

# OpenScope Call for *In Vivo* Neurophysiology Experiments in Mice

**Opportunity Number:** U24NS113646-RFP-2021

**Purpose:** The OpenScope program is soliciting proposals for experiments to be carried out using the “Allen Brain Observatory” *in vivo* imaging and electrophysiology platforms in the brains of mice. Data will be collected by skilled operators at the Allen Institute and will be packaged in a standardized format and distributed to external teams for their own analysis. In the current call for proposals, recordings will be targeted to the visual pathways of mice passively viewing a stimulus monitor (future calls for proposals may include more brain regions and/or active behavioral tasks). We anticipate selecting three projects in 2021 distributed across Allen Brain Observatory rigs: Neuropixels electrophysiology and two-photon imaging. 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

**Posted:** July 30th, 2021

**Letter of Intent Due:** September 22nd, 2021 (5 pm Pacific)

**Full Proposal Due:** November 22nd, 2021 (5 pm Pacific)

**Eligibility:** This opportunity is available to anyone, provided they are not currently 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 through an 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 encouraged to get in touch with [openscope@alleninstitute.org](mailto: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

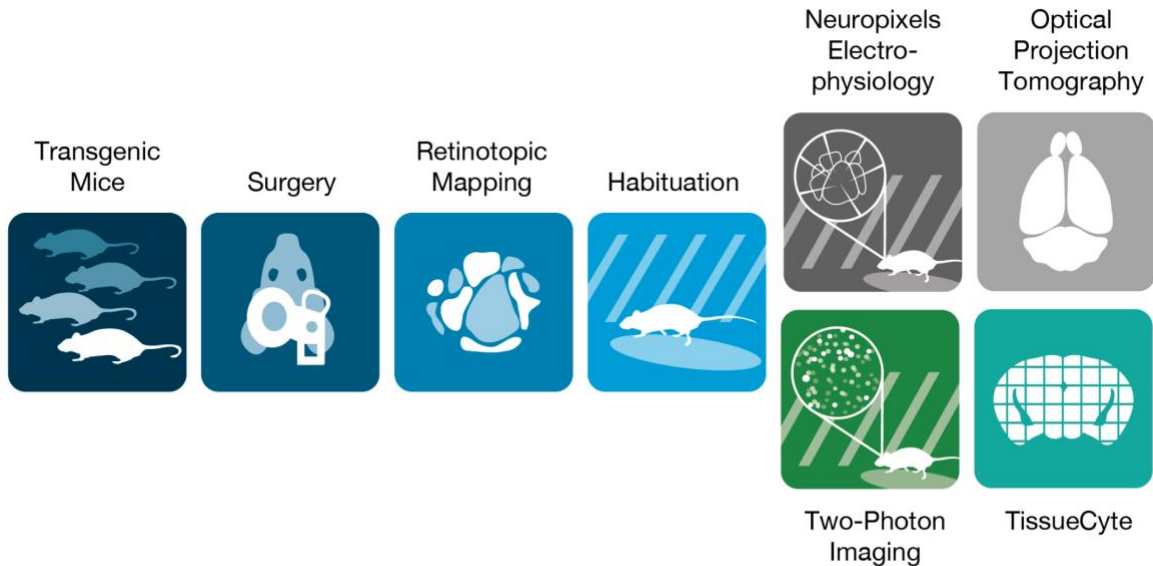
Launched in 2016, the Allen Brain Observatory consists of a set of standardized protocols, rigs, and quality control procedures for recording large-scale neural activity from the brains of awake mice. The original platform (based on single-plane two-photon microscopes) was used to survey over 60,000 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](https://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, 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.

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 and Neuropixels electrophysiology platforms. Each application can leverage either imaging or electrophysiology; proposals that require both platforms are not possible at this point. External use of the Allen Institute pipeline is best thought of as code deployment where only software modifications are possible. To guarantee the highest level of standardization and data quality, we will only use our existing validated hardware.

