CRCNS.org bf-3 data description
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Recordings from ventral pallidum neurons in male rats undergoing fear discrimination

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Summary

These data were collected from the ventral pallidum of 14 adult male Long Evans rats using 16 wire drivable microelectrode bundles. Data include 435 single units recorded from 194 behavior sessions. Fear discrimination sessions consisted of 16 trials with a mean inter-trial interval of 3.5 min. Auditory cues were 10 s in duration and consisted of repeating motifs of a broadband click, phaser, or trumpet. Each cue was associated with a unique probability of foot shock (0.5 mA, 0.5 s): danger, p = 1.00; uncertainty, p = 0.25; and safety, p = 0.00. Auditory identity was counterbalanced across rats. Foot shock was administered 2 s following the termination of the auditory cue on danger and uncertainty shock trials. The order of trial type presentation was randomly determined by the behavioral program, and differed for each rat, each session. The microelectrode bundle was advanced in ~84 μm steps every other day to record from new units during the following session.

For three of the fourteen rats, the electrode bundle was centered at +0.36 mm from bregma, for six of the fourteen rats, the electrode bundle was centered at 0.00 mm from bregma, and the other five rats had electrodes centered at -0.36 mm from bregma.

Data included are the result of spike sorting. Sample scripts used to visualize firing and behavior data from our main data file have been provided. These data have been published in *Communications Biology* and the complete methods are available with that publication.

Conditions for using the data

Publications created through usage of the data should cite the paper in which they are first reported:

> Moaddab M, Ray MH and McDannald MA. (2021) Ventral pallidum neurons dynamically signal relative threat. Commun Biol 4, 43. https://doi.org/10.1038/s42003-020-01554-4

And also cite the dataset using:

> Mahsa Moaddab and Michael A. McDannald (2021); Recordings from ventral pallidum neurons in male rats undergoing fear discrimination. CRCNS.org https://doi.org/10.6080/K0HH6H8V

Methods

Experimental subjects

A total of 14 adult male Long Evans rats, weighing 250–275 g were obtained from Long Evans breeders maintained in the Boston College Animal Care Facility. The rats were single-housed on a 12 h light/dark cycle (lights on at 7:00 a.m.) with free access to water. Rats were maintained at 85% of their free-feeding body weight with standard laboratory chow (18% Protein Rodent Diet #2018, Harlan Teklad Global Diets, Madison, WI), except during surgery and post-surgery recovery. All protocols were approved by the Boston College Animal Care and Use Committee and all experiments were carried out in accordance with the NIH guidelines regarding the care and use of rats for experimental procedures.

Electrode assembly

Microelectrodes consisted of a drivable bundle of sixteen 25.4 µm diameter Formvar-Insulated Nichrome wires (761500, A-M Systems, Carlsborg, WA) within a 27-gauge cannula (B000FN3M7K, Amazon Supply) and two 127 µm diameter PFA-coated, annealed strength stainless-steel ground wires (791400, A-M Systems, Carlsborg, WA). All wires were electrically connected to a nano-strip Omnetics connector (A79042-001, Omnetics Connector Corp., Minneapolis, MN) on a custom 24-contact, individually routed and gold immersed circuit board (San Francisco Circuits, San Mateo, CA). Sixteen individual recording wires were soldered to individual channels of an Omnetics connector. The sixteen wire bundle was integrated into a microdrive permitting advancement in ~42 µm increments.

