to thank the several graduate students in the laboratory for their effort in completing this experiment, including Kylene Shuler, Justin Pastore, Tyler Bailey and John H. Spiegel III. This research was supported by grants to E.K. from DoD/ARL W911NF-23-1-0145, Brain and Behavior Research Foundation, and NIH/NIMH 5R01MH106617. Research was also supported in part by the National Science Foundation under CAREER grant no. IIS 2046086.

REFERENCES

[1] Sen, B., & Parhi, K. K. (2017, March). Extraction of common task signals and spatial maps from group fMRI using a PARAFAC-based tensor decomposition technique. In 2017 IEEE International Conference on Acoustics, Speech and Signal Processing (ICASSP) (pp. 1113-1117). IEEE.

[2] Williams, A. H., Kim, T. H., Wang, F., Vyas, S., Ryu, S. I., Shenoy, K. V., ... & Ganguli, S. (2018). Unsupervised discovery of demixed, low-dimensional neural dynamics across multiple timescales through tensor component analysis. Neuron, 98(6), 1099-1115.

[3] Corches, A., Hiroto, A., Bailey, T. W., Speigel, J. H., Pastore, J., Mayford, M., & Korzus, E. (2019). Differential fear conditioning generates prefrontal neural ensembles of safety signals. Behavioural Brain Research, 360, 169–184. https://doi.org/10.1016/j.bbr.2018.11.042

[4] Vieira, P. A., Corches, A., Lovelace, J. W., Westbrook, K. B., Mendoza, M., & Korzus, E. (2015). Prefrontal NMDA receptors expressed in excitatory neurons control fear discrimination and fear extinction. Neurobiology of learning and memory, 119, 52-62.

[5] Ghosh, K. K., Burns, L. D., Cocker, E. D., Nimmerjahn, A., Ziv, Y., Gamal, A. E., & Schnitzer, M. J. (2011). Miniaturized integration of a fluorescence microscope. Nature methods, 8(10), 871-878.

[6] Cai, D. J., Aharoni, D., Shuman, T., Shobe, J., Biane, J., Song, W., ... & Silva, A. J. (2016). A shared neural ensemble links distinct contextual memories encoded close in time. Nature, 534(7605), 115-118.

[7] Pnevmatikakis, E. A., & Giovannucci, A. (2017). NoRMCorre: An online algorithm for piecewise rigid motion correction of calcium imaging data. Journal of neuroscience methods, 291, 83-94.

[8] Zhou, P., Resendez, S. L., Rodriguez-Romaguera, J., Jimenez, J. C., Neufeld, S. Q., Giovannucci, A., Friedrich, J., Pnevmatikakis, E. A., Stuber, G. D., Hen, R., Kheirbek, M. A., Sabatini, B. L., Kass, R. E., & Paninski, L. (2018). Efficient and accurate extraction of in vivo calcium signals from microendoscopic video data. ELife, 7. https://doi.org/10.7554/elife.28728

[9] Sheintuch, L., Rubin, A., Brande-Eilat, N., Geva, N., Sadeh, N., Pinchasof, O., & Ziv, Y. (2017). Tracking the same neurons across multiple days in CA2+ Imaging Data. Cell Reports, 21(4), 1102–1115. https://doi.org/10.1016/j.celrep.2017.10.013

[10] Kolda, T. G., & Bader, B. W. (2009). Tensor decompositions and applications. SIAM Review, 51(3), 455–500. https://doi.org/10.1137/07070111x

[11] Papalexakis, E. E., Faloutsos, C., & Sidiropoulos, N. D. (2016). Tensors for data mining and data fusion: Models, applications, and scalable algorithms. ACM Transactions on Intelligent Systems and Technology (TIST), 8(2), 1-44.

[12] Brett W. Bader, Tamara G. Kolda and others, Tensor Toolbox for MATLAB, Version 3.6, www.tensortoolbox.org, September 28, 2023.

[13] Papalexakis, E. E., & Faloutsos, C. (2015). Fast efficient and scalable core consistency diagnostic for the PARAFAC decomposition for big sparse tensors. 2015 IEEE International Conference on Acoustics, Speech and Signal Processing (ICASSP). https://doi.org/10.1109/icassp.2015.7179011

[14] Bro, R., & Kiers, H. A. (2003). A new efficient method for determining the number of components in PARAFAC models. Journal of Chemometrics: A Journal of the Chemometrics Society, 17(5), 274-286.

[15] Fornito, A., Zalesky, A., & Bullmore, E. (2016). Fundamentals of brain network analysis. Elsevier Academic Press.

[16] Rubinov, M. (2015-2016), Community Louvain code. University of Cambridge.

[17] Traag, V. A., Waltman, L., & Van Eck, N. J. (2019). From Louvain to Leiden: guaranteeing well-connected communities. Scientific reports, 9(1), 5233.

[18] Canteras, N. S., Resstel, L. B., Bertoglio, L. J., de Pádua Carobrez, A., & Guimaraes, F. S. (2010). Neuroanatomy of anxiety. Behavioral neurobiology of anxiety and its treatment, 77-96.

[19] Haber, S. N., Liu, H., Seidlitz, J., & Bullmore, E. (2022). Prefrontal connectomics: from anatomy to human imaging. Neuropsychopharmacology, 47(1), 20-40.

[20] Rolnick, D., Aspuru-Guzik, A., Beery, S., Dilkina, B., Donti, P. L., Ghassemi, M., ... & White, A. (2024). Application-Driven Innovation in Machine Learning. arXiv preprint arXiv:2403.17381.