OpenScope Call for *In Vivo* Neurophysiology Experiments in Mice

Opportunity Number: U24NS113646-RFP-2024

Purpose: The OpenScope program is soliciting proposals for experiments to be carried out using the Allen Brain Observatory *in vivo* electrophysiology and two-photon imaging platforms in the brains of mice. Data from the proposed experiments will be collected by skilled operators at the Allen Institute and will be packaged in a standardized format and distributed to selected teams for their own analysis.

We anticipate selecting three to four projects this cycle. The projects will be distributed across two Allen Brain Observatory rigs: Neuropixels electrophysiology or two-photon imaging. Recordings from either rig will be performed in brain areas of mice viewing a stimulus monitor. In this year's call for proposals, mice can be either passively viewing a screen (similar to the 2021 RFP) or performing a detection of visual change task (added in the 2022 RFP).

The resulting cellular data (spiking activity and segmented ROI) and meta-data will be delivered to the applicant team for their own subsequent analysis and publication. Experiments should be designed to address fundamental questions related to the function of the mammalian neocortex and associated structures in health or disease.

Key dates

Letter of Intent Due: September 10th, 2024 (5 pm Pacific)
Full Proposal Due: November 12th, 2024 (5 pm Pacific)

Eligibility: This opportunity is available to national and international applicants at any career level or type of institution, provided they are not employed by the Allen institute.

Note: This is not a funding opportunity; no money will be distributed to selected applicants. Instead, a selected proposal provides access to fully funded data collection activities at the Allen Institute (funded by the NIH Brain Initiative U24 grant awarded directly to the Allen Institute). This award also provides support for one team member to take one trip to the Allen Institute. No monetary support for activities outside the Allen Institute is provided to selected applicants. Therefore, each applicant must ensure they have the resources and funding to execute all other portions of their proposed work, including a data analysis plan and expected efforts towards publication (first on bioRxiv and subsequently in a peer-reviewed scientific journal).

Contact information: Applicants are highly encouraged to get in touch with openscope@alleninstitute.org to seek advice throughout the application process. Communication with the OpenScope team is strongly recommended to confirm that proposals comply with the technical capabilities of the Allen Brain Observatory platforms.

Background

Launched in 2016, the Allen Brain Observatory consists of a set of standardized protocols, rigs, and quality control procedures for recording large-scale neural activity from the brains of awake mice. The original platform (based on single-plane two-photon microscopes) was used to survey over 60,000 neurons from 14 different transgenic mouse lines and six cortical visual areas (de Vries et al., 2020). A free, searchable summary of this survey (available at observatory.brain-map.org) allows researchers to explore neuronal responses to diverse visual stimuli in an unbiased way. Subsequent surveys have added recordings of spiking activity of ca. 100,000 neurons with Neuropixels probes (Siegle et al., 2021) as well as physiological recordings in the context of a visually guided behavior task (Garrett et al., 2020).

While this survey-style approach has yielded valuable datasets, it should be combined with more focused, small-scale experiments to unravel the complexity of the brain. Thoroughly evaluating models of cortical function necessitates targeted experiments using novel stimulus sets and/or recordings from specific cell populations. To that end (and thanks to funding from a NIH BRAIN Initiative U24 award), we are providing external scientists with the opportunity to leverage the Allen Institute's cutting-edge data generation platforms to generate data sets that these scientists can then further analyze. The primary goals of this program, called "OpenScope," are:

- To test hypotheses concerning neuronal function using large-scale measurements of neuronal activity in awake mice.

- To establish a new mode of knowledge generation in systems neuroscience, analogous to observatories in astronomy and particle accelerators in high-energy physics. These "brain observatories" will feature open designs and standardized operating procedures, rigs, processing pipelines and data and meta-data formats, allowing clinical and basic research neuroscientists to test emerging theories on state-of-the-art instrumentation and under standardized conditions, yielding reproducible data.

In the current call for proposals, we will consider hypothesis-driven experiments that address important open questions in the domain of mammalian cortical computation and which fit within the constraints of our data collection platforms. We believe that OpenScope will help scientists accelerate their research timelines and ultimately pave the way for a new way of making discoveries in systems neuroscience.

Experimental Capabilities

All data collection will be performed on the Allen Brain Observatory two-photon imaging or Neuropixels electrophysiology platforms. Each application can leverage either two-photon imaging or Neuropixels electrophysiology; proposals that require both platforms are not possible

at this point. To guarantee the highest level of standardization and data quality, we will only use our existing validated hardware.

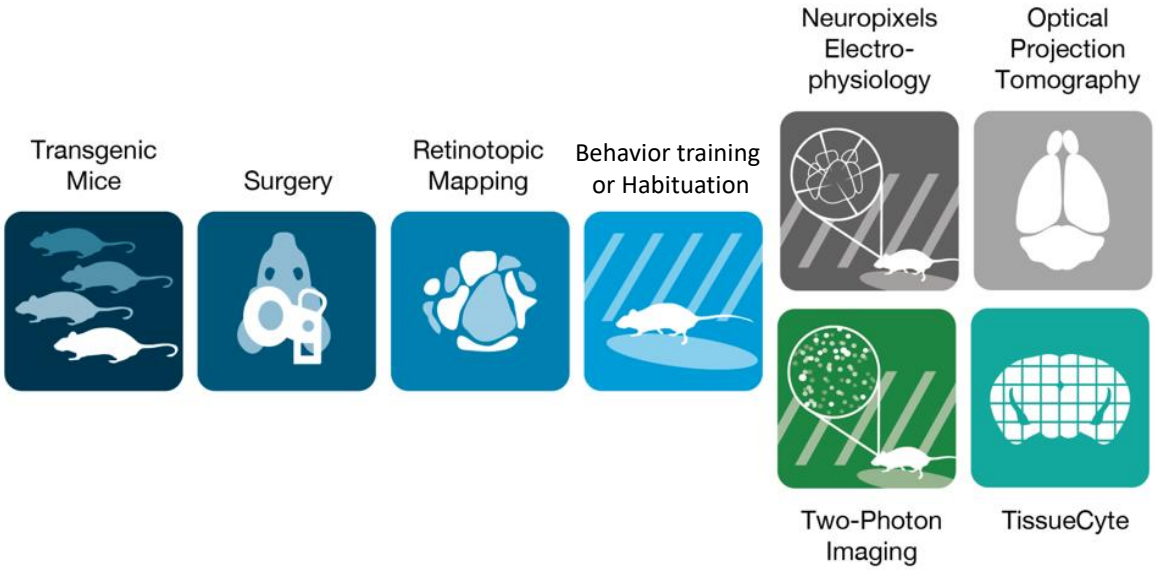
Projects this RFP supports:
. Possible experiments include:

Neuropixels recordings

- Single session recordings only (no chronic recordings)
- Recordings from brain areas within our standard 5 mm craniotomy window (see the list of brain areas below) or using a larger custom implant to access non-visual areas.
- A set of visual or non-visual recordings areas (see Neuropixels section)
- Mice passively viewing a screen
- Mice trained on the detection of visual change task (see Appendix). We will train mice with our standard protocol and allow modifications of the behavioral protocol during the recording day(s).

Two-photon mesoscopic imaging

- Chronic recordings
- Visual brain areas (see list in the two-photon section below)
- A set of new non-visual recordings areas across the dorsal cortex are now possible (see optical section).
- Mice passively viewing a screen
- Mice trained on the detection of change task (see Appendix). We will train mice with our standard protocol and allow modifications of the behavioral protocol during the recording day(s).



Experimental Methods

Surgery: A titanium headframe is secured to the mouse skull, and a craniotomy is drilled over the cortex and replaced with a coverslip.

For two-photon imaging, a single 5 mm glass coverslip is positioned over the visual cortex or a large custom glass implant over the entirety of the left dorsal cortex (a variant of the crystal skull).

For Neuropixels electrophysiology, two types of surgical preparations are available: a 5mm window that focuses on the visual cortex, or a larger craniotomy that enables broader cortical access. This second surgery gives access to cortical regions on the dorsal part of the left hemisphere. This surgery involves a plastic skull implant that has holes through which individual probes can be inserted and is more technically challenging than the 5mm window.

Retinotopic mapping: Intrinsic signal imaging is used to identify the boundaries and retinotopic layout of major cortical visual areas.

Habituation: Mice are gradually acclimated to head fixation and visual stimuli over the course of two weeks.

Behavior: Mice can be trained to perform a go/no-go visual change detection task in which they report when they perceive a stimulus change (see Appendix). The identity and timing of the visual stimuli, in addition to a few other parameters, can be modified by the experimenter.

Data collection: Physiological data is collected from awake mice viewing a visual stimulus monitor. In parallel, one eye camera, body camera, and face camera can be used to monitor mouse behavior. Pupil size and gaze location are automatically extracted from the eye camera video. Mice are free to run on a rotating disk, the position of which is also tracked throughout the experiment.

***Ex vivo* imaging:** Post-mortem brains for each mouse are either imaged using a TissueCyte system (2P imaging) or optical projection tomography (electrophysiology). In the electrophysiology experiments, this data is used to precisely register each recorded neuron to a 3D location in the Allen Mouse Common Coordinate Framework.

Data packaging: Datasets are packaged as standardized NWB files and uploaded to DANDI data archive (https://dandiarchive.org/). Additional metadata (for example, raw physiology data or behavior videos) are available upon request via an AWS S3 bucket.

Pilot experiments: A small pilot dataset will be used to validate key components of the experimental design. The pilot will be collaboratively designed by the Allen Institute and the

external project team. It is intended to facilitate the success of eventual “production” experiments, not to increase the overall size of the dataset.

Visual and behavior stimuli

Behavior and visual stimulation are provided by selected teams and must fulfill the following requirements:

- Programmed in Python. We will provide all necessary software dependencies to the selected teams.
- For passively viewing sessions, as many different visual stimulus protocols as needed can be presented during the experiments, although we encourage as simple an experimental design as possible to address the scientific question.

For behavior sessions, **Appendix I** (Behavior training task at the Allen Institute**)** describes the details of the Allen Institute behavior pipeline [Garrett M, et. al] and possible modifications for this call. **Applicants are encouraged to contact** openscope@alleninstitute.org

All visual stimuli will be presented on a 51.8 x 32.4 cm monitor placed 15 cm from the mouse’s right eye. The visual stimuli cover a 120º x 95º span of the mouse’s right visual hemifield and are warped to ensure visual angles are consistent across the entire screen.

Stimuli must be programmed in Python using the PsychoPy library. The following stimulus types have been previously implemented on our rigs:

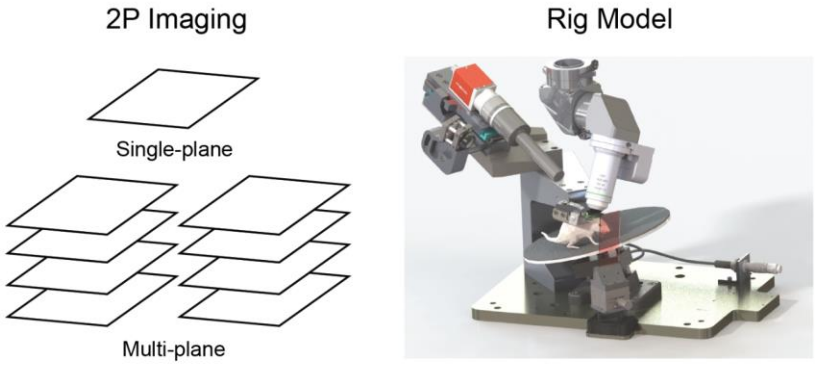
- Natural movies (presented at 30 Hz)
- Images of natural or artificial scenes
- Drifting gratings (with varying direction, spatial frequency, temporal frequency, and contrast)
- Static gratings (with varying orientation, spatial frequency, phase, and contrast)
- Gabor patches (used for receptive field mapping)
- Locally sparse noise (used for receptive field mapping)
- Full-field flashes
- Dot motion (with varying direction, speed, dot size, and density)
- Detection of change task

We will provide all necessary software dependencies to the selected applicant teams to validate their stimuli before deployment.

- For passively viewing sessions, as many different visual stimulus protocols as needed can be presented during the experiment, within the limit of each session duration (see below for imaging- and electrophysiology-specific recording times). However, we encourage teams to use the **simplest possible experimental design** that addresses the scientific question at hand.
- For behavior sessions, **Appendix I** describes the details of the Allen Institute behavior pipeline [Garrett M, et. al] and possible modifications for this call. We will consider

applications that add simple auditory cues. **Applicants are encouraged to contact** openscope@alleninstitute.org **to seek advice if a particular modification is not listed.**

Two-Photon Imaging Platform



Recording devices:

- Single-plane microscopes can sample at 30 Hz from one 400 x 400 µm field of view per session.
- Multi-plane microscopes can sample at 10 Hz from 8 different 400 x 400 µm fields of view at a time. Each field of view encompasses one area and one depth, with a maximum of 4 simultaneously recorded areas. Planes are positioned in pairs as in Orlova, Tsyboulski, Najafi et al, 2020. See **Appendix II – Imaging experimental variants** for more details.

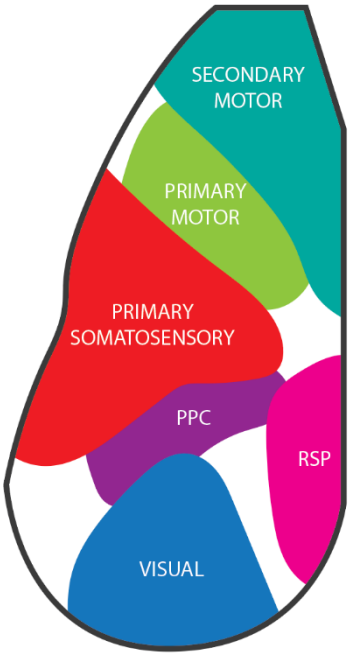
When choosing between single-plane or multi-plane imaging, each project should carefully consider their experimental needs. For example, cell matching across many sessions will be more accurate with single-plane imaging, while multi-plane imaging will provide more simultaneously recorded cells per session.

Available brain areas:

There are two possible rig configurations with different surgical preparations.

- Configuration 1: A 5mm window over the visual cortex. Retinotopic targets are available in the following visual cortical areas: V1, LM, PM, AL, AM, RL. Targeting of other brain areas within the 5 mm window is possible, provided no objective collisions occur. Those targets may be specified by their location relative to retinotopically mapped visual areas.

- Configuration 2: A left dorsal skull implant. This is using a laser-cut, bended glass coverslip. This configuration allows targeting of cortical regions beyond the visual cortex. The accessible regions include dorsal cortical areas such as the visual cortex, the somatosensory cortex and the motor cortex. Areas will be targeted based on offset from the center of V1 and vascular anatomy, as measured using intrinsic imaging (see image below). We will have sufficient precision to target areas listed on this figure but not sub-nuclei. We encourage teams to to **contact** openscope@alleninstitute.org **to seek advice with this newer implant.**



Experiment duration: Each session may be up to 70 minutes in duration. Individual neurons can be reliably tracked across a maximum of 4 sessions.

Cohort size: Up to 90 sessions can be collected across a maximum of 10 mice. Mice will be 70 to 120 days old upon recordings of neuronal activity.

Transgenic mice: Any Cre-driver mouse line currently used in the Allen Brain Observatory is available, along with intermediate driver and reporter lines; see **Appendix IV – Cre lines** for a table of mouse lines and associated details.

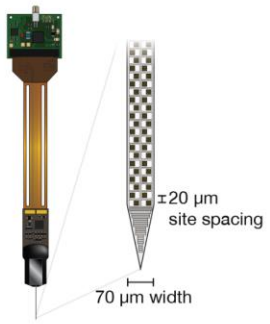
Data processing: Data will be processed with our automated pipeline, including motion correction, cell segmentation, demixing, neuropil subtraction, ΔF/F normalization, and session-to-session cellular alignment. Calcium fluorescence traces will be synchronized with visual stimuli prior to packaging in NWB files. Further analysis will have to be performed by the awarded team.

See **Appendix III – Data generation plan** for example experimental designs for single-plane and multi-plane two photon imaging.

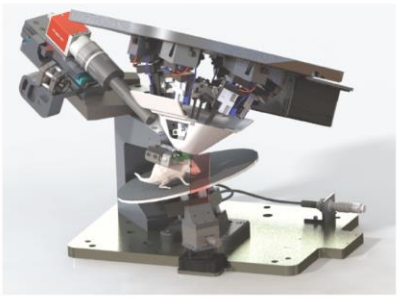
One year after data collection ended: The resulting data will be under an optional embargo for the first year to give the applicant teams advanced access. After that year, the embargo will be lifted and the datasets publicly released on DANDI in the form of NWB files.

Neuropixels Electrophysiology Platform

Neuropixels Probes



Rig Model



Recording devices: Neuropixels 1.0 probes (Jun et al., 2017) contain 384 recording sites distributed across 3.84 mm of a 70 µm wide shank. Each site is sampled at both 30 kHz (AP band) and 2.5 kHz (LFP band).

Available brain areas: Electrophysiological recordings will use standardized rigs with six independently movable Neuropixels probes. There are two possible rig configurations with different surgical preparations.

- Configuration 1: A 5mm window over the visual cortex. Each probe can be targeted to a retinotopically aligned sub-region of the following cortical visual areas: V1, LM, AL, RL, AM, and PM. The probes also typically record from CA1, CA3, and DG in hippocampus, LGN and LP in the thalamus, and APN in the midbrain. For subcortical areas, precise targeting is not available, and recordings from these areas are not guaranteed in every experiment.
- Configuration 2: A left dorsal skull implant. This configuration allows targeting of cortical regions beyond the visual cortex. The accessible regions include: VIS, RSP, S1 (except lateral part), M1, M2, ACA. Some subcortical regions can also be targeted in this configuration including LGN, LP, SC (except medial SC), STRd, and CA1.

Experiment duration: All visual stimuli, including any spontaneous intervals, must fit within a 2-hour block.

Cohort size: Experiments can be performed so as to deliver up to 10 validated mice data, with one session per mouse.

Transgenic mice: Experiments can optionally be carried out in mice expressing ChR2 in Parvalbumin (PV)-positive or Somatostatin (SST)-positive neurons. This makes it possible to identify these neurons during an “opto-tagging” interval performed at the end of each session. It is not currently possible to deliver light pulses to activate ChR2+ neurons in conjunction with visual stimulation. See **Appendix IV – Cre lines** for a table of mouse lines and associated details.

Pharmocological drug injection: We will consider in vivo drug injections (intraperitoneal

or subcutaneous injections) that are compatible with neuronal recordings. We encourage to reach out the OpenScope team (openscope@alleninstitute.org) to discuss specific drugs and protocols possible.

Data processing: Raw continuous data is processed by the Kilosort spike sorting algorithm, which extracts times and cluster IDs for all spikes in the dataset. Artifactual "noise" clusters are removed from the Kilosort outputs, and a battery of quality metrics are computed for the remaining clusters. The spike times for each "unit" are synchronized to the visual stimuli and packaged in NWB files along with their associated quality metrics, to facilitate automated selection of units to include for analysis. No manual curation is performed on the units prior to packaging. LFP data is also available for each experiment. Further analysis will have to be performed by the awarded team.

See **Appendix III – Data generation plan** for example experimental designs for Neuropixels electrophysiology.

One year after data collection ended: Collected datasets will be publicly released on DANDI in the form of NWB files.

Application Instructions

Applications follow a two-stage process:

1. Applicants submit a two-page **Letter of Intent** (due September 10th, 2024) that briefly describes their proposed hypothesis and experimental plan.

2. After evaluating the Letters of Intent, a maximum of 15 teams will be asked to submit a six-page **Full Proposal** (due November 12th, 2024) that includes a detailed description of the experiments to be run by the Allen Institute, as well as the analysis to be carried out by the project team.

Up to three to four Full Proposals will be selected (acceptance rate of ~20%).

Letter of Intent

The Letter of Intent consists of three sections:

1. Motivation – Describe the hypothesis to be tested and the current state of knowledge related to this topic. Specify 1-2 aims the proposal will address.
2. Experimental Design – Describe the experimental design and how it addresses the hypothesis at hand. This section should clearly indicate the platform that will be used (single-plane imaging, multi-plane imaging, or Neuropixels electrophysiology).

3. Analysis Plan – Briefly describe how the newly generated dataset will be used to test the proposed hypothesis. Briefly describe your personnel commitments to this data analysis if awarded (without mentioning names, for instance "PhD student: 100% effort", "Postdoc: 50% effort", etc.)

Formatting Guidelines

- Total length should not exceed 2 pages (including figures). No supplemental data that exceeds the 2-page limit will be reviewed.
- A bibliography may be provided and is not included in the 2-page limit.
- Please name the file using the following convention: 2024_LOI_[Project_title]
- The Letter of Intent should be submitted in PDF format
- For additional formatting details (font size, color, type density, citations, orientation, paper size and margins), follow the guidelines provided by the NIH (https://grants.nih.gov/grants/how-to-apply-application-guide/format-and-write/format-attachments.htm)

Important: Reviewers will be blinded to the identity of the applicant, collaborators, and their organizations. Applications that identify the applicant, collaborators, or their organizations in the main text of the proposal will be rejected for noncompliance. A document describing common blinding mistakes is included in **Appendix V – Blinding mistakes**.

Selection Process

Each Letter of Intent will be evaluated based on the quality of the hypothesis and the feasibility of running the experimental design and the associated analysis, given the capabilities of the Allen Brain Observatory.

A team of blinded internal Allen Institute staff will first review the LOIs for compliance with the RFP and technical feasibility on Allen Institute pipelines. Technically feasible LOIs will then be sent to blinded external reviewers outside of the Allen Institute, who will rank the proposals on scientific merit. Technically feasible LOIs with the highest scientific merit scores will be advanced to the next round. A maximum of 15 LOIs will be selected for full proposal invitations.

Importantly, proposals should offer a good fit between the experimental and scientific needs. A balanced application will not necessarily leverage all available platform capabilities or recording bandwidth but will instead propose the minimal dataset required to answer the question at hand. The anticipated scientific impact will be used to rank LOI proposals, if necessary, to keep our full proposal acceptance rate at around 20% or higher. A maxiumum of 15 LOIs will be selected for full proposal invitations.

