Zapit: a random-access optogenetics platform for head-fixed mouse behavior

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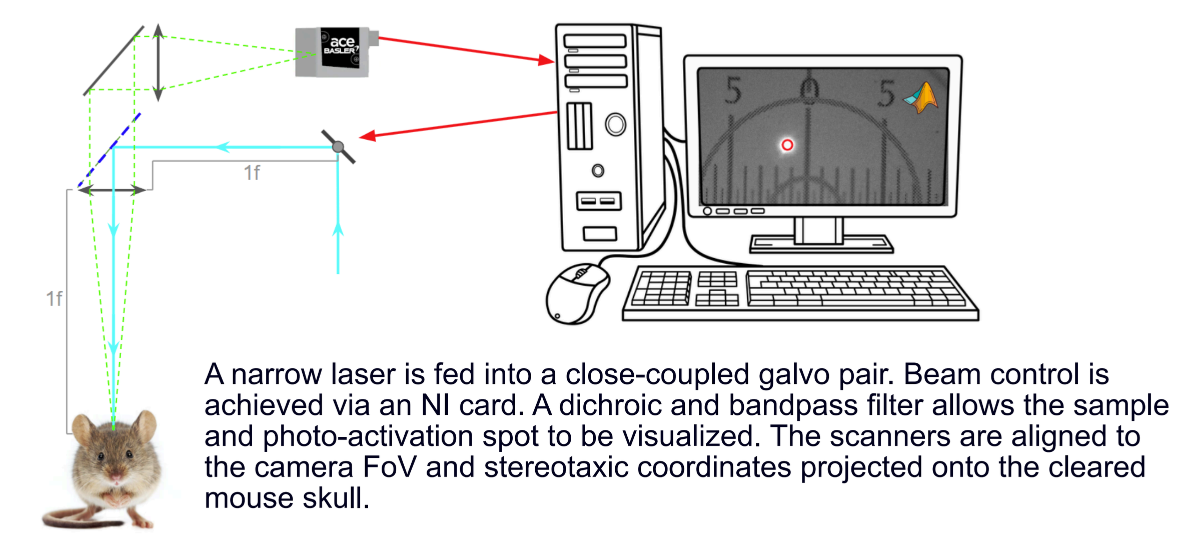
ABSTRACT

Zapit is a scanner-based photo-stimulation solution for head-fixed mouse optogenetics. The system directs a focused laser spot to stereotaxically defined locations on the dorsal brain surface with a latency of under 2 ms.

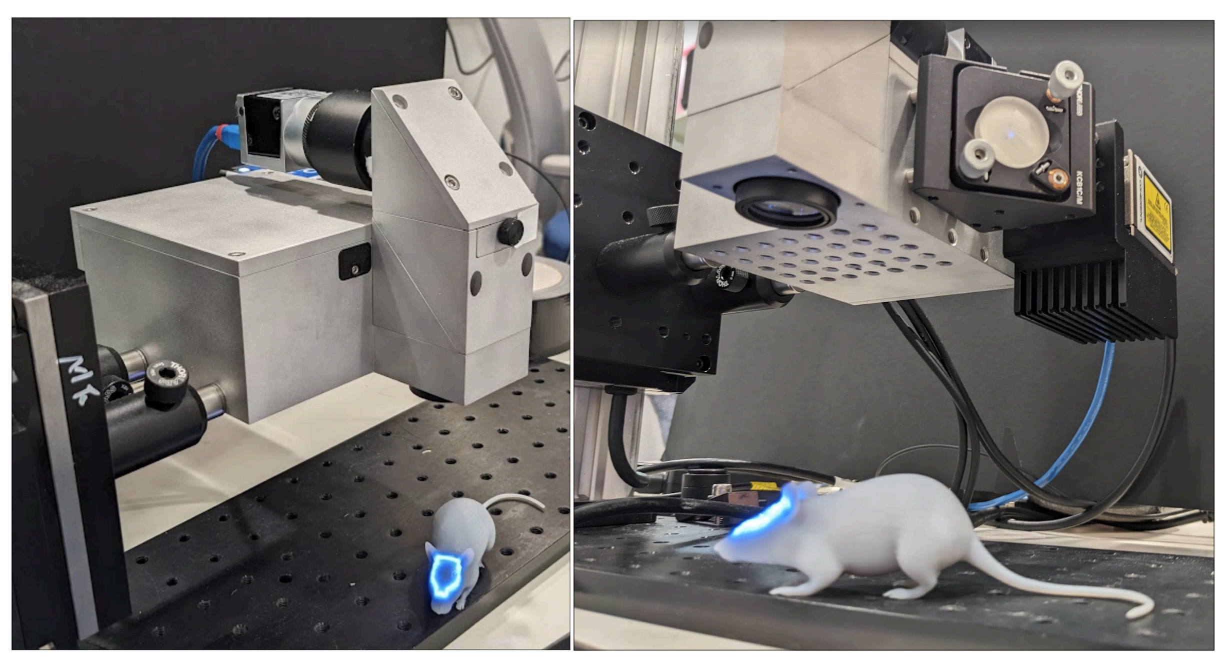
Zapit comprises a hardware design, a fully featured GUI for calibration and stimulus configuration, and an API for control during experiments. The core software is written in MATLAB for Windows but experiments can be run via a different programming language: we provide a shared memory Python bridge to the MATLAB API, and a cross-platform TCP/IP interface. Stimulus triggering can be done via TTL to ensure latency considerations never come into play.

Zapit is open source and developed in the open. We provide CAD models for machining a compact sealed enclosure but a functioning system can be assembled using off the shelf parts from companies like ThorLabs.

How it works

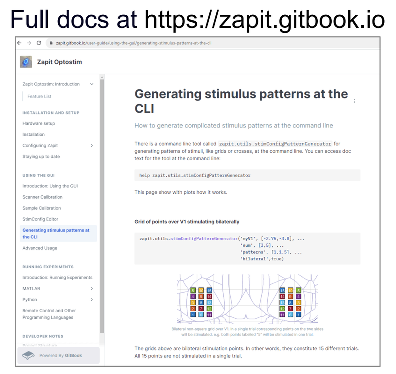


A narrow laser is fed into a close-coupled galvo pair. Beam control is achieved via an NI card. A dichroic and bandpass filter allows the sample and photo-activation spot to be visualized. The scanners are aligned to the camera FoV and stereotaxic coordinates projected onto the cleared mouse skull.

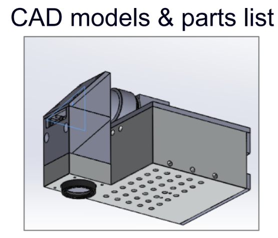


Supported, Documented, Well-Maintained

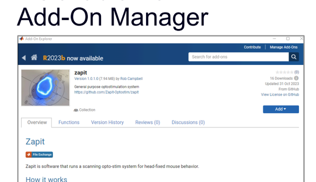
Full docs at https://zapit.gitbook.io



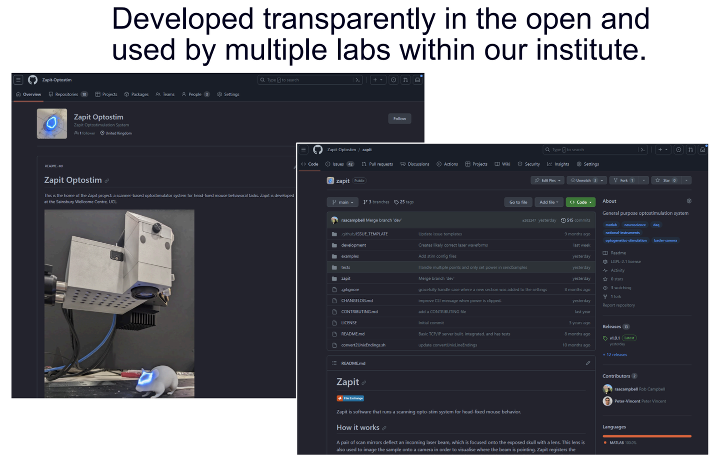
CAD models & parts list



Installable via MATLAB Add-On Manager

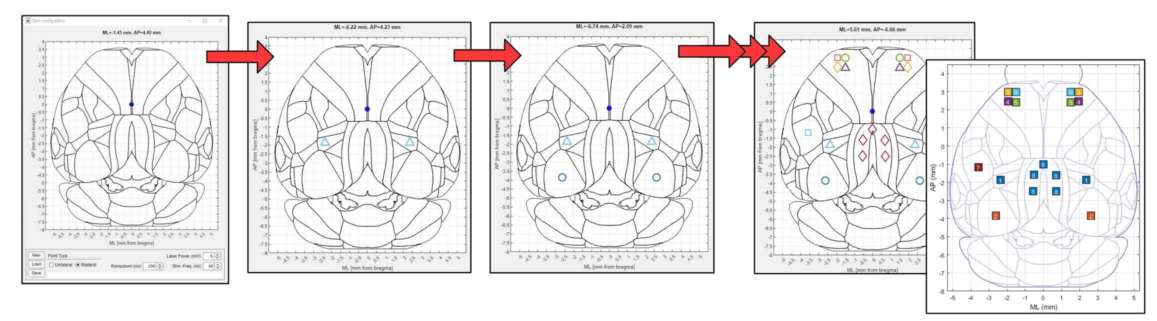


Developed transparently in the open and used by multiple labs within our institute.

