CRCNS Data Sharing

Large-scale neuronal recordings in primary visual cortex
USERS GUIDE

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Users guide

Large-scale neuronal recordings in cat and monkey primary visual cortex were made with multi-site silicon electrode arrays that enable simultaneous recording from more than a hundred single units at once. This document details the experimental procedures and contains information for understanding the data files and formats for the freely available data distributed by the CRCNS Data Sharing Initiative. For further background information see the `summary.pdf`.

Multi-site electrode arrays (polytrodes)

Polytrodes for these recordings were made by the Center for Neural Communication Technology at the University of Michigan, to a variety of specifications. They can now be obtained commercially from NeuroNexus Technologies, Ann Arbor MI (www.neuronexustech.com).

These particular high-density 54-channel polytrodes are single-shank planar silicon electrode arrays designed for *in vivo* large scale extracellular recordings. They are passive recording devices (i.e. no on-chip electronics), and come in a number of two- and three-column designs, collinear and staggered site arrangements, with different inter-site spacing (Figure 1A), to achieve a good trade-off between adequate sampling and isolation of individual neurons (which requires spikes to appear on multiple sites), and traversal of as much brain as possible with a finite number of sites. The type of polytrode used in a specific recording is indicated in the appropriate data directory.

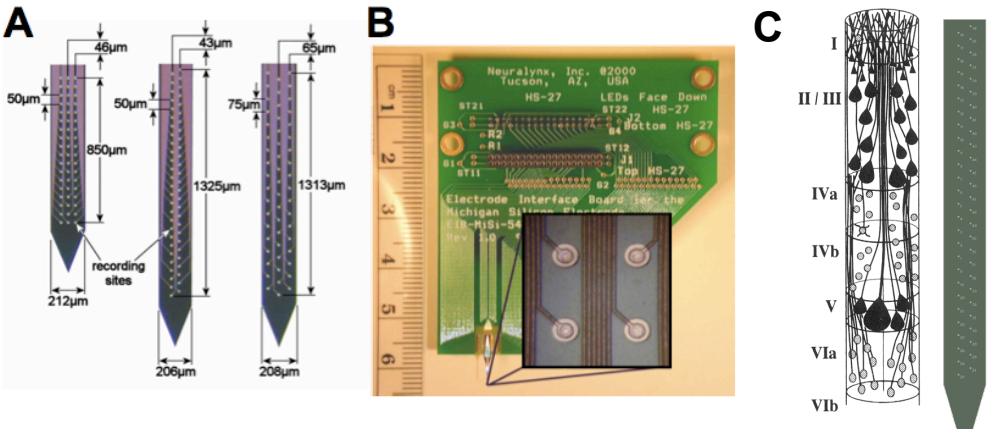


Figure 1 (A) Photomicrographs of three different polytrode designs. The polytrode shanks have planar recording sites spaced 43-75µm apart in two or three columns. Polytrodes with denser site spacing (54µmap1b) provide highly detailed field recordings, and span ~1mm. The longer two column polytrodes (54µmap2b) traverse 1.3~1.7mm, enabling simultaneous recordings of units from entire cortical columns in primary visual cortex. All polytrodes are 15µm thick, 199-212µm wide (depending on the design), with 15µm diameter recording sites made of pure iridium. (B) A polytrode shown here bonded to the headstage interface board used in the cat recordings. The exposed recording sites are visible in the inset. (C) 2a polytrode schematic, with 65µm spaced sites, in relation to the cortical layers.