Full Proposal

The Full Proposal should consist of four sections:

1. Outline and Motivation (1-2 pages including figures)
    a. Describe the current state of knowledge in the field related to the proposed question/hypothesis.
    b. Specify 1-2 aims the proposal will address.
    c. (Optional but recommended) Describe a preliminary analysis that was carried out on public data from the Allen Brain Observatory, and explain why the currently available datasets are insufficient for addressing the question at hand.
2. Experimental Design (1-2 pages including figures)
    a. Describe the experimental design and how it will provide insight into the proposed question/hypothesis. Care should be taken to address all potential outcomes, including a null result.
    b. Describe the rationale of the experimental design broken down by aim(s).
3. Analysis Plan (1-2 pages including figures)
    a. Describe the metrics and analysis steps that will be used, separated by aim.
    b. As in the “Motivation” section, preliminary analysis on available Allen Brain Observatory data will strongly support the feasibility of the analysis plan.
    c. Briefly describe your personnel commitments to this analysis if awarded (without mentioning names, for instance "PhD student: 100% effort", "Postdoc: 50% effort", etc.)
4. Diversity statement (1/2 page, max 150 words)
    a. Describe how awarding your team will support diversity and inclusion in neuroscience. Your statement could identify how awarding your project helps to grow and develop under-served communities in experimental neuroscience. This can include theoretical scientists will less access to experimental methods and/or groups and universities with minimal access to neuroscience infrastructure.
    b. While you write this paragraph, keep attention to not revealing your team identity to maintain blinding of your proposal.

In addition, each graduate student and postdoc member of an applicant team *must* supply a **letter of support** from a lab head at your home institution indicating that they are eligible to apply for this opportunity and that their institution will support them in meeting the deliverables if their team is selected (see below for an approximate project timeline).

Formatting Guidelines

- Total page count should not exceed 6 pages (including figures). No supplemental data that exceeds the 6-page limit will be reviewed.
- A bibliography may be provided and is not included in the 6-page limit.
- Please name the file using the following convention: 2023_FULL_[Project_title]
- The proposal should be submitted in PDF format
- For additional formatting details (font size, color, type density, citations, orientation, paper size and margins), follow the guidelines provided by the NIH (https://grants.nih.gov/grants/how-to-apply-application-guide/format-and-write/format-attachments.htm)

Important: Reviewers scoring individual proposals will be blinded to the identity of the applicant, collaborators, and their organizations. Applications that identify the applicant, collaborators, or their organizations in the main text of the proposal will be rejected for noncompliance. A document describing common blinding mistakes is included in **Appendix V – Blinding mistakes**.

Selection Process

The Full Proposals will be scored based on four criteria:

1. Scientific impact of the proposed question/hypothesis
2. Quality of experimental design and feasibility of implementation
3. Quality of data analysis plan
4. Potential to expand access and representation in the Systems Neuroscience community

Applications that depend on experiments that do not fit within the technical capabilities of the call will not be eligible for selection. Within these boundaries, the technical complexity of proposals will be taken into account and weighted along with their scientific impact. We encourage applicants to consider proposals with the a high chance of experimental success.

Full proposals will be reviewed by blinded internal Allen Institute reviewers, who will again evaluate the projects for technical feasibility on Allen Institute pipelines. These internal reviewers are scoring the likelihood that the experimental methods, as described, will be successfully completed within the proposed time and resources.

The internal technical risk score will be balanced with an external review of Scientific Merit. Blinded reviewers external to the Allen Institute will evaluate the proposals for scientific impact, ability of the experimental plan to answer the proposed hypothesis, and potential for the applicant to expand community access and representation.

Top proposals will be sent forward to the Steering Committee for the selection of the final 3 awards. The Steering Committee considers overall balance of the three selected projects, including the balance of technical feasibility to scientific merit, and the diversity of projects across the community. The final selection by the OpenScope Steering Committee may be partially unblinded to ensure a balance of selected proposals across the community and institutions.

Up to three Full Proposals will be selected. All applicants will be notified of the decision about their proposal.

Project Timeline

Below is a draft timeline for executing selected projects; the exact schedule will be determined at the drafting of the Collaborative Research Agreement (CRA) before the winning project start date. Applicants should notify their relevant legal team about their OpenScope application well before the selection decision to avoid any delays in reviewing and signing the CRA. Successful completion of each project requires awarded teams meet the agreed upon schedule (including timelines for teams to supply visual stimulus code to the Allen Institute):

- **January 2025 - February 2025:**
    - Virtual project kickoff meeting; initiate a collaboration agreement with the applicants' institution.
    - Required transgenic mouse line(s) are selected for breeding.
    - External teams will work with the OpenScope team to draft a document outlining a small set of pilot experiments. As part of this effort, selected teams will provide initial visual stimulation code for testing. The goal of this pilot is to test and iterate the visual stimulation code and important aspects of the experimental design, as well as key components of the analysis plan.

- **March 2025 - July 2025:** Upon completion of the pilot project, external teams will execute their analysis plan and provide an updated experimental design and simulation code for the final data collection effort.

- **January 2025 - December 2025:** One member of each external team will be invited for a one-week visit to the Allen Institute to shadow the data collection effort and be introduced to our data formats and data processing pipeline. The precise visit date will be chosen in collaboration with the Allen Institute to have the best impact for the success of the project.

- **March 2025 - March 2026:** Production datasets will be shared with external teams as early as possible in the data collection process through shared online repositories (AWS, DANDI). Datasets will be shared with application teams as NWB files are uploaded to the Cloud (https://gui.dandiarchive.org/#/), Unless requested to be immediately shared, files will be embargoed from public view on DANDI for one year.

- **2025 – 2026:** External teams will be responsible for execution of the data analysis plan in the year following data collection. Teams will provide a written report outlining the results of the analyses to the Allen Institute, along with commented analysis code used to generate all individual figures. This report will be used to evaluate the future of the program, as well as to plan publication of this work with external teams.

- **One year after data collection ended:** Embargo will be lifted. Collected datasets will be publicly released on DANDI.

- **Late 2026:** The outcome of this work will be published in high-impact journals in collaboration with the Allen Institute. Collaborator(s) are expected to lead publication of their results along with contributing members at the Allen Institute. In parallel to a scientific publication, we will encourage the team to consider a data publication such as (Gillon, C. J. *et al*. *Sci Data* **10**, 287 (2023). This will broaden the impact of their dataset when it is released.

Confidentiality Notice

The Allen Institute will treat all applications as confidential. Information in the proposals will not be shared beyond the Allen Institute and the scientific review panel.