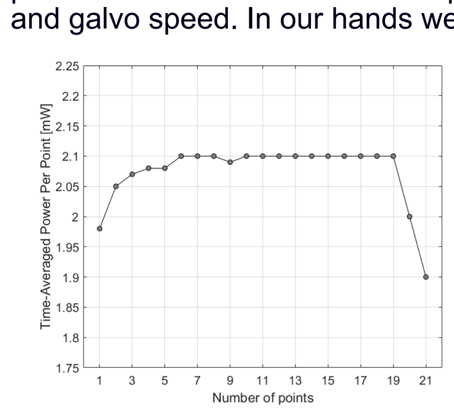


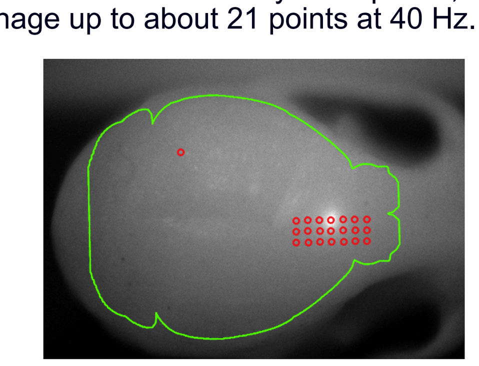
Stimuli are defined in stereotaxic space using a GUI

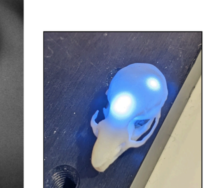
Stimuli are defined as groups of points that will be co-stimulated in a single trial. Stimulus repetition rate is nominally 40 Hz.



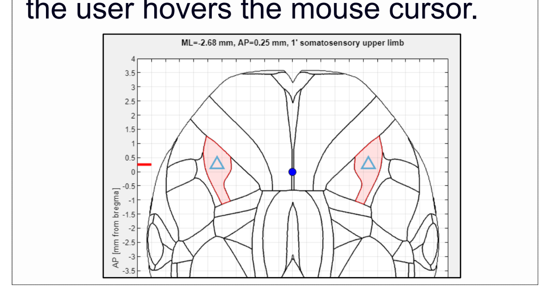
A single trial can consist of anywhere between 1 point up to a maximum of roughly 25 points. The maximum number of points in a trial is limited by laser power, repetition rate, and galvo speed. In our hands we manage up to about 21 points at 40 Hz.



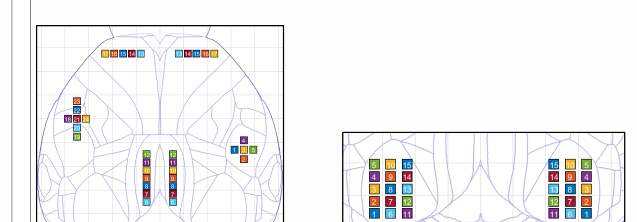




The GUI reports brain-area name as the user hovers the mouse cursor.

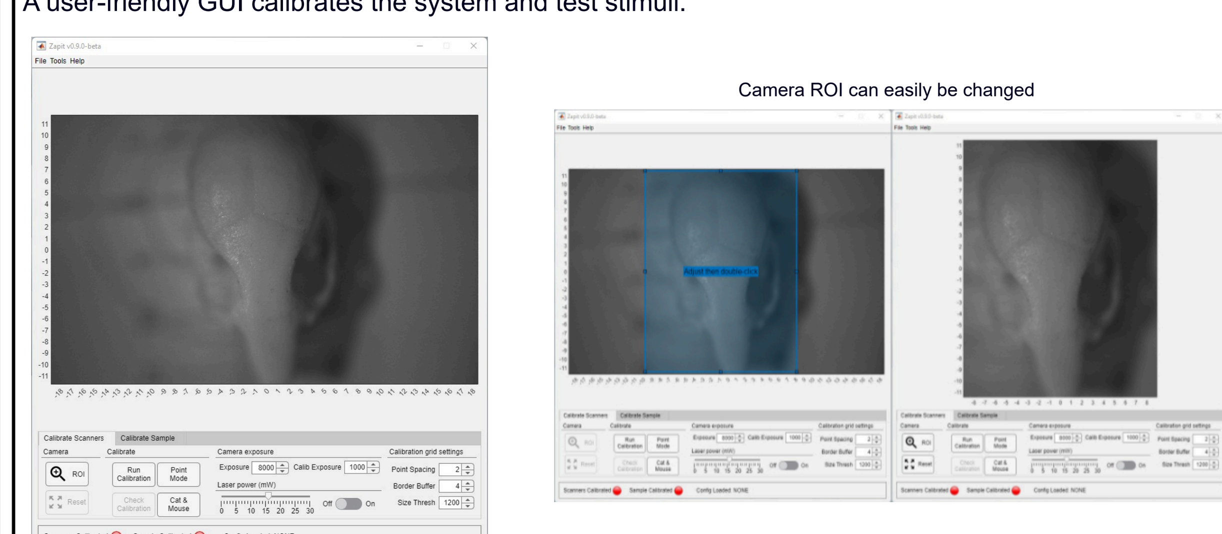


Command-line tools can be used to build regular patterns of stimulation points.