Cat surgical & recording procedures

Surgical preparation for acute electrophysiology. Adult cats of either sex were prepared for acute electrophysiological recordings in accordance with guidelines established by the Canadian Council for Animal Care. For the initial surgery cats were anesthetized with an i.v. bolus of sodium thiopental (2.5% w/v) to effect, with booster injections administered as needed. Either intubation or a tracheotomy was performed, and the cat was placed in a stereotaxic frame and connected to temperature, BP, ECG, EEG, pO_2, and end-tidal CO_2 monitors. Pressure points and wounds were infiltrated with the local anesthetics Lidocaine (5%) and Marcaine (bupivacaine hydrochloride, 0.25%). Dexamethasone (0.3 mg i.m.) was given to prevent brain edema. Intravenous injections of anesthetics were discontinued, and surgical anesthesia was maintained by artificial ventilation with a mixture of 70% N_2O and 1.5% isoflurane in oxygen. Core body temperature was maintained near 38°C, tidal $SpCO_2$ and pO_2 were stabilized at 40 mmHg and 99~100% respectively, by varying the respiration rate. A 5 × 10 mm craniotomy was made over cortical areas 17 and 18. With the aid of a surgical microscope a small area of dura was carefully reflected. At this point paralysis was induced with pancuronium bromide (2 mg/kg) and maintained throughout the experiment by continuous i.v. infusion of pancuronium (0.2 mg/kg/hr) dissolved in lactated Ringers with 5% dextrose, delivered at a rate of 3 mls/kg/hr. Pupils were dilated with topical atropine (5%) and nictitating membranes were retracted with phenylephrine eye-drops (10%). Using reverse ophthalmoscopy, rigid gas-permeable contact lenses (Harbour City Contact Lens Services, Nanaimo BC) of appropriate radius of curvature and power were used to focus both eyes on the stimulus display monitor positioned 50cm in front of the cat. Additional drops of phenylephrine and atropine were periodically applied as required.

Data acquisition. Extracellular neural activity in the primary visual cortex (area 17), referenced to a platinum wire loop positioned above the dura, was buffered by two unity gain headstage pre-amplifiers (HS-27s, Neuralynx, AZ), amplified 5000x and bandpass filtered (500Hz-6kHz) with a multichannel amplifier (FA-I-64, MultiChannel Systems, Germany). A custom-made patch box (Multichannel Systems) enabled a selection of the polytrode channels to be relayed into ten amplifier channels having band-pass filters appropriate for recording LFPs (0.1-150Hz). These 64 signals were continuously sampled with 12 bit resolution by two synchronized 32-channel ADC cards (DT3010s, Data Translation, MA) at 25kHz/channel (downsampled to 1kHz/channel for the LFP-filtered channels).

The aggregate noise of the system with the polytrode in phosphate buffered saline was 3–4μVrms (20~30μVpp); *in vivo,* with additive background neural activity, the 'noise' level was 5~8μVrms (35~56μVpp). Isolatable spikes were typically in the range 50~250μVpp, with occasional spikes as large as 1.8mVpp. Offline the spike waveform data were upsampled four-fold to 100kHz using Nyquist interpolation (Blanche and Swindale 2006).

Primate surgical & recording procedures

Experiments were done on awake, head-restrained adult male rhesus monkeys (*macaca mulatta*). Monkeys were trained via standard operant conditioning techniques to maintain fixation on a fixation spot in exchange for a juice reward.

Surgical procedure for implantation of head post and recording chambers. After completion of the fixation training, animals were implanted with a Ultem (General Electric Plastics) headpost and plastic (MRI compatible CILUX) recording chambers (Crist, Hagerstown MD) 20mm in diameter. For surgery initial anesthesia was induced by i.m. injection of ketamine (10 mg/kg), supplemented with xylazine (0.04 mg/kg), and maintained with 0.5-3% isofluorane in oxygen/nitrous oxide (30/70) after tracheal intubation. Ceramic bone screws, the head post and recording chamber were then fixed and interconnected by dental acrylic cement. Bilateral cranial reference electrodes were also implanted and wired to external ports embedded in the dental acrylic for connection with the pre-amplifiers. Surgical procedures were conducted under aseptic conditions. Post-operative treatment included prophylactic administration of antibiotics and a recovery period of six weeks. All surgical procedures and experimental techniques were performed in accordance with the NIH Guide for Care and Use of Laboratory Animals and the regulations for the welfare of experimental animals issued by the Federal Government of Germany approved by the local authorities.

Data acquisition. The polytrode was bonded to a head-stage printed circuit board designed for use in the confines of the recording cylinder (MultiChannel Systems, Reutlingen, Germany). Polytrode signals, referenced to one of the cranial electrodes, were fed to two 32 channel headstage pre-amplifiers (MPA-32I, MultiChannel Systems), then to a 64 channel amplifier (FA-I-64, Multichannel Systems). All channels had broad-band filter settings appropriate for recording both LFP and single units (0.1Hz–6KHz). Signals were continuously sampled and streamed to hard disk at 16-bit resolution with a PC-based high-speed signal acquisition card (16AISS64, General Standards, MA). The sampling rate was 25 kHz per channel and time stamps were accurate to the nearest microsecond.

