MIGHTEX

HEAD-FIXED SINGLE-CELL CALCIUM IMAGING OF THE VENTRAL PALLIDUM WITH OASIS FIBERSCOPE

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Application Note

Broadly, the Kravitz Lab seeks to understand the neural circuits coordinating complex behaviors, such as those involved in decision making, reward seeking, and ingestion. Recently, we have been studying the circuits that regulate hedonic feeding, which is thought to be a driver of weight gain in diet induced obesity. We have been interested in the *ventral pallidum*, a structure that plays a key role in encoding valence and motivation associated with consummatory phases of feeding behavior [1–4]. Recent studies using optogenetic activation strategies demonstrated that activation of GABAergic VP neurons (VP^{GABA}) induces voracious feeding for fats in sated animals, suggesting that these neurons play a selective role in hedonic feeding [5]. However, our lab seeks to understand the *endogenous dynamics* of VP^{GABA} neurons, and how they modulate palatable reward consumption.

We used the Mightex OASIS system to record calcium dynamics of VP^{GABA} neurons in awake, head-fixed mice implanted with Gradient Refractive Index (GRIN) Lenses to deliver excitation light deep into the brain. By recording single neuron dynamics, we observed proportionally similar numbers of activated and inhibited neurons during reward consumption (high-calorie *Ensure* reward), suggesting that VP^{GABA} neurons have heterogeneous responses. Interestingly, a subpopulation of VP^{GABA} neurons is highly responsive to palatable reward consumption and might be a mechanistic target for suppressing hedonic feeding that underlies dietinduced obesity.

The Mightex OASIS implant's integration with scientific-grade camera hardware (pco sCMOS) enabled these single-cell resolution recordings through flexible control of spatial resolution, sampling rates, and illumination duty cycle. The Mightex PolyScan4 acquisition software facilitated reliable synchronization of calcium imaging with behavioral timestamps, allowing precise alignment of neural activity with licking microstructure and consumption duration. Using open-source toolboxes designed for one-photon endoscopic calcium imaging, we were able to integrate the OASIS system into a complete pipeline, from acquisition to alignment of neural dynamics with behavior (**Figure 1**, see references for specific tools, 6-12). An example movie is attached showing normalized calcium dynamics (average frame dF/F) (Video 1). Following source extraction, we can align single cell calcium dynamics to behaviors of interest that have been synchronized during acquisition using PolyEcho DAQ (**Figure 2**). Here, we show individual example cells as well as the behavior-averaged cell response aligned to the average behavior during a single recording session. Additionally, the PolyEcho and Polyscan4 ecosystem imparts the ability to synchronize illumination with the shutter exposure, which enables decreased photobleaching and multi-color illumination control (**Figure 3A,B**).

During our experiments, we appreciated the easy-to-understand Polyscan4 acquisition GUI, which provides real-time feedback during recording sessions, and post-acquisition playback of recording sessions (**Figure 3C**). The GUI also allows users to easily set the parameters (exposure, gain, sampling rate) of both imaging and behavior cameras. Together, the Mightex equipment ecosystem (OASIS, PolyEcho DAQ, Mightex SS Camera, PolyScan4 software) provide users a flexible solution for synchronizing imaging with behaviors of interest.

Mightex Equipment Used

a. OASIS Fiberscope
b. BLS-Series 470nm LED and LED Controller
c. PolyEcho Intelligent Control Module 12-channel variant
d. USB3.0 Monochrome 1.5MP CMOS Camera (12 bit)
e. PolyScan4 Acquisition Software (Pro-license required for PCO sCMOS support)

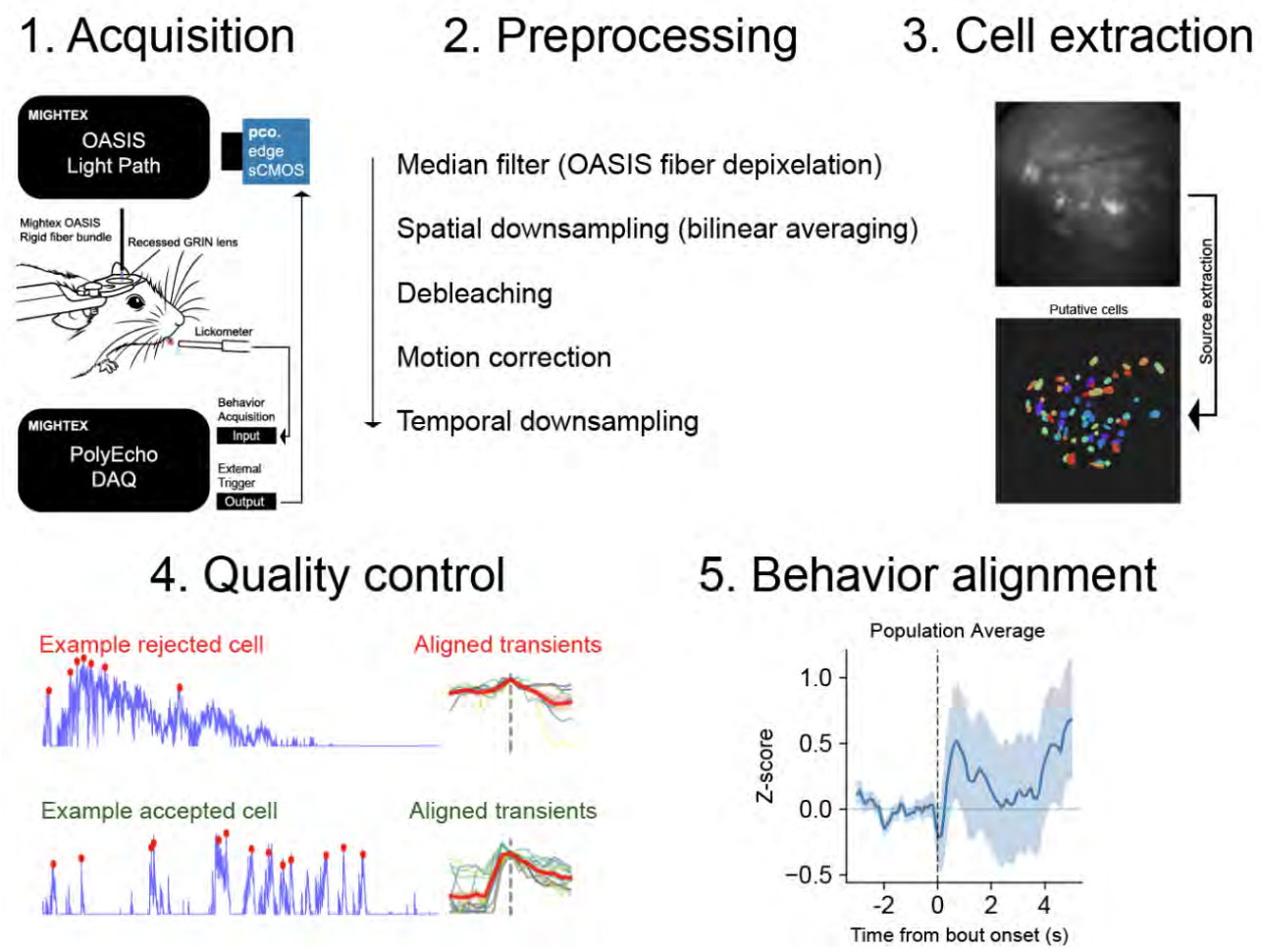

Figure 1: *Pipeline overview using OASIS Fiberscope.*

During acquisition, external behavioral timestamps (licking, task-related events) generated by Arduino microcontrollers are sent into the PolyEcho digital acquisition (DAQ) box and synchronized with frame capture of the imaging camera (PCO Edge sCMOS) and behavior camera (Mightex USB3.0 CMOS Camera). Additionally, the PolyEcho DAQ acts as a pulse generator to trigger both cameras at a desired frame rate (20hz, 60hz, respectively). The exposure state of the imaging camera is also used to control the illumination LED used to excite the calcium indicator (more acquisition details are described in Figure 2). After acquiring imaging movies, they are preprocessed in the indicated sequence, using open-source toolboxes (see references) to achieve those steps. After