The following procedures are shared across all platforms:

**Surgery:** A titanium headframe is secured to the mouse skull, and a 5 mm craniotomy is drilled over the left visual cortex and replaced with a glass coverslip. In electrophysiology experiments, this coverslip is removed and replaced with a perforated plastic window prior to recording.

**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.

**Data collection:** Physiological data is collected from awake mice passively 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 Stimuli

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 120° x 95° 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 (see example code in **Appendix – Example stimulus code**). 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)

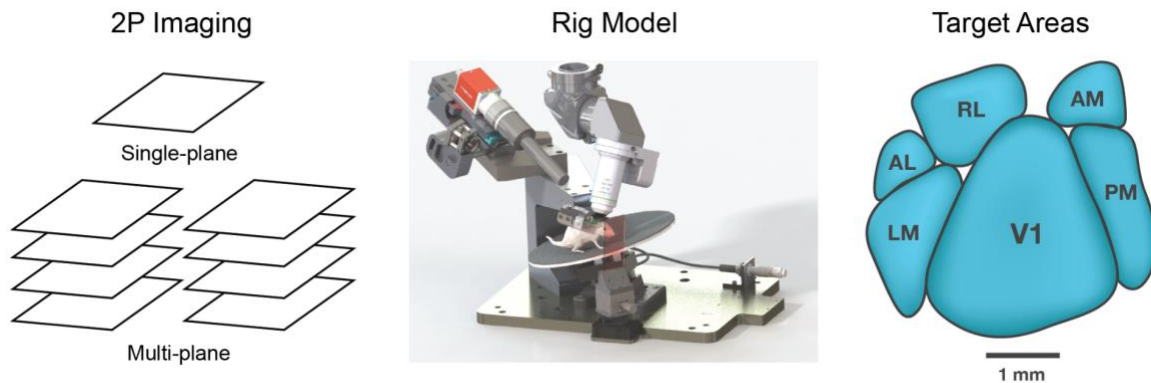
We will provide all necessary software dependencies to the selected applicant teams to validate their stimuli before deployment.

Importantly, stimuli must be shown with an **entirely “open-loop” design**. No information collected during the experiment can be used to update stimulus parameters.

Within the limit of each session duration (see below for imaging- and electrophysiology-specific recording times), as many different visual stimuli as necessary can be presented. However, we

encourage teams to use the **simplest possible experimental design** that addresses the scientific question at hand.

## Two-Photon Imaging Platform



### Recording devices:

- Single-plane microscopes can sample at 30 Hz from one 400 x 400  $\mu\text{m}$  field of view per session.
- Multi-plane microscopes can sample at 10 Hz from 8 different 400 x 400  $\mu\text{m}$  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 – 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:** 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.

**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 – 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,  $\Delta F/F$  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 – Data generation plan** for example experimental designs for single-plane and multi-plane two photon imaging.

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

## Neuropixels Electrophysiology Platform



**Recording devices:** Neuropixels 1.0 probes (Jun et al., 2017) contain 384 recording sites distributed across 3.84 mm of a  $70 \mu\text{m}$  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. 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.

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

**Cohort size:** Experiments can be performed on up to 10 mice, 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 – Cre lines** for a table of mouse lines and associated details.

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

**One year after data collection ended:** Embargo will be lifted. 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 22nd, 2021) 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 22nd, 2021) 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 Full Proposals will be selected (acceptance rate of ~20%).

### Letter of Intent

The Letter of Intent should consist of two sections:

1. Motivation – Describe the hypothesis to be tested and the current state of knowledge related to this topic and 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 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.
- Please name the file using the following convention: 2021\_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 – 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.

Importantly, proposals should offer a good fit between the experiments and the 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 20% or higher.

## Full Proposal

The Full Proposal should consist of three 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.)

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 does not need to be included in the 6-page limit.
- Please name the file using the following convention: 2021\_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 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 – Blinding mistakes**.