The aggregate noise of the system with the polytrode in phosphate buffered saline was 2–3μVrms (15~20μVpp); *in vivo,* with additive background neural activity, the noise level was 5~8μVrms (35~56μVpp). Isolatable spikes were typically in the range 50~300μVpp, with occasional spikes as large as 3mVpp. Offline the spike waveform data were upsampled four-fold to 100kHz using Nyquist interpolation (Blanche and Swindale 2006).

Electrophysiology procedures (both species). Polytrodes are extremely flexible and cannot penetrate the dura mater without fracturing, nor usually the pia mater without excessive dimpling of the brain. It was thus necessary to reflect the dura and make a tiny incision (~250μm long) in the pia using an angled slit

knife intended for ophthalmic microsurgery (ClearCut™ 3.2mm, Alcon Surgical). The polytrode could then be inserted without noticeable depression of the brain.

While viewing the exposed surface of the brain through a surgical microscope, the polytrode was slowly advanced into the cortex with a micromanipulator until the top sites were ~200μm below the surface. The usual practice was to record at a single fixed depth per penetration, either traversing a cortical column by inserting vertically in the crown of a gyrus, or down the medial bank of a lateral gyrus for trans-columnar recordings. After insertion the craniotomy was filled with agar (2.5% in artificial-CSF) to diminish brain movements. Spike amplitudes were often attenuated or even abolished following movement of the polytrode, presumably due to loss of electrical coupling with the neuropil. Following insertion of the electrode into the brain there was a waiting period of 10~45 mins to allow the electrode to stabilize.

Prior to making recordings any faulty recording sites were shorted to ground to avoid electrical artifacts. During recording, spike waveforms were displayed in 1ms edge-triggered epochs in the same layout as the recording sites to aid visualization of the neural activity across the probe. LFP and EEG signals were displayed on scrolling charts and a current source density (CSD) profile derived from the LFPs provided real-time information about the depth and alignment of the polytrode in relation to the cortical laminae (Figure 3). An audio monitor was connected to the amplified channels, and auditory feedback used to assess visual responsiveness and the net receptive field position of the recorded units.

Visual stimulation

Visual stimuli were presented on an Iiyama HM903DT monitor at a resolution of 800 x 600 pixels and a refresh rate of 200Hz. Software-based gamma correction was used to linearize the screen luminance output (mean luminance 52 cd/m^2, peak luminance 105 cd/m^2 , black level ≤0.01 cd/m^2). The recording laboratory was dimly lit, measured with a photometer to have less than 3 cd/m^2 ambient light. Neural responses were evoked with a wide range of visual stimuli, including sinusoidal drifting gratings of differing orientation, spatial and temporal frequency, and contrast; spatiotemporal noise stimuli generated with varying statistics; flashed spots, bars and gratings; and dynamic natural scene movies (www.ini.uzh.ch/~peterk/ProjectFrameset.html) that simulate retinal stimulation under natural viewing conditions (Figure 2).

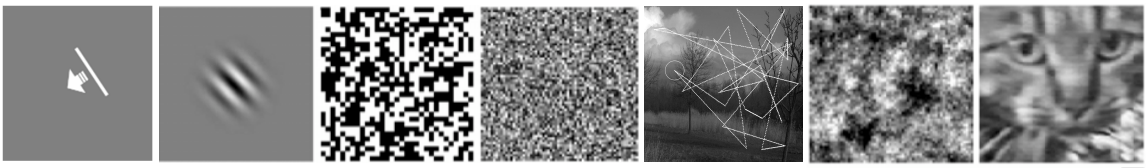


Figure 2 A sample of the set of visual stimuli used to characterize neuronal responses. From left to right: oriented drifting bar; sinusoidal grating patch; m-sequence white noise; Gaussian white noise; simulated saccade movie; spatiotemporal pink noise; dynamic natural scene movie (scale = 1.5 deg).

All stimuli were generated using the freely available open-source package Vision Egg (http://www.visionegg.org) with an extension library called 'dimstim' (http://www.ece.ualberta.ca/~mspacek/Dimstim_2005-06-08.zip). Being built around an interpreted language makes it relatively easy to replicate the original stimuli by executing the scripts contained in the experiment meta-files. Details of specific stimuli are provided in the corresponding data files.