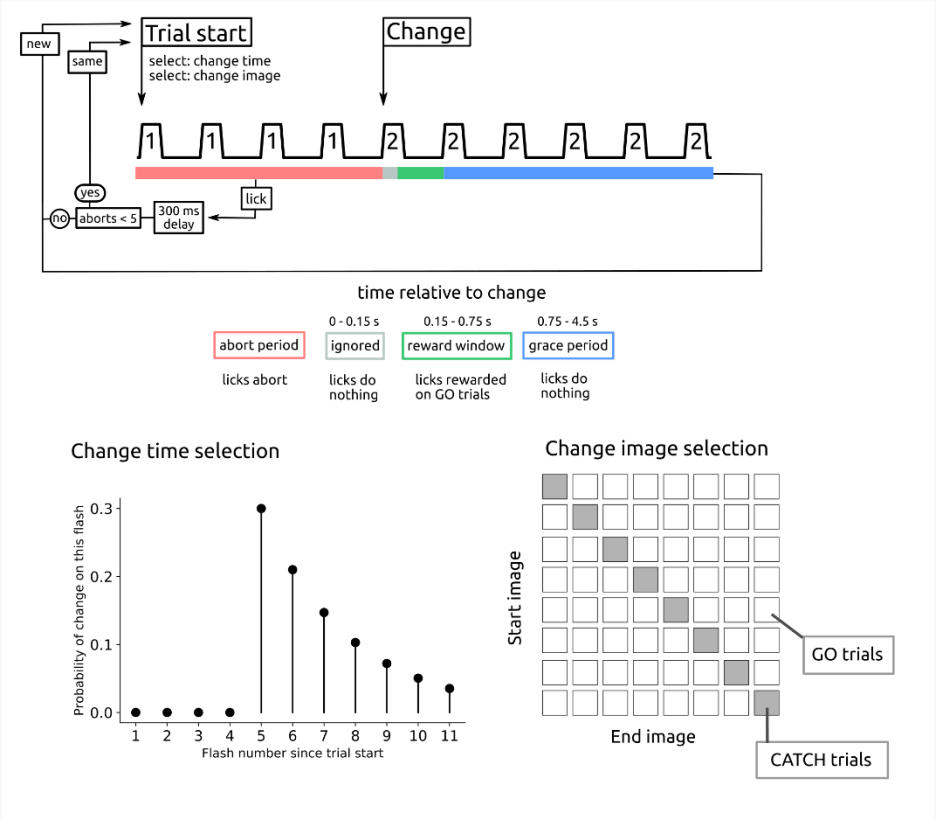
If you would like to have an explicit confidentiality agreement associated with your proposal, please reach out to openscope@alleninstitute.org prior to submission.

APPENDIX I - BEHAVIORAL TASK AT THE ALLEN INSTITUTE.

At the Allen Institute we have developed a behavioral task we call "Detection of Change" that motivates mice to lick in response to changes in visual stimuli. This task uses the "go, no-go" paradigm where animals should withhold responding until they perceive a change.

We can measure performance in this task by comparing the correct response rate to stimulus changes (during “go” trials), and the false-alarm rate when the stimulus does not change (during “catch” trials). Animal performance is monitored each day, and we can automatically transition animals from easy to difficult versions of this task using their daily performance metrics.

Below is a flow-chart of the task logic during a single behavioral trial where an image changes (from '1' to '2'). While task logic is most easily described on a trial-by-trial basis, from the animal's perspective there is no indication when trials start or end; animals simply perceive a continuous series of visual stimuli that change pseudo-randomly according to the parameters of the task logic.



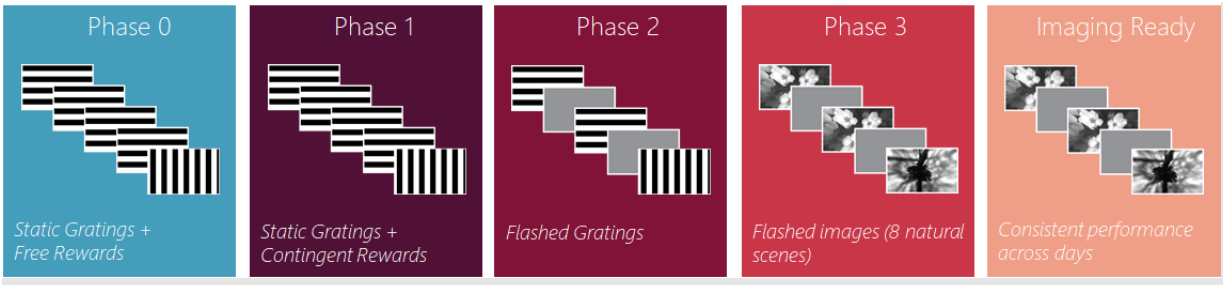
For additional information, we encourage investigators to consult the online materials describing the Allen Institute Visual Behavior – 2P pipeline:

https://portal.brain-map.org/explore/circuits/visual-behavior-2p

Also, please see the following study that uses this detection of change task and explores stimulus novelty responses in the visual cortex: https://elifesciences.org/articles/50340

Training Procedure

Animal training occurs during 4 stages, each adding a level of complexity to the previous stage.



Habituation:

Habituation occurs for 5 days before stage 0 training. It consists of increasingly long periods of head-restraint to the headframe and running wheel while in the behavioral training environment.

Stage 0:

The purpose of stage 0 is to present the lick spout and water rewards for the first time. Mice are given a single 15-minute session with a static grating that randomly changes orientation (from 0 to 90 degrees), and water rewards are given on every change; thus, rewards are not contingent on licking.

Stage 1:

The purpose of stage 1 is to learn task rules using an easy discrimination between two static gratings that differ in orientation by 90 degrees. At the start of each session, animals receive warm-up trials (lick-non-contingent rewards) that teaches licking behavior. After the warm-up trials, animals are required to lick in response to a change stimulus in order to get rewards. Performance must be above a threshold (peak d' > 2 in a 100 trial rolling window) for 2 of 3 consecutive days to exit stage 1.

Stage 2:

In stage 2 a 500ms grey period is introduced between 250 ms stimulus presentations. A flashing stimulus makes the perception of change more difficult because it requires a visual short-term memory of the previous stimulus. Graduation to stage 3 happens after performance in 2 of 3 consecutive days (potentially including days in stage 1) is above a threshold (peak d' > 2 in a 100 trial rolling window).

Stage 3:

In stage 3, the visual stimuli change from gratings to natural images, and stimulus flashing is maintained. Graduation to stage 4, where reward volume is decreased, occurs automatically after 3 sessions.

Stage 4:

The only difference between stage 3 and stage 4 is that the reward volume decreases, which typically increases the number of trials a mouse will perform before losing motivation.

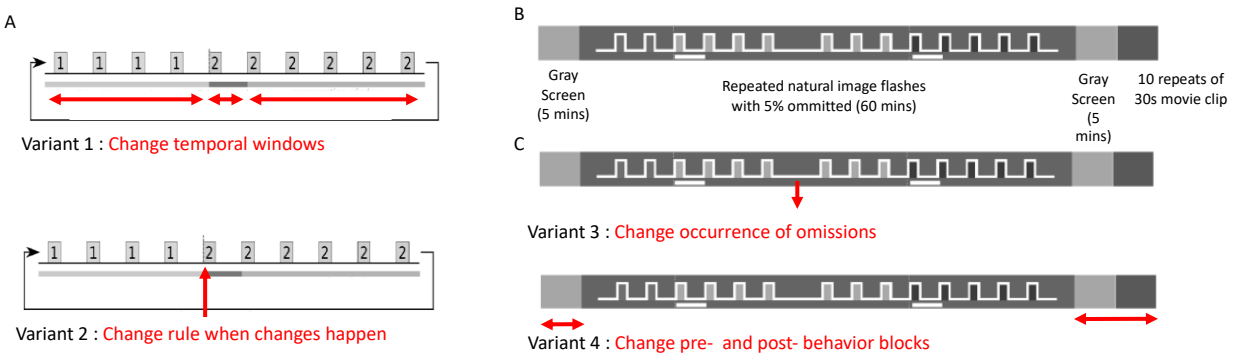
Imaging ready:

Mice are ready to be recorded on a physiological rig when they meet an engagement criterion as well as a performance criterion. For at least three days, they habituate to the environment of the physiological rig by being head-fixed to the headframe and running wheel while viewing stage-4 flashing visual stimuli. After habituation, imaging sessions may begin.