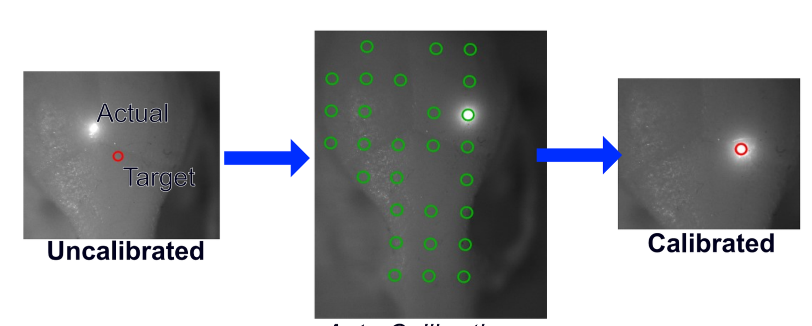
Zapit MATLAB GUI

A user-friendly GUI calibrates the system and test stimuli.



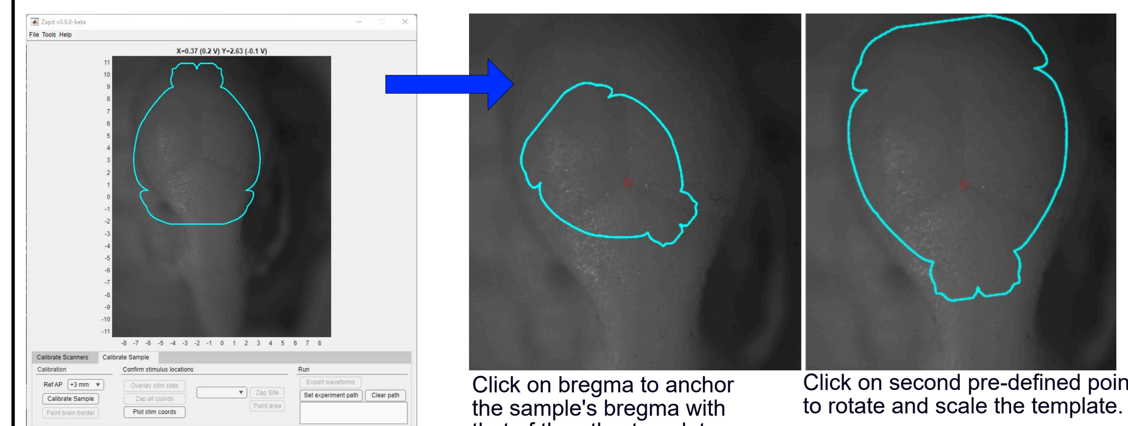
Scanner calibration

A single-click initiates a calibration procedure, aligning the beam position with the camera. After calibration the beam can be pointed at any location in the image with a mouse click.



Stereotaxic Calibration

A two-click procedure registers a brain outline using skull landmarks.

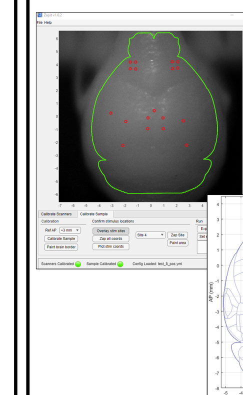


Click on bregma to anchor the sample's bregma with that of the atlas template.

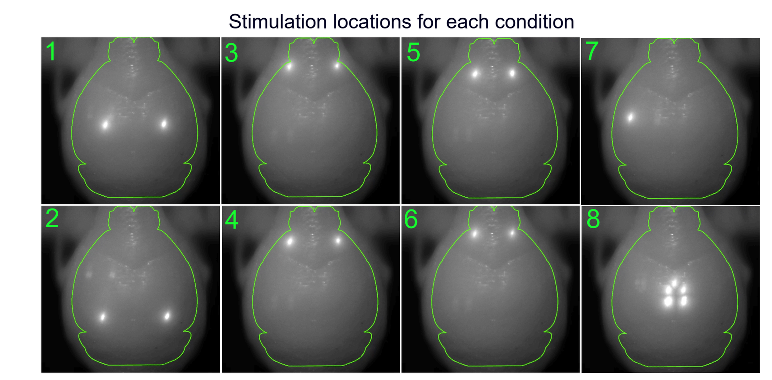
Click on second pre-defined point to rotate and scale the template.