Surgery

Stereotaxic surgery was performed aseptic conditions under isoflurane anesthesia (1-5% in oxygen). Carprofen (5 mg/kg, i.p.) and lactated ringer's solution (10 mL, s.c.) were administered preoperatively. The skull was scoured in a crosshatch pattern with a scalpel blade to increase efficacy of implant adhesion. Six screws were installed in the skull to further stabilize the connection between the skull, electrode assembly and a protective head cap. A 1.4 mm diameter craniotomy was performed to remove a circular skull section centered on the implant site and the underlying dura was removed to expose the cortex. Nichrome recording wires were freshly cut with surgical scissors to extend ~2.0 mm beyond the cannula. Just before implant, current was delivered to each recording wire in a saline bath, stripping each tip of its formvar insulation. Current was supplied by a 12 V lantern battery and each Omnetics connector contact was stimulated for 2 s using a lead. Machine grease was placed by the cannula and on the microdrive. For implantation dorsal to the VP, the electrode assembly was slowly advanced (~100 µm/min) to the following coordinates: -0.08 mm form bregma, -2.05 mm lateral from midline, and -6.95 mm ventral from the cortex. Once in place, stripped ends of both ground wires were wrapped around two screws in order to ground the electrode. The microdrive base and a protective head cap were cemented on top of the skull using orthodontic resin (C 22-05-98, Pearson Dental Supply, Sylmar, CA), and the Omnetics connector was affixed to the head cap.

Behavior apparatus

All experiments were conducted in two, identical sound-attenuated enclosures that each housed a Pavlovian fear discrimination chamber with aluminum front and back walls retrofitted with clear plastic covers, clear acrylic sides and top, and a stainless steel grid floor. Each grid floor bar was electrically connected to an aversive shock generator (Med Associates, St. Albans, VT) through a grounding device. This permitted the floor to be grounded at all times except during shock delivery. An external food cup and a central nose poke opening, equipped with infrared photocells were present on one wall. Auditory stimuli were presented through two speakers mounted on the ceiling of enclosure. Behavior chambers were modified to allow for free movement of the electrophysiology cable during behavior; plastic funnels were epoxied to the top of the behavior chambers with the larger end facing down, and the tops of the chambers were cut to the opening of the funnel.

Nose poke acquisition

Experimental procedure started with two days of pre-exposure in the home cage where rats received the pellets (Bio-Serv, Flemington, NJ) used for rewarded nose poking. Rats were then shaped to nose poke for pellet delivery in the behavior chamber using a fixed ratio schedule in which one nose poke yielded one pellet until they reached at least 50 nose pokes. Over the next 5 days, rats were placed on variable interval (VI) schedules in which nose pokes were reinforced on average every 30 s (VI-30, day 1), or 60 s (VI-60, days 2 through 5). For fear discrimination sessions, nose pokes were reinforced on a VI-60 schedule independent of auditory cue or foot shock presentation.

Fear discrimination

Prior to surgery, each rat received eight 54-minutes Pavlovian fear discrimination sessions. Each session consisted of 16 trials, with a mean inter-trial interval of 3.5 min. Auditory cues were 10 s in duration and consisted of repeating motifs of a broadband click, phaser, or trumpet (listen or download: http://mcdannaldlab.org/resources/ardbark). Each cue was associated with a unique probability of foot shock (0.5 mA, 0.5 s): danger, p=1.00; uncertainty, p=0.25; and safety, p=0.00. Auditory identity was counterbalanced across rats. For danger and uncertainty shock trials, foot shock was administered 2 s following the termination of the auditory cue. This was done in order to observe possible neural activity during the delay period is not driven by an explicit cue. A single session consisted of four danger trials, two uncertainty shock trials, six uncertainty omission trials, and four safety trials. The order of trial type presentation was randomly determined by the behavioral program, and differed for each rat, each session. After the eighth discrimination session, rats were given full food and implanted with drivable microelectrode bundles. Following surgical recovery, discrimination resumed with single-unit recording. The microelectrode bundles were advanced in ~42-84 μm steps every other day to record from new units during the following session.

Histology

Rats were deeply anesthetized using isoflurane and current from a 6 V battery was passed through 4 of the 16 nichrome electrode wires. Rats were transcardially perfused with 0.9% biological saline and 4% paraformaldehyde in a 0.2 M Potassium Phosphate

Buffered solution. Brains were extracted and post-fixed in a 10% neutral-buffered formalin solution for 24 h, stored in 10% sucrose/formalin, frozen at -80°C and sectioned via sliding microtome. In order to identify VP boundaries, we performed immunohistochemistry for substance P (primary antibody, rabbit anti-substance P, 1:100, Immunostar, Hudson, WI; secondary antibody, Alexa Fluor 594 donkey anti-rabbit, Jackson ImmunoResearch Laboratories, West Grove, PA), and NeuroTraceTM (1:200, Thermo Fisher Scientific, Waltham, MA). Sections were mounted on coated glass slides, coverslipped with Vectashield mounting medium without DAPI (Vector Laboratories, Burlingame, CA), and imaged using a fluorescent microscope (Axio Imager Z2, Zeiss, Thornwood, NY).