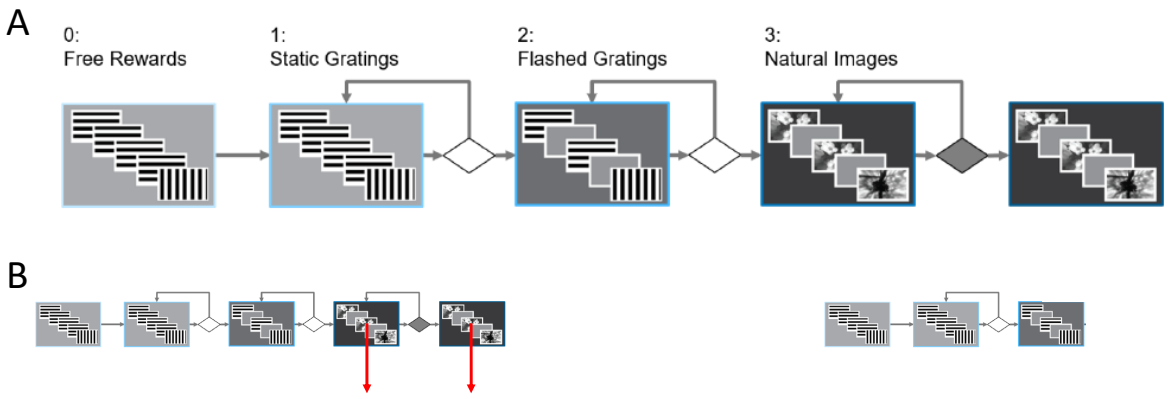
Guidelines and advice for task modification

When designing an OpenScope project using the Change-Detection task, investigators have the option to change one or more behavioral parameters (**see the 2 figures in this section**). Some changes to this task are untested by Allen Institute scientists and may result in a degradation or complete loss of animal performance. It is our experience that some changes result in a loss of motivation, and others cause a switch to a behavioral strategy that is not visually-guided, where licking may be either random or dictated by timing. If investigators wish to propose task modifications, we ask them to follow some guidelines:

1. Investigators may choose to move the animal to imaging at any point after stage 0, however, we *strongly* encourage investigators to train animals at least through task learning (typically complete by the end of stage 1 or midway through stage 2).
2. We will habituate mice on the physiological rig using stimuli from the last stage of behavioral training, and we will begin imaging once stable behavioral performance is achieved under the microscope.
3. Any modifications to either task parameters or stimulus parameters (see parameter list below) is best done after the minimum 3-days habituation to the physiological rig.
4. Most behavioral logic parameters are fixed at the session level; one cannot randomize them trial-by-trial. For instance, it is not currently possible to randomly vary the change-time probability distribution by randomly choosing a "change_time_scale".
5. If grating stimuli are chosen, most stimulus parameters can be varied trial-by-trial.
6. It is possible to radically modify the task with small parameter tweaks, such as by making the change-time deterministic (for instance, if change_flashes_min=change_flashes_max). We warn investigators that some such modifications will result in a task where vision is no longer necessary to gain reward.



Temporal structure of the change detection task. A. The temporal windows of the task can be varied as well as the rules that determine when the changes happen. **B.** The current task includes unexpected omission events for which an image is replaced by a gray screen**.** In addition, the main behavior block is padded with gray screen blocks and a sequence of natural movle clips. **C**. Example variants include changes to the omission rate and/or the pre and post stimulus blocks.



A. General steps involved in training mice to perform the change detection task implemented at the Allen Institute. 4 phases automatically gate the graduation of mice based on standardized performance metrics. **B.** Potential general variant of the task. We recommend keeping the initial two stages of the behavior with gratings as they establish the general task structure. 2 general groups of variants are described in this figure. Additional variants involving changing the temporal structure of the task are described in **the previous figure**.

Example proposal task-flows

When proposing a behavioral task-flow for each experimental group, we ask that you use a format like the examples below:

Example 1:

In this simple example, training occurs through stage 4 and the image-set is changed while under the physiological rig.

	stage 1	stage 2	stage 3	stage 4	imaging habituation	imaging day 1	imaging day 2	imaging day 3	imaging day 4	imaging day 5	imaging day 6	imaging day 7	imaging day 8
Parameters	default	default	default	default	default	default	default	default	image matrix B	image matrix B	image matrix B	image matrix C	image matrix C

Example 2:

In this simple example, training occurs through stage 2 and grating orientation is changed while under the physiological rig.

	stage 1	stage 2	stage 3	stage 4	imaging habituation	imaging day 1	imaging day 2	imaging day 3	imaging day 4	imaging day 5	imaging day 6	imaging day 7	imaging day 8
Parameters	default	default	na	na	habituate using stage 2	stage 2 default	stage 2 default	stage 2 default	stage 2, 'sf' = 0.08 or 0.04	stage 2, 'sf' = 0.16 or 0.04	stage 2, 'sf' = 0.16 or 0.08	stage 2 default	stage 2 default

Default task parameters during training

Below is a table of most parameters that govern a Change Detection behavioral session.

We have greyed out parameters that we think should not be modified. With the exception of stage 0, all behavioral sessions will last 60 minutes. Note that all stimuli have a 20-35 ms monitor display lag.

Stage 1:

behavioral logic params

parameter key	default value	description
warm_up_trials	5	This controls the number of free rewards (rewards delivered following the change, not contingent on licking) at the beginning of the session. Designed to remind the animal of existence/position of the lick spout and the overall task structure.
abort_on_early_response	True	If True, the trial is restarted if the animal responds before the change. Aborts are designed to limit guess licking

catch_frequency	0.25	Probability of choosing catch trial probability of drawing catch trial: stimulus does not change
response_window	[0.15, 1.0]	Window following change in which lick is rewarded (min,max)after change, start/stop of reward availability
reward_volume	0.01	Volume in mL per reward. Not variable during session.
pre_change_time	2.25	Minimum time after trial start before which a change can be drawn. Reducing this to zero will eliminate much of the time penalty imposed on guessing; the mouse will be able to see a change (and thus get a reward) much sooner after a guess. However, if this is non-zero it is a fixed interval that the animal can learn, and it encourages timed-guessing behavior.
stimulus_window	6.0	This is the duration of the change_time_dist: the length of time when a change-stimulus can occur. This is not used in flashed change-detection, instead the start and stop of the stimulus window is set by change_flashes_min and change_flashes_max
change_time_dist	exponential	Distribution from which change times are drawn. This is exponential for all non-flashed stimuli (e.g. stage 0 or 1), geometric distribution is used for discrete (flashed) stimuli. Exponential distributions are censored by the length of the stimulus_window
change_time_scale	2.0	Parameter governing distribution (if exponential change_time_dist, this specifies its mean in seconds).

visual stimulus params

parameter key	default value	description
class	grating	Psychopy class of stimulus. stage 2 should avoid psychophysically demanding stimuli, but this can be accomplished with images other than gratings
sf	0.04	Spatial frequency
tex	sqr	Texture
size	(200,150)	Extent of stimulus window in degrees

phase	0.25	Phase of grating
groups	{vertical:[0,180], horizontal:[90,270]}	The parameter that changes can be specified here. We change only orientation by 90 degrees, but this can be changed to any other psychopy parameter that determines the stimulus shown (contrast, speed, spatial-frequency, etc).