Stereotaxic Calibration: stimulus presentation

Once calibrated we can stimulate in stereotaxic space: presenting points in arbitrary patterns and locations modulated at 40 Hz. Stimuli can be tested interactively using the GUI.

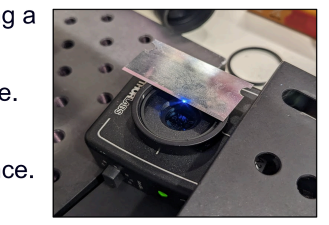


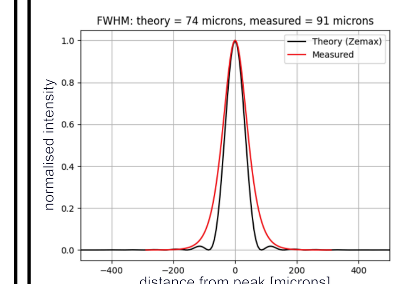
Points loaded into the Zapit GUI



Spot Size (PSF)

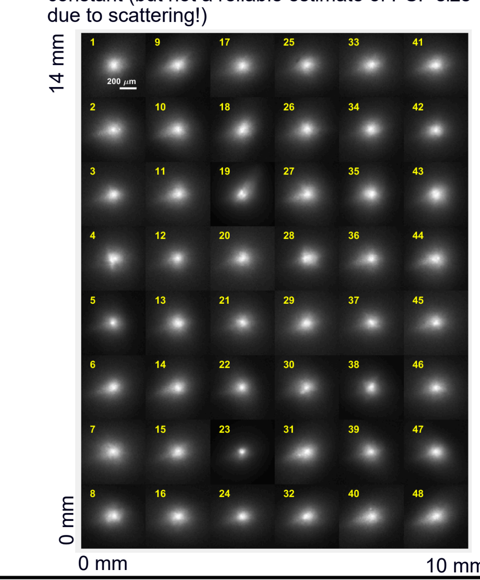
Measure 1-D PSF by translating a focused laser spot across the edge of a razor blade with a photodiode beneath blade edge. This generates a cumulative density function (CDF) of light intensity as a function of distance. The CDF can be turned into a PSF.





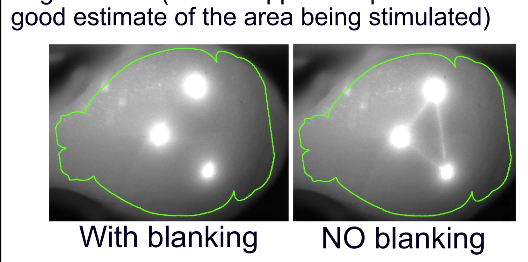
The beam has a measured full-width half max of roughly 60 microns. This agrees with optical modeling. Radius of photoactivation *will be larger than this* due to scattering. Stimulation radius will depend on laser power.

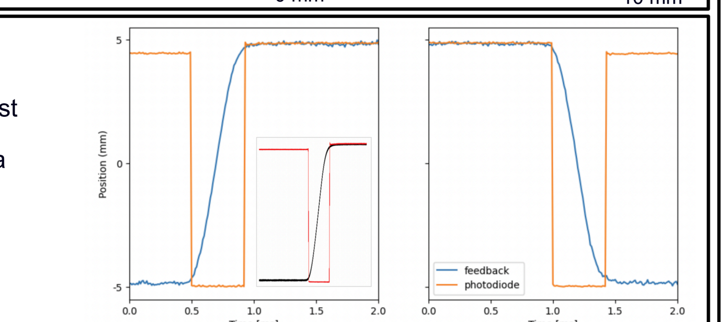
Imaged spot shape and size across the FoV is constant (but not a reliable estimate of PSF size due to scattering!)



Beam blanking and reliability

The beam must be switched off ("blanked") whilst moving between points to avoid stimulating off-target tissue. (NOTE: apparent spot size is not a good estimate of the area being stimulated)





The beam is configured to move smoothly from one point to the next in 0.3 ms. The actual beam position is shown by the blue trace. Whilst the beam is in motion the laser power is set to 0 mW (orange trace). Large figures show a single trial. Small inset figure shows 100 overlaid trails to demonstrate consistency.