Online estimation of recording depth

Generalized activation of the optic pathway via direct thalamic or photic stimulation evokes a characteristic laminar activation pattern in the primary visual cortex that can be revealed by CSD analysis (Nicholson and Freeman 1975; Mitzdorf and Singer 1978). CSD analysis was exploited here to confirm the depth of the polytrode in the cortex. Evoked responses to brief flashed stimuli (full frame, 10ms duration) were averaged, and the one dimensional CSD was computed from the second spatial derivative of LFPs from vertically aligned (translaminar) sites:

$$\frac{\delta^2\phi}{\delta z^2} \approx \frac{\phi_{(z+n\Delta z)} - 2\phi_{(z)} + \phi_{(z-n\Delta z)}}{(n\Delta z)^2}$$ (Nicholson and Freeman 1975)

where φ was the average field potential, z was the electrode site coordinate perpendicular to the layers, Δz was the sampling interval (100-150μm depending on the polytrode), and nΔz was the differentiation grid (typically n=2).

Monkey eye tracking

Binocular eye position was monitored at 500Hz with an Eyelink II infrared eye tracking system (SR Research, Ontario, Canada). According to manufacturer's specifications this system measures gaze position with an accuracy better than 0.2°. Precision is noise limited to <0.01°. During recording the monkey was required to maintain fixation on a fixation spot within a ±0.5° virtual fixation window centered on the screen, otherwise the stimulus and data collection was paused. Blinks or gaze deviations outside the fixation window for less than 70ms were ignored. Data from the eye tracking computer was synchronized with the neural data acquisition and stimulus computers with millisecond precision.