Stage 2:

behavioral logic params

parameter key	default value	description
free_reward_trials	10	These are free (non lick-contingent) rewards that are given after N miss trials. They are used to remind the animal of the task during periods of inactivity
catch_frequency	0.25	The probability that a change will not occur at the change time. A response during a catch trial is counted as a "false alarm", and withholding response to a catch trial will be counted as a "correct-reject".
failure_repeats	5	Aborted trials are repeated up to N times. These trials have the same change image at the same change time. This only triggers on aborts, and not trials that reached the change time.
reward_volume	0.01	Volume of "HIT" trial rewards in mL
auto_reward_vol	0.005	Volume of warm-up trial free rewards, and of free-reward trial rewards
warm_up_trials	5	Controls the number of free rewards (rewards delivered following the change, not contingent on licking) at the beginning of the session. Designed to remind the animal of existence/position of the lick spout and the overall task structure.
timeout_duration	0.3	A fixed delay after an early lick. A small value (0.3 sec) is typically used.
periodic_flash	(0.25, 0.5)	(flash duration, inter-flash interval), in seconds. We can't reliably give short duration flashes, so 1, 2 or 3 frame flashes, which are at the psychophysical limit of detection, will be unreliable. However, image draw time is accurately recorded for post-hoc analysis.
response_window	(0.15, 0.75)	(min, max) time after change when a response is counted as a HIT or False_Alarm, in seconds

end_after_response	True	Trials can end one of two ways: they can end after response, which means that trials end at a fixed time after the change time. Alternatively, trials can end after a fixed duration that does not depend on the change time.
end_after_response_sec	3.5	Trial ends N seconds after a change
change_time_dist	geometric	Distribution from which change times are drawn: exponential for all non-flashed stimuli (e.g. stage 0 or 1), geometric distribution is used for discrete (flashed) stimuli
change_time_scale	0.3	The coefficient for the geometric distribution
change_flashes_min	4	Minimum number of stimulus flashes before a change-time can occur. Reducing this to zero will eliminate much of the time penalty imposed on guessing; the mouse will be able to see a change (and thus get a reward) much sooner after a guess. However, if this is non-zero it is a fixed interval that the animal can learn, and it encourages timed-guessing behavior.
change_flashes_max	12	Maximum number of stimulus flashes before a change-time can occur. This censors the end of the geometric distribution. Change times drawn outside this limit cause a resampling of the distribution until a change-time is drawn that is beneath this limit.
abort_on_early_response	True	If True, the trial is restarted if the animal responds before the change. Aborts are designed to limit guess licking

visual stimulus params

parameter key	default value	description
class	grating	Psychopy class of stimulus
sf	0.04	Spatial frequency
tex	sqr	Texture
size	(200,150)	Extent of stimulus window in degrees
phase	0.25	Phase of grating

groups	{vertical:[0,180], horizontal:[90,270]}	The parameter that changes is specified here. We change only orientation by 90 degrees, but this can be changed to any other psychopy parameter that determines the stimulus shown (contrast, speed, spatial-frequency, etc).

Stage 3:

behavioral logic params

parameter key	default value	description
free_reward_trials	10	These are free (non lick-contingent) rewards that are given after N miss trials. They are used to remind the animal of the task during periods of inactivity
catch_frequency	null (if even matrix sampling is used this defaults to 1/8 = 0.125)	The probability that a change will not occur at the change time. A response during a catch trial is counted as a "false alarm", and withholding response to a catch trial will be counted as a "correct-reject".
failure_repeats	5	aborted trials are repeated up to N times. These trials have the same change image at the same change time. This only triggers on aborts, and not trials that reached the change time.
reward_volume	0.01	volume of "HIT" trial rewards in mL
auto_reward_vol	0.005	volume of warm-up trial free rewards, and of free-reward trial rewards
warm_up_trials	5	controls the number of free rewards (rewards delivered following the change, not contingent on licking) at the beginning of the session. Designed to remind the animal of existence/position of the lick spout and the overall task structure.
timeout_duration	0.3	a fixed delay after an early lick. A small value (0.3 sec) is typically used.
periodic_flash	(0.25, 0.5)	(flash duration, inter-flash interval), in seconds. We can't reliably give short duration flashes, so 1, 2 or 3 frame flashes, which are at the psychophysical limit of detection, will be unreliable. However, image draw time is accurately recorded for post-hoc analysis.

response_window	(0.15, 0.75)	(min, max) time after change when a response is counted as a HIT or False_Alarm, in seconds
end_after_response	True	trials can end one of two ways: they can end after response, which means that trials end at a fixed time after the change time. Alternatively, trials can end after a fixed duration that does not depend on the change time.
end_after_response_sec	3.5	trial ends N seconds after a change
change_time_dist	geometric	distribution from which change times are drawn: exponential for all non-flashed stimuli (e.g. stage 0 or 1), geometric distribution is used for discrete (flashed) stimuli
change_time_scale	0.3	the coefficient for the geometric distribution
change_flashes_min	4	Minimum number of stimulus flashes before a change-time can occur. Reducing this to zero will eliminate much of the time penalty imposed on guessing; the mouse will be able to see a change (and thus get a reward) much sooner after a guess. However, if this is non-zero it is a fixed interval that the animal can learn, and it encourages timed-guessing behavior.
change_flashes_max	12	Maximum number of stimulus flashes before a change-time can occur. This censors the end of the geometric distribution. Change times at are drawn outside this limit cause a resampling of the distribution until a change-time is drawn that is beneath this limit.

visual stimulus params

parameter key	default value	description
image stimulus	Image Set A	Image Set A is the default set of 8 images. Investigators can propose to use any set of images of arbitrary content and size

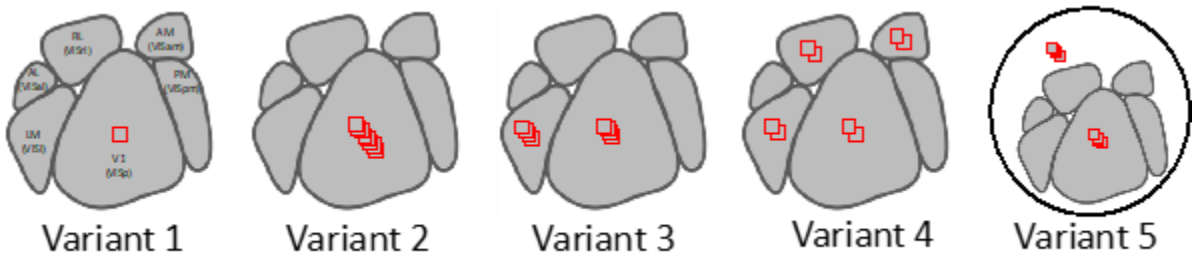
sampling	even	The method of sampling the matrix of image transitions ('even', 'random'). If 'even', change matrix is sampled evenly (i.e., every possible combination of images, including same-to-same) is sampled pseudorandomly). If "even" this overrides catch probability, and the catch probability becomes the probability of choosing the transition matrix diagonal (1/N where there are N images).

Stage 4:

behavioral logic params

reward_volume	0.007	Volume of "HIT" trial rewards. This is the only param that is different between stage 3 and stage 4

APPENDIX II - IMAGING EXPERIMENTAL VARIANTS



Possible variants using the two-photon imaging platform: single plane imaging (Variant 1), full cortical column imaging (8 planes in one area, Variant 2), dual area imaging (4 planes in each, Variant 3), 4 areas with two planes each (Variant 4), areas recorded beyond the visual cortex within the 5 mm window (Variant 5).