Running an experiment: the basics

Once calibration is complete the GUI is no longer needed. Stimuli are presented as trials during experiments using a command-line API. The basic example to the left presents each stimulus for one second using software triggering at the **MATLAB** command line.

```
for ii = 1:length(hZP.stimConfig.stimLocations)
    % Does not wait for a hardware trigger: starts right away
    hZP.sendSamples('conditionNum',ii,'hardwareTriggered',false)
    pause(1)
    hZP.stopOptoStim
    pause(0.3) % To allow the ramp-down to happen
end
```

Code snippet to the right shows how to present basic stimuli in **Python** using a shared memory bridge to MATLAB. Anything that can be achieved at the MATLAB command line can be done from Python. We provide a pip-installable package to achieve this.

```
import zapit_python_bridge.bridge as zpb
from time import sleep

hZP = zpb.bridge()
hZP.send_samples(conditionNum=-1, hardwareTriggered=False)
sleep(0.75)
hZP.stop_opto_stim()
sleep(0.5)
```

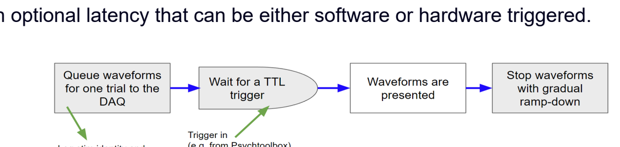
We supply a **TCP/IP** client/server interface for Zapit. This allows Zapit to be controlled by *any* PC on the local network running *any* programming language. We supply example code for MATLAB and Bonsai.

```
% Start the client, specifying the IP address of the Zapit server
client = zapit_tcp_bridge.TCPclient('ip','122.14.143.200');
client.connect;

% present the last stimulus condition for a short period
nCond = client.getNumConditions;
client.sendSamples('conditionNumber',nCond, 'hardwareTriggered', false);
pause(3)
client.stopOptoStim;

% Disconnect
delete(client)
```

Stimulus paradigms

Stimuli **ramp down in intensity** over a 250 ms time period to avoid rebound effects. Stimuli can be presented in a number of regimes:

1. Software triggered onsets. Open-ended duration. Software triggered offsets.
2. Hardware (TTL) triggered onsets with open-ended duration and software triggered offsets.
3. Fixed duration stimuli with optional latency that can be either software or hardware triggered.

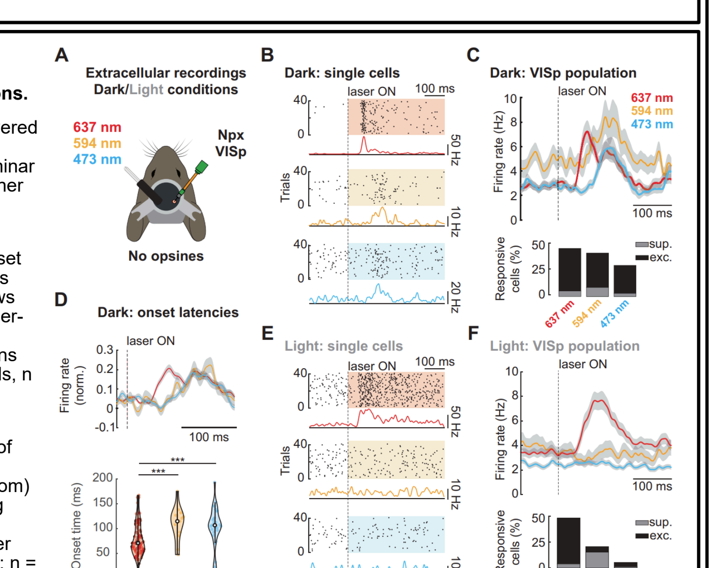


Fiber illumination in the brain strongly activates the visual cortex of wild-type mice in ambient darkness and shows wavelength-specific adaptation under ambient light conditions.

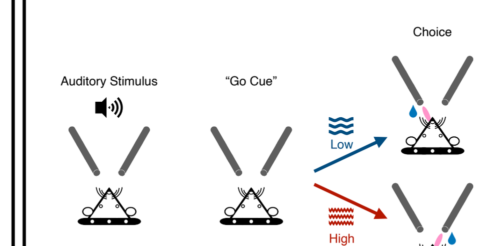
Red (673 nm), orange (594 nm) or blue (473 nm) laser was delivered via an optical fiber implanted in the left primary visual cortex of awake head-fixed mice. All laser powers under 10 mW. Translaminar Neuropixels recordings were performed at contralateral V1 in either complete darkness (**B**-**D**) or under light conditions (**E** & **F**).

In the dark, single-unit spike raster plots (B) and time-averaged population spike density functions (C) are aligned to stimulus onset and show an obvious response at all wavelengths. D (Top) shows average normalized spike density functions and D (Bottom) shows violin plots displaying onset latencies of red, orange and blue laser-evoked responses. C Bottom: Percentage of excited (black) and suppressed (gray) units for red, orange and blue laser stimulations (n = 249 cells, n = 3 mice; n = 58 cells, n = 1 mouse; n = 162 cells, n = 2 mice).