pre-processing, we are left with a downsampled, motion corrected movie that is ready for cell extraction (multiple open-source algorithms exist, see references). As there is no perfect cell extraction, a quality check is performed to remove cells that exhibit dynamics inconsistent with the calcium indicator (jGCaMP7s). Finally, cells that pass quality control are used for alignment with behavior timestamps captured at the acquisition stage.

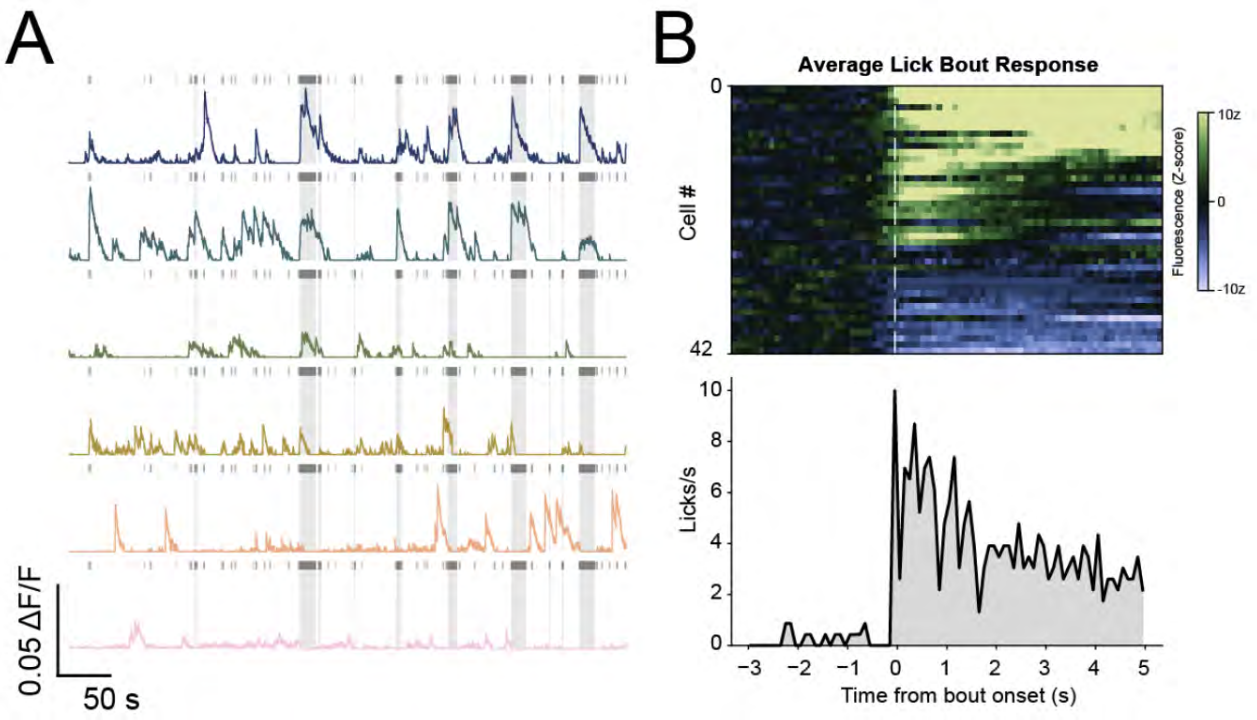

Figure 2: *Polyscan4 synchronized behavior alignment with extracted cells.*

After recording, we use a pipeline of open-source tools (see references) designed for the analysis of 1-photon imaging movies [6–12]. To deal with neuropil signal contamination, the movie is first spatially filtered then motion corrected. Additionally, we employ spatial and temporal downsampling to reduce movie

sizes prior to signal extraction. In **Figure 2A**, we show representative single cell dynamics (N=6) aligned to licking behavior which was recorded using the PolyEcho DAQ. Cells are sorted in descending order of their response to licking, where highly activated cells are displayed on top and inhibited cells are displayed on the bottom. In the middle, these cells are partially modulated by licking. Single raster ticks represent single licks, whereas shaded gray regions represent lick bouts (extended bursts of licking). In **Figure 2B**, we show a heatmap of the average lick bout response, where each row represents a single extracted cell from a recording session. These cells are sorted in the same manner, from highest to lowest responders. In the bottom panel below the heatmap, the average licking behavior is shown that is aligned to t=0, which indicates the start of the licking bout.

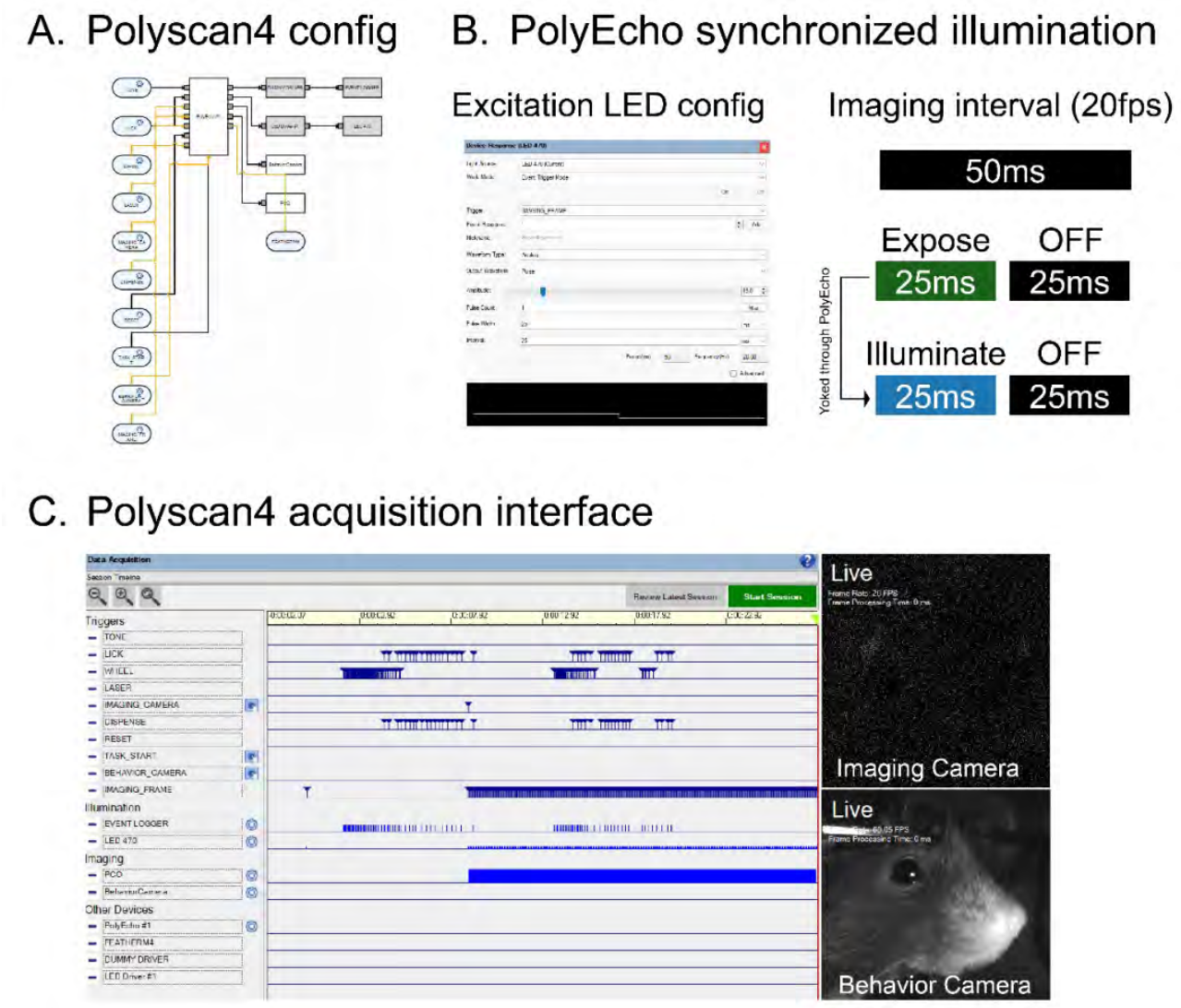

Figure 3: *Imaging and behavior acquisition configuration using PolyScan4.*

The Polyscan4 wiring diagram must first be configured prior to initiating a recording session, shown in **Figure 3A**. Here, the user sets the desired input/output control of various hardware and software triggers, which are physically connected to and integrated by the PolyEcho DAQ. We are using the 12-channel digital inputs to synchronize different behaviors of interest – those timestamps are generated externally by an Arduino microcontroller (Adafruit Feather M4 SAMD51 Board) which we programmed to run our behavior task (head-fixed reward licking). The timestamps of the events configured in this wiring diagram are exported as CSV files (wide-format), which can be used for later analysis.

After configuring the wiring diagram, the user is allowed to configure the desired response profile of different triggers, shown in **Figure 3B**. Here, we use a digital output from the PolyEcho as a pulse generator to command a desired frame rate of the imaging camera (20hz) but simultaneously use those pulses to synchronize the trigger of an excitation LED of wavelength suitable for the calcium indicator (470nm, jGCaMP7s). The dialog window on the