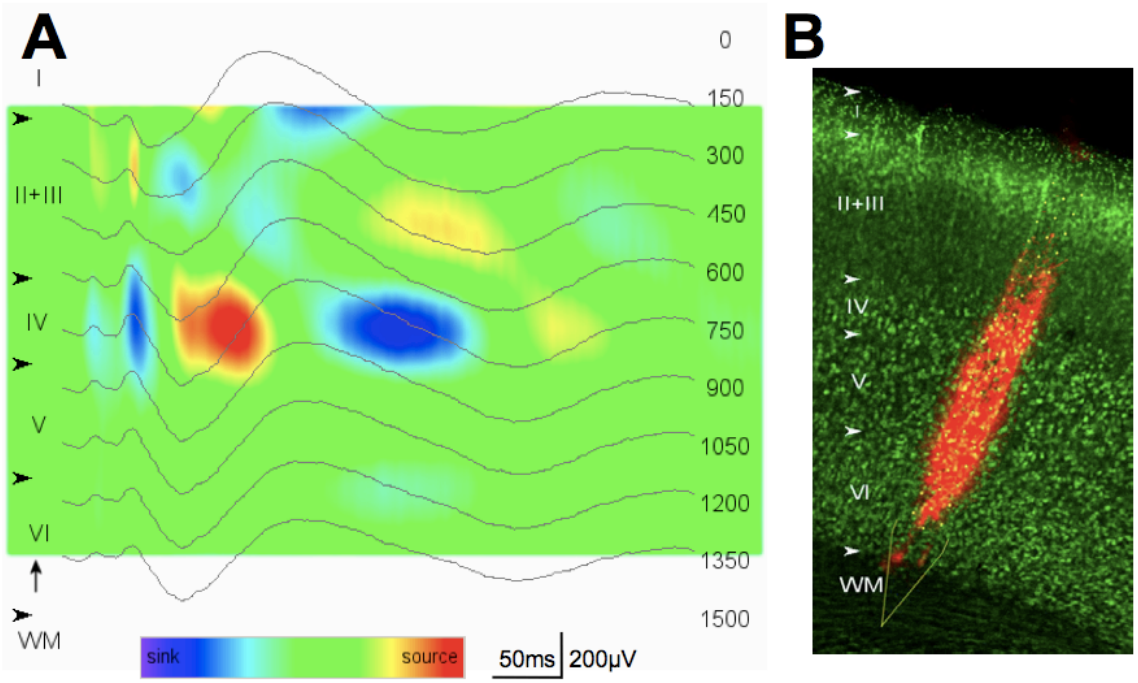


Figure 3 (A) Photic stimulation evokes characteristic LFP responses in the visual cortex (average traces, n = 400), from which the CSD profile was derived (color map). Prominent current sinks and sources in upper layer II/III and IV can be used to identify the depth of the polytrode with respect to the cortical layers (arrows). In this example the top and bottom polytrode sites were 150µm and 1350µm deep, respectively (cortical depth in µm is indicated to the right of the figure). The flash (vertical arrow) duration was 10ms, LFPs were sampled (Δz) every 150µm, and for the differentiation grid n=2. (B) Histological reconstruction of the electrode track confirmed the vertical alignment and depth of the polytrode. The tip was visible in the white matter of the adjacent brain section (Blanche et al., 2005).

Spike sorting

Because of the lack of available software or published algorithms suitable for use with polytrodes we developed our own techniques for spike sorting. The method works as follows: there is an initial threshold-based event detection stage where a large sample of candidate spike waveforms is identified. These are clustered, first using a divisive clustering algorithm, then refined with an extended *k*-means clustering algorithm, to generate a set of spike waveform templates. These templates are then fit to the voltage waveforms at each point in time and an RMS error value is calculated for each point in the voltage record. A spike is identified if there is a local minimum in the error value and if it falls below a user-set threshold. Histograms of the values of local error minima are used to determine the threshold for each unit, and to ensure that the distribution of minima does not overlap the tail of the distribution of larger values resulting from fits to noises and spike events that are near-neighbors in terms of spike shape but nevertheless not the same unit.

Following sorting, additional checks are done to check classification accuracy. These include measures of similarity across all pairs of templates, to ensure they are distinct, and calculations of spike train autocorrelograms, which will fail to

show a clean trough of zero height with a width of a few msecs around $\Delta t=0$ if spikes are being misclassified. Extensive tests have been carried out to investigate the method's ability to detect and correctly classify spikes, and to characterize sensitivity to temporal overlap (Blanche et al., 2008). These tests have shown that the method can reliably isolate spikes with unitary signal-to-noise ratios, and that error rates in identification are low in absolute terms and also lower than other published methods.

Data overview

The shared portion of these data have been grouped into two datasets:

Dataset 1: Multi-neuron evoked responses in primary visual cortex

- spike times of ten (10) simultaneously recorded cells from a single penetration in anaesthetized cat area 17 (trans-columnar down the medial bank) in response to oriented drifting bars, spatiotemporal white noise, and a dynamic natural scene movie.
- spike times of ten (10) simultaneously recorded cells from a single penetration in awake monkey V1 during a fixation paradigm, in response to stimuli comparable to the cat experiments.
- 2D eye tracking data in the case of the monkey recording.
- code and movie frames for re-generating the stimuli, and stimulus meta-data.
- additional meta-data detailing the experimental setup, data acquisition, signal processing, spike sorting, etc.

The receptive fields of all neurons were within 8° of the area centralis (or fovea).

Dataset 2: Multi-neuron spontaneous activity in visual cortex

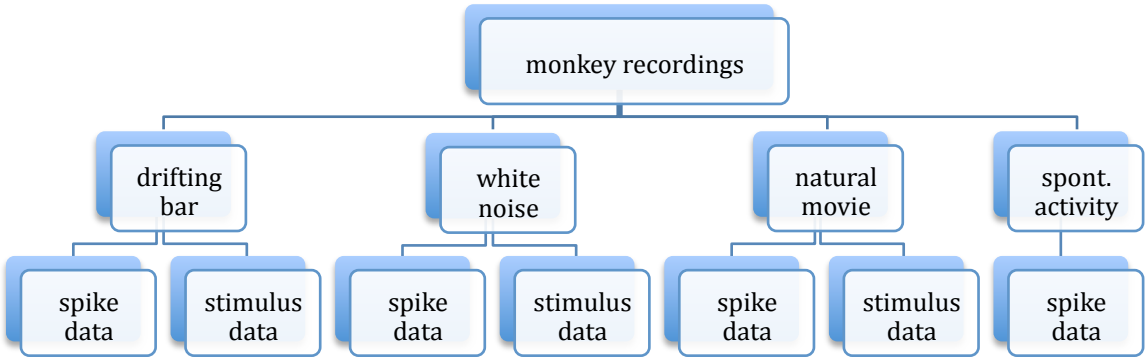
- spike times of 25~50 simultaneously recorded neurons, from two recordings in cat (down the medial bank in area 17 and a vertical penetration in area 18), and monkey primary visual cortex, ~200 neurons in total.
- 2D eye tracking data in the case of the monkey recording.
- additional meta-data detailing the experimental setup, data acquisition, signal processing, spike sorting, etc.