Under ambient light conditions the (E & F) a visually evoked excitation is only found for 637 nm stimulation. (E) Raster plots of spikes of individual units in response to 40 or 80 repetitions (40 displayed) of 1 second of red (top), orange (middle) or blue (bottom) laser stimulation and corresponding PSTH representing the firing rate averaged across the trials. F Bottom: Percentage of excited (black) and suppressed (gray) units for red, orange and blue laser stimulations (n = 218 cells, n = 3 mice; n = 58 cells, n = 1 mouse; n = 120 cells, n = 2 mice).

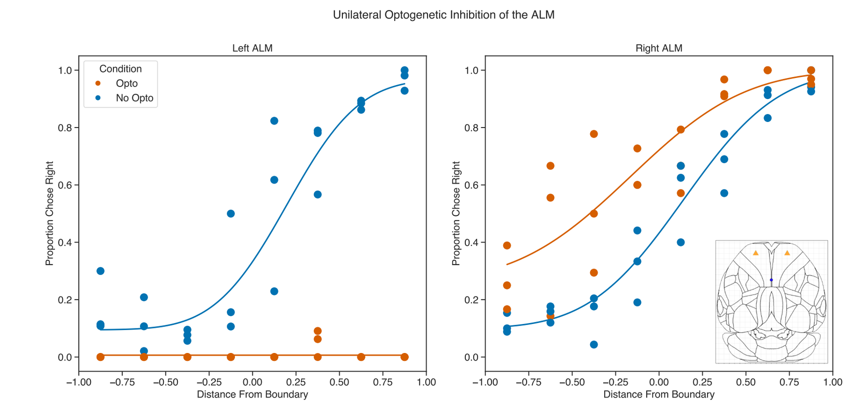


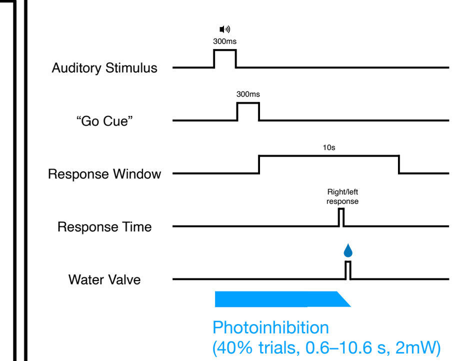
ALM inactivation produces an ipsilateral choice bias.



Schematic of the sound intensity categorisation task. Mice were head fixed and presented with an auditory stimulus (High/Loud) or (Low/Quiet) on either side of an intensity boundary.

After a 300 ms “Go-Cue” (or delay) mice indicated their choice ("Low" or "High") by licking either the left or right lick spout. Water reward was given following correct responses, mice were punished with a timeout following incorrect choices.