APPENDIX III - EXAMPLE DATA GENERATION PLAN TABLE FOR A PROPOSED SET OF EXPERIMENTS

WITH SINGLE-PLANE TWO-PHOTON IMAGING:

Stimulus	Depth	Cre-line	Visual Area	Number of mice	Cell matching?
Stim 1	Layer 2/3	Slc17a7-Cre; Camk2a-tTA; Ai93(TITL-GCaMP6f)	VISp	5	Parent
Stim 2			VISl	5	To Stim 1
Stim 3			VISpm	5	To Stim 1
Stim 1	Layer 4	Rorb-IRES2-Cre; Camk2a-tTA; Ai93(TITL-GCaMP6f)	VISp	5	Parent
Stim 2			VISl	5	To Stim 1
Stim 3			VISpm	5	To Stim 1

MULTI-PLANE TWO-PHOTON IMAGING:

Stimulus	Cre-line	Visual Area / Depth	Number of mice
Stim 1	Slc17a7-Cre; Camk2a-tTA; Ai93(TITL-GCaMP6f)	VISp / Layer I + 2/3+ 4 + 5	10
		VISl / Layer I + 2/3+ 4 + 5	
Stim 2	Slc17a7-Cre; Camk2a-tTA; Ai93(TITL-GCaMP6f)	VISp / Layer I + 2/3+ 4 + 5	10
		VISl / Layer I + 2/3+ 4 + 5	
Stim 3	Slc17a7-Cre; Camk2a-tTA; Ai93(TITL-GCaMP6f)	VISp/ Layer I + 2/3+ 4 + 5	10
		VISl/ Layer I + 2/3+ 4 + 5	

NEUROPIXELS EXPERIMENTS:

Stimulus	Mouse line	Probe	Probes entering Visual Area	Priority (Recording with all 6 probes can have lower yields)	Number of mice
Stim 1	C57BL/6J	Probe 1	VISp	Essential	5
		Probe 2	VISl	Essential	
		Probe 3	VISpm	Essential	
		Probe 4	VISrl	Bonus	
		Probe 5	VISam	Bonus	
		Probe 6	VISal	Bonus	
Stim 2 with opto-tagging	Pv-Cre; Ai32	Probe 1	VISp	Essential	5
		Probe 2	VISl	Essential	
		Probe 3	VISpm	Essential	
		Probe 4	VISrl	Bonus	
		Probe 5	VISam	Bonus	
		Probe 6	VISal	Bonus	

APPENDIX IV - AVAILABLE CRE LINES AND AREAS/LAYERS AVAILABLE FOR IMAGING.

This table provides a list of available areas and layers for imaging for each line. Abbreviations: i, inhibitory neurons; e, excitatory neurons. The asterisk indicates that expression was noted but not available for imaging due to depth and density. For those lines available online in the Allen Brain Observatory, a link is provided to the protein expression in the brain.

Mouse Line	Areas and Layers								Link
	VISp				VISl, VISpm, VISal, VISrl, VISam				
Cux2-CreERT2;Camk2a-tTA; Ai93(TITL-GCaMP6f)	2/3e	4e			2/3e	4e			Protein
Fezf2-Cre; Ai148(TIT2L- GCaMP6f -ICL-tTA2)			5e	*			5e	*	
Nr5a1-Cre; Camk2a-tTA; Ai93(TITL-GCaMP6f)		4e				4e			Protein
Ntsr1-Cre_GN220; Ai148(TIT2L- GCaMP6f -ICL-tTA2)				6e				6	
Rbp4-Cre_KL100;Camk2a- tTA; Ai93(TITL-GCaMP6f)			5e				5e		Protein
Rorb-IRES2-Cre;Camk2a-tTA; Ai93(TITL-GCaMP6f)		4e		*		4e		*	Protein
Scnn1a-Tg3-Cre; Camk2a-tTA; Ai93(TITL-GCaMP6f)		4e				-			Protein
Slc17a7-Cre; Camk2a-tTA; Ai93(TITL-GCaMP6f)	2/3e	4e	5e	*	2/3e	4e	5e	*	
SST-Cre; Ai148(TIT2L- GCaMP6f -ICL-tTA2)	2/3i	4i	5i	6i	2/3i	4i	5i	6i	
Tlx3-Cre_PL56; Ai148(TIT2L- GCaMP6f -ICL-tTA2)			5e	*			5e	*	
VIP-Cre; Ai148(TIT2L- GCaMP6f -ICL-tTA2)	2/3i	4i			2/3i	4i			
PV-Cre; Ai162(TIT2L- **GCaMP6s** -ICL-tTA2)	2/3i	4i	5i	6i	2/3i	4i	5i	6i	

APPENDIX V - COMMON BLINDING MISTAKES AND HOW TO AVOID THEM.

We perform a "blinded" review, in which identities of the applicant, collaborators, and their organizations are concealed from reviewers, for the letter of intent and full application stages. All applicants should carefully review the Request for Proposals to determine which documents must be stripped of all identifying information. Applications or letters of intent that contain identifying information in the LOI or proposal text will be administratively rejected. A few common blinding mistakes, and techniques to avoid them, are described below. This is not an exhaustive list, and applicants should thoroughly review all documents prior to submission to remove identifying information.

1. **Avoid identification of personnel or laboratories through references.**
   Refrain from using words such as "I," "we," and "our" in the text, particularly when references will be cited. Do not refer to published work in a way that reveals any connection with the applicant or collaborators on the proposal.
   - Common Mistake 1: "We recently developed a method to purify XYZ cells from ABC tissue and successfully established the first PDQ assay (Reference)," where the reference cited is a publication co-authored by a member of the proposal team.
   - Common Mistake 2: "Our laboratory has previously reported that Z protein phosphorylates B protein on Serine 370 (Reference)," where the reference cited is a publication co-authored by a member of the proposal team.
   - Common Mistake 3: "The applicant is uniquely positioned to conduct serotyping experiments due to experience with similar work (Reference)," where the reference cited is a publication co-authored by a member of the proposal team.

   Do not include highlighting such as bold, underlined, or italicized fonts that identify certain publications as authored by the applicant or a member of the research team in the References Cited section. Do not include references to "in press" manuscripts, as they are not part of the public domain.
2. **Avoid inclusion of organization names or acronyms in blinded documents.**
   Review all documents that are required to be blinded to ensure that no organization names or acronyms are listedwithin. This includes the applicant's organization, as well as the organization(s) of any collaborators.
   - Common Mistake 5: "Samples will be collected from patients recruited from the population available at Big State University (BSU) Hospital
   - Common Mistake 6: "Tissue sections will be paraffin-embedded and sectioned by the BSU Tissue Histology Core facility."
3. **Check that no computer account metadata was submitted along with pdf files.** The Document Information Dictionary contains a number of optional entries, which Microsoft Word automatically fills in. Title, Author, Subject, Creator, and others. These can be seen by looking at Document Properties (File-> Properties->Summary on Mac OS)
4. **Avoid inclusion of the applicant's name or that of other personnel in blinded documents.**

   Review all documents that are required to be blinded to ensure that no names are listed within. This includes the applicant or collaborators who will be involved in the proposed project. Do not provide names of people you have collaborated with

on other projects, even if they are not involved in the proposed project, as this may lead to identification of study personnel.

- Common Mistake 10: “The reagent was provided by Dr. Jane Doe, who has agreed to consult on this project,” regardless of whether Dr. Doe is included as a collaborator.
- Common Mistake 11: “The cells will be grown and subjected to irradiation in Dr. Smith’s laboratory,” regardless of whether Dr. Smith is included as a collaborator.
- Common Mistake 12: “Our collaborator, Dr. John Doe, has demonstrated uptake of the drug by the nanoparticles (Reference),” regardless of whether Dr. Doe is included as a collaborator.

Ensure that names are absent from all headers, footers, titles, and figure legends.