The total duration of the spontaneous recordings is ~9 minutes.

At a later date the unprocessed, continuously sampled waveforms from these recordings, ~1hr in duration, will be released. Additional stimuli and neurons may also be added at a later date.

Data files & formats

The data are organized into several sub-directories and files. The directory hierarchy for the monkey data is shown here:



An identical directory structure exists for the cat data. The directory names should be fairly self-explanatory. The top level directory identifies the species. The second level groups recordings by stimulus type, including recordings of spontaneous activity. The third level directory groups data by modality.

The `spike_data` directories contain five different file types:

`txx.spk` files contain spike times for an individual neuron, indexed by template id (e.g. `t00.spk`, `t18.spk`, etc.). Timestamps are in microseconds with 10μs precision and stored as binary 64-bit signed integers.

`spk_info.txt` is a text file with additional metadata describing the `.spk` files.

`polytrode_xx.pas` is a text file with metadata describing the polytrode used in the recordings, and specifies the mapping between channel number and electrode site coordinates (in microns). The file is Object Pascal code, but it should be trivial to parse and extract the relevant metadata.

`txx.tem` contain the multi-channel spike waveform template for an individual spike-sorted unit, indexed by template id (e.g. `t00`, `t18`, `t26`). The template comprises a 1ms average spike waveform from each channel in the electrode array, up-sampled to 100kHz, and stored as 54x100 32-bit real numbers (in millivolts). The data are stored in ascending order by channel, using the channel numbering scheme defined in `polytrode_x.pas`.

`.tif` image files: `polytrode2a.tif` is an image of the probe showing the recording site configuration; `spk_templates_olay.tif` shows the templates used for spike sorting.

The `stimulus_data` directories contain (up to) six different file types:

`.din` files contain the timing information for the stimulus, one entry for every vertical frame refresh of the stimulus CRT (i.e. 200Hz, so every 5ms). These are binary files comprising consecutive pairs of 64-bit timestamps/64-bit condition indices. For the drifting bar stimulus, the condition index indicates the orientation of the bar; refer to the relevant `.py` file for the mapping between condition index and bar orientation. For the spatiotemporal white noise and dynamic natural scene movies the condition index refers to the (zero-based) frame index of the `.m` file (see below) that was displayed on that refresh. Since the movie frames were updated at 50Hz, and the CRT refresh was 200Hz, there are four repeating entries per frame. As with the spike times, all timestamps are in microseconds, stored as 64-bit signed integers.

`stim_info.txt` is a text file with metadata describing the `.din` file.

`.py` files are text files with additional metadata describing the stimulus. They are actual Python scripts that, if executed in conjunction with Vision Egg & DimStim, will display the original stimulus presented in the recording session.

`.m` files contain the stimulus movie sequences. Both the spatiotemporal white noise and the dynamic natural scene movies are 64 x 64 pixels, with a resolution of 0.2^{o}/pixel, subtending 12.8^{o} of visual angle. Pixels are 8 bit grayscale, stored uncompressed as bytes (unsigned integers). The movies were displayed at a frame rate of 50Hz, and run for 2 minutes each, thus each movie sequence is 6000 frames long. Note that there is no movie for the drifting bar stimulus; this was generated 'on the fly' by Vision Egg.

`.edf` files are native Eyelink II files and contain binocular eye-tracking data for the monkey recordings, sampled every 2ms (500Hz).