left is how the exposure of the camera is yoked to the illumination trigger. Because we are using an analog output to the LED that receives a digital trigger from the camera, we can set a custom pulse (25ms ON, 25ms OFF, 50% duty cycle) and also use the voltage of the analog system to control the LED intensity. This configuration enables a reduction in photobleaching by only exciting the indicator when the imaging camera is exposing, thereby minimizing photobleaching. You can configure this behavior to control multiple LED light sources, to enable multi-color imaging of two fluorescent indicators.

When the user begins the session, PolyScan displays the collected timestamps in their relative order, as well as displays the different live-feeds of configured cameras – in this example you can see the live feeds of the imaging and behavior cameras simultaneously. After completion of the session, the user can use the review session functionality to view and play back all the timestamps that were captured.

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More details on key references for preprocessing and cell-extraction of imaging datasets:

[6] **Ca**lcium **Im**aging **An**alysis (CaImAn) – a Python and MATLAB pipeline for preprocessing and cell extraction suitable for 1-photon and 2-photon datasets. extraction suitable for 1-photon and 2-photon datasets.

[7] EXTRACT – cell extraction algorithm that was used for Mightex OASIS acquired movies (cell extraction).

[8] More details on the EXTRACT algorithm.

[9] **Ca**lcium **I**maging **A**nalysis (CIAtah) – a preprocessing, spatial filtering, and motion correction pipeline suitable for 1-photon and 2-photon datasets. Has a useful GUI for visualization of each step of preprocessing. Used for Mightex OASIS acquired movies (preprocessing).

[10] NoRMCorre – Non-rigid (and rigid) Motion Correction algorithm with Python and MATLAB implementations. Used for Mightex OASIS acquired movies (preprocessing/motion correction).

[11] More details on subpixel imaging registration algorithms.

[12] Book chapter for the execution of surgery, acquisition, and analysis of single-cell imaging datasets.