Single-unit data acquisition

During recording sessions, a 1x amplifying headstage connected the Omnetics connector to the commutator via a shielded recording cable (Headstage: 40684-020 & Cable: 91809-017, Plexon Inc., Dallas TX). Analog neural activity was digitized and high-pass filtered via amplifier to remove low-frequency artifacts and sent to the Ominplex D acquisition system (Plexon Inc., Dallas TX). Behavioral events (cues, shocks, nose pokes and pellet deliveries) were controlled and recorded by a computer running Med Associates software. Timestamped events from Med Associates were sent to Ominplex D acquisition system via a dedicated interface module (DIG-716B). The result was a single file (.pl2) containing all time stamps for recording and behavior. Single units were sorted offline with a template-based spike-sorting algorithm (Offline Sorter V3, Plexon Inc., Dallas TX). Timestamped spikes and events (cues, shocks, nose pokes and pellet deliveries) were extracted and analyzed with statistical routines in Matlab (Natick, MA).

Data files organization

VP cube data structure organization (found in cubeFile folder)

Data structure is 3D CUBE ROW x COLUMN x Z (R:C:Z)
Z is always the # of neurons (n = 435)
16 rows reflect the 16 trials per session.
Rows 1-4, danger (p = 1.00)
Rows 5-6, uncertainty shock (p = 0.25)
Rows 7-12, uncertainty no-shock
Rows 13-16, safety (p = 0.00)

1. VPcube.name (1 x 22 x 435)
   - First four characters are animal ID
   - Next six characters are date spike was acquired
   - Epr25a is the name of our behavior program
   - SPK and the rest of the string indicate the spike number recorded from the given session
2. VPcube.wave
   - mean waveform values for each trial type based on 32 points, sampled over 0.0008 seconds
3. VPcube.tag
4. VPcube.poke = nose poke data
   .base = baseline nose poke rate 2 s prior to cue presentation

.cue = mean nose poke rate during cue presentation
.s1 = nose poke rate during cue presentation + 20 s prior to onset and post cue offset in 1 s bins
.ms500 = same as. s1 but in 500ms bins

5. VPcube.cer = suppression ratio data for nose pokes
   .cue = mean suppression ratio during 10 s cue presentation
   .s1 and .ms500 reflect bin sizes as in VPcube.poke
6. VPcube.fire = firing data
   firing (CV, CS, raw, diff, and z) at diff intervals aligned to cue presentation
7. VPcube.half duration & .amplituderatio = waveform characteristics for each spike.

Data format

Each of the 435 single units has its own .mat file (found in matFiles folder), the file type created and recognized by matlab. Each .m file is structured identically and the structure is described in the data file organization heading. For our analyses, we construct a 3-D matrix composed of all units (found in cubeFile folder). To do this, place all .m files in a folder, then open the cubeAnalysis script. Specify the location of the .m file folder inside the script as well as the folder to save to. The output of the cubeAnalysis script is a single file that contains firing information for all neurons. The analysisScript permits the user to select neurontypes or analyze all neurons. The analysisScript must point to the folder containing this file. Basic code to calculate and plot population firing, as well as perform linear regression are provided.

How to get started

This dataset is designed for use with MATLAB. Start by determining the appropriate selection criteria for your question. Next, plot the selected activity to reveal population level responding. Reference our sample code to see an example of plotting cue-associated behavior, population firing and a basic regression analysis.

How to get help

To get help with the data set post any questions on the forum at CRCNS.org.

Change history

Version 0.6 (Feb 3, 2021) – Initial version.