`.eye` files are tab-delimited text files exported from the original `.edf` files. The data are arranged in columns, for example:

```
.
23799536        400.4   296.1  2268.0          399.6   298.4  2358.0
MSG   23799537 Stimulus started
23799538        400.1   296.1  2267.0          398.9   299.0  2358.0
MSG   23799540       0        0
23799540        400.5   296.3  2268.0          399.0   299.1  2355.0
MSG   23799542       0        1
23799542        400.8   296.4  2265.0          399.0   299.1  2352.0
23799544        400.5   296.3  2267.0          399.1   299.3  2351.0
23799546        400.2   296.3  2268.0          399.2   299.5  2351.0
MSG   23799547       0        2
23799548        400.5   295.9  2268.0          399.0   299.6  2350.0
23799550        400.7   296.4  2270.0          399.0   299.1  2350.0
MSG   23799552       0        3
23799552        400.5   296.8  2270.0          398.9   299.1  2350.0
.
.
```

Entries that start with a number are organized as follows:

	---------- Left eye ----------			---------- Right eye ----------		
Timestamp	XPos	YPos	Pupil area	XPos	YPos	Pupil area
23799538	400.1	296.1	2267.0	398.9	299.0	2358.0

The timestamps are in milliseconds, but are not synchronized with the clock used to generate timestamps for the spikes or stimulus frames. The XPos and YPos are given in pixel screen coordinates, and the pupil area is measured in pixels^2. In the example shown the monkey's gaze was centered on the middle of the screen, at 400, 300.

In order to synchronize the eye-tracking records with the stimulus and neural data acquisition computers, messages were sent to the eye-tracking computer from the stimulus computer on every screen refresh:

MSG 23799547 0 2

where the first number is the Eyelink II timestamp (in ms), and the second number is the condition index (as in the `.din` files). The third number increments with every screen refresh that the condition index (i.e. stimulus) didn't change. In this way it is possible to align the timebase of the Eyelink II data with that of the stimulus and spikes.

Other events, such as the start and end of the stimulus runs, were also relayed to the eye-tracking computer, for example:

MSG 23799537 Stimulus started

Periodically a drift correction procedure was run, whereby the stimulus was paused and a quick 1-point calibration of the eye tracking system was performed. Drift corrections are identified by the following entries:

MSG 23799460 DRIFTCORRECT LR LEFT at 400,300 OFFSET 0.58 deg. -12.8,16.6 pix.
MSG 23799460 DRIFTCORRECT LR RIGHT at 400,300 OFFSET 0.44 deg. 6.7,14.4 pix.

The Xpos and Ypos entries for both eyes are offset appropriately follow the drift correction procedure.

Saccades, fixations, and loss of tracking when the monkey blinked or looked outside the screen were also detected and flagged by the Eyelink II system, for example:

SSACC L 20910792

indicates the onset of a left eye saccade. For further information and useful utilities for processing and parsing the Eyelink data files, refer to the SR Research support website (https://www.sr-support.com/forums/).

Terms of Use

These data are provided free of charge and without warranty. There are no restrictions placed on its use, however if the data are used in a published academic work or for teaching purposes, both the Data Sharing Initiative and the relevant laboratory where the data were obtained must be cited in the Methods or Acknowledgement section. For the cat data, the appropriate attribution is: *Neural data were recorded by Tim Blanche in the laboratory of Nicholas Swindale, University of British Columbia, and downloaded from the NSF-funded CRCNS Data Sharing website*. For the monkey data, the appropriate attribution is: *Neural data were recorded by Tim Blanche in the laboratory of Winrich Freiwald, University of Bremen, and downloaded from the NSF-funded CRCNS Data Sharing website*.

References

A fuller description of the polytrodes and experimental methods can be found in these publications:

Blanche TJ, Spacek MA, Hetke JF, Swindale NV (2005) Polytrodes: high density silicon electrode arrays for large scale multiunit recording. *J. Neurophys. 93 (5): 2987-3000*.

Blanche TJ & Swindale NV (2006) Nyquist interpolation improves neuron yield in multiunit recordings. *J. Neuro. Methods 155 (1): 81-91*.

Blanche TJ, Godfrey K, Douglas RM & Swindale NV (2008) Spike sorting for polytrodes. J. Neurophys. *submitted*.

Mitzdorf U, Singer W. (1978) Prominent excitatory pathways in the cat visual cortex (A 17 and A 18): a current source density analysis of electrically evoked potentials. *Exp Brain Res.; 33: 371-94*.

Nicholson C, Freeman JA. (1975) Theory of current source-density analysis and determination of conductivity tensor for anuran cerebellum. *J. Neurophys.; 38: 356-68*.

Wegener D, Freiwald WA, Kreiter AK (2004) The influence of sustained selective attention on stimulus selectivity in macaque area MT. *J Neurosci 24: 6106-6114.*