### Selection Process

The Full Proposals will be scored based on three criteria:

1. Impact of the proposed question/hypothesis
2. Quality of experimental design and feasibility of implementation
3. Quality of data analysis plan

Applications that depend on experiments that do not fit within the technical capabilities of the call will not be eligible for selection.

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

## Project Timeline

Potential applicants should ensure that they will be able to comply with the following timeline, if their proposal is accepted:

- **January 2021 - February 2022:**
  - Virtual project kickoff meeting; initiate a collaboration agreement with the applicants' academic 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, 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 2022 - May 2022:** 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 2022 - December 2022:** 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 2022 - February 2023:** 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.
- **2022 - 2023:** 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.

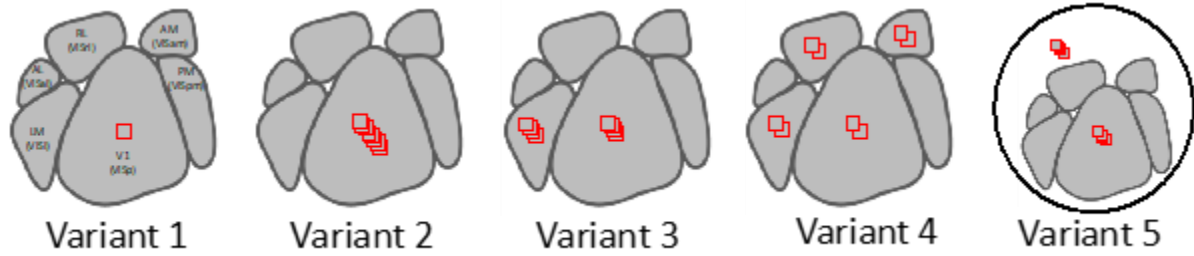
- **Late 2023:** the outcome of this work will be published in high-impact journals in collaboration with the Allen Institute. Collaborator(s) are expected to lead publication of their results along with contributing members at the Allen Institute.

## 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](mailto:openscope@alleninstitute.org) prior to submission.

## APPENDIX - 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 - EXAMPLE DATA GENERATION PLAN TABLE FOR A PROPOSED SET OF EXPERIMENT 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			VISI	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			VISI	5	To Stim 1
Stim 3			VISpm	5	To Stim 1

**APPENDIX - EXAMPLE DATA GENERATION PLAN TABLE FOR A PROPOSED SET OF EXPERIMENT WITH MULTI-PLANE TWO-PHOTON IMAGING**

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

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

<b>Stimulus</b>	<b>Mouse line</b>	<b>Probe</b>	<b>Probes entering Visual Area</b>	<b>Priority (Recording with all 6 probes can have lower yields)</b>	<b>Number of mice</b>
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 - 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			<a href="#">Protein</a>
Fezf2-Cre; Ai148(TIT2L- GCaMP6f -ICL-tTA2)			5e	*			5e	*	
Nr5a1-Cre; Camk2a-tTA; Ai93(TITL-GCaMP6f)		4e				4e			<a href="#">Protein</a>
Ntsr1-Cre_GN220; Ai148(TIT2L- GCaMP6f -ICL-tTA2)				6e				6	
Rbp4-Cre_KL100;Camk2a- tTA; Ai93(TITL-GCaMP6f)			5e				5e		<a href="#">Protein</a>
Rorb-IRES2-Cre;Camk2a-tTA; Ai93(TITL-GCaMP6f)		4e		*		4e		*	<a href="#">Protein</a>
Scnn1a-Tg3-Cre; Camk2a-tTA; Ai93(TITL-GCaMP6f)		4e				-			<a href="#">Protein</a>
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- <b>GCaMP6s</b> -ICL-tTA2)	2/3i	4i	5i	6i	2/3i	4i	5i	6i	

## APPENDIX - 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 listed within. 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. ***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.**