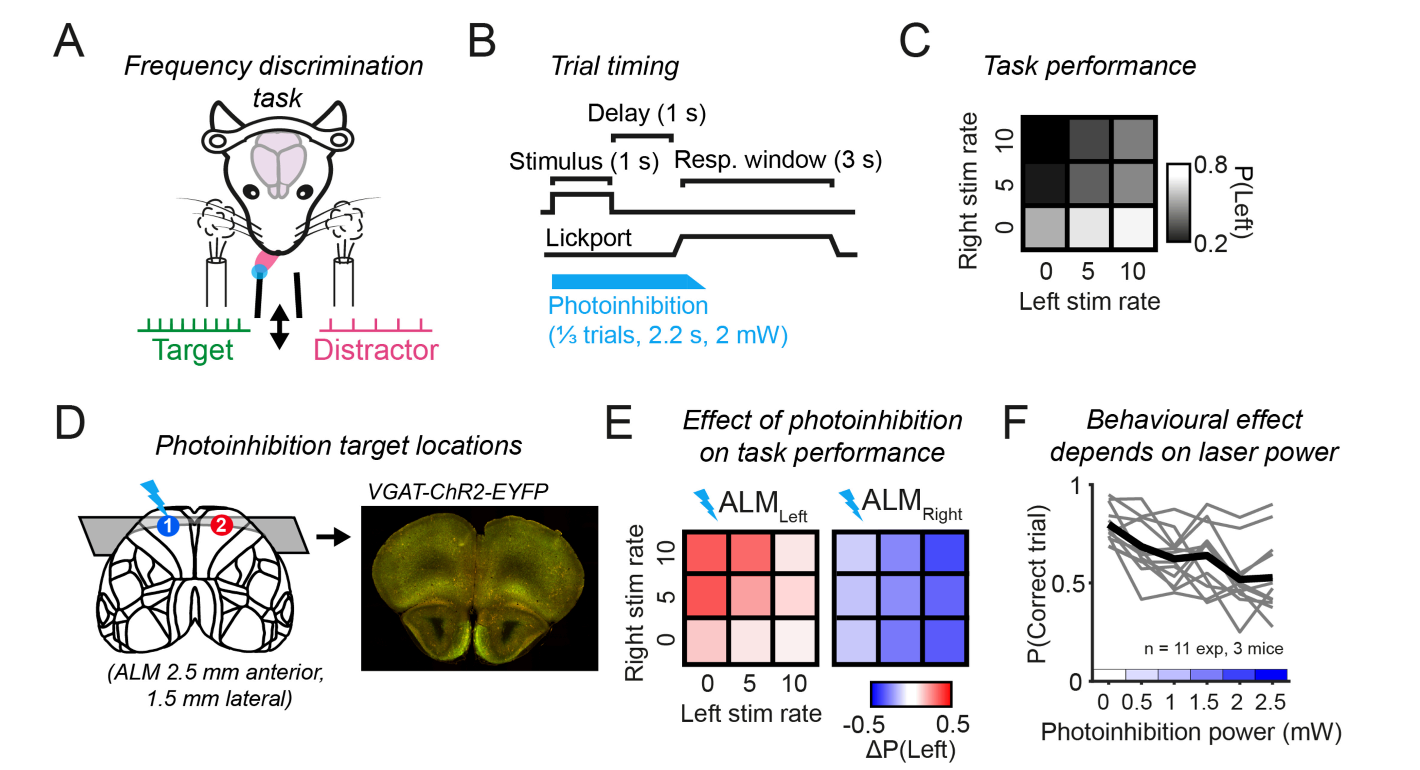




Timeline of events during a successful trial of the 2-AFC task. Photoinhibition (0.6–10.6 s + 250 ms ramp down, 40 Hz rate, 2 mW) was delivered from the start of the auditory stimulus until either the mouse made a response or until the end of the response window on the 40% of trials that contained optical stimulation.

Unilateral photoinhibition of the left and right Anterior Lateral Motor cortex in separate trials (ALM, 2.5mm anterior, 1.5 mm lateral to bregma -- inset figure) causes an ipsilateral choice bias in a VGAT-ChR2 mouse, with the data points showing the proportion of right choices as a function of the relative distance of the stimulus to the boundary for individual sessions, and the lines showing the psychometric fits. Successive sessions of ALM-Left sessions (N = 3) were run. Photoinhibition trials are shown in orange, control trials in blue (Total trial counts: 375 ALM opto trials; 869 ALM no opto trials).

ALM inhibition drives ipsilateral choice bias during a delayed-response directional licking task



(A) Mice performed a bilateral whisker-guided frequency discrimination task and reported choices via left/right licking. Trains of air-puffs were delivered to the left and right whiskers, with the target lickport response cued by the side receiving the faster stimulus rate.

(B) Trial timing schematic. The whisker stimulus presentation period (1 s) was followed by a short delay epoch (1 s). After the delay, a motorised stage moved the lickport forwards cuing the start of the response window. Photoinhibition (2.2 s + 250 ms rampdown, 40 Hz rate, 2 mW time-averaged) was delivered during the stimulus and delay epochs on a third of trials.

(C) Discrimination performance on control trials. Mice received 9 trial-types corresponding to different combinations of left and right stimuli. Data show the performance, quantified as the probability of reporting a left lick choice, concatenated across 15 experiments in 3 mice (Total trial count = 3149).

(D) Optogenetic photoinhibition was targeted to left and right anterior lateral motor cortex (ALM) in VGAT-ChR2-EYFP expressing mice.

(E) Behavioural effect of lateralised photoinhibition of ALM quantified as the difference in P(Left) on photoinhibition trials and control trials. The colour map depicts increases in P(Left) in red, and decreases in P(Left) in blue. Data are concatenated across 15 experiments in 3 mice (Total trial count = 747 ALM left trials; 740 ALM right trials).

(F) We assessed the tendency for contralateral photoinhibition of ALM to impair performance on unilateral whisker trials as a function of laser power.

Differential behavioral responses can be elicited by stimulating brain areas located right next to each other

We expressed channelrhodopsin in inhibitory neurons (VGAT-ChR2-YFP). Mice performed a head-fixed visual psychophysics task, indicating choices with licking responses. Psychophysical curves could be reliably impacted by laser stimulation. The stimulation was highly location specific, with locations under a mm apart having different effects. These show the effect of two light powers: 2 mW and 4 mW time-averaged, as well as the effect of photo-inhibiting a control area (S1) where nothing happens to show that the mice are not affected by/cant see the light (we used a masking light on every trial)