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Multi-fiber photometry in head-fixed mice performing a dual-cue delay Pavlovian association

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Abstract

This protocol outlines a dual-cue delay Pavlovian association and multi-fiber photometry recordings in head-fixed mice. Mice are first placed on a controlled water schedule and habituated to head fixation on a spherical treadmill. During the conditioning phase, they learn to associate visual and auditory cues with water rewards, followed by a partial extinction phase where reward probabilities are downshifted.

Behavioural responses, such as licking and movement, are tracked and synchronized with photometry data to assess acetylcholine, dopamine and glutamate release dynamics using genetically encoded sensors (**see Protocol: Stereotaxic injections of viral vectors and chronic optical fiber implants in mouse brains**) during the learning and extinction of Pavlovian associations.

A custom-built microscope with precise excitation and detection systems is used to monitor fluorescence signals from genetically encoded sensors. Imaging is synchronized with behavioural data collection, and data processing is performed to ensure accurate analysis.

Materials

Equipment:

- Spherical Styrofoam Treadmill (Smoothfoam, 8in diameter)
- Optical Computer Mice (Logitech G203)
- Acquisition Board (NIDAQ, PCIe 6343)
- Solenoid Valve (# 161T012, Neptune Research)
- Camera used to capture orofacial movements (Blackfly S USB3, BFS-U3-16S2M-CS, Teledyne Flir)
- LED lights (Thor labs, M470L3, 470 nm)
- Micromanipulator (Newport Model 96067-XYZ-R)
- **Bandpass Filter** (Chroma, No 525/50m)
- Tube lens (Thor labs, No TTL165-A)
- Hamamatsu Camera (Hamamatsu, Orca Fusion BT Gen III)
- Vibration isolation table (Newport)
- Programmable digital acquisition card (NIDAQ, National Instruments PCIe 6343)

Software:

- **HCImage live** (HCImage live, Hamamatsu)
- MATLAB

Troubleshooting

Before start

Genetically encoded sensors to monitor acetylcholine, dopamine, and glutamate dynamics, as well as tetanus toxin light chain (TelC) to suppress acetylcholine, were injected in the anterior dorsal medial striatum of ChAT-IRES-Cre (B6;129S6-Chattm2(cre)Lowl/J, JAX stock number 006410) mice following the **Protocol: Stereotaxic injections of viral vectors and chronic optical fiber implants in mouse brains**.

Habituation & Behaviour Setup

1 One week before starting the Pavlovian conditioning training and photometry recordings, place mice on a water schedule, receiving 1 mL of water per day, and maintain them at 80-85% of their initial body weight for the duration of the experiments.

2 Three to four days prior to training, habituate mice to head fixation on a spherical styrofoam treadmill (**Dombeck et al., 2010**). Details of the behavioural setup are described in **Vu et al., 2024**.

2.1 Allow mice to freely move on the treadmill during all experiments.

2.2 Measure the ball rotation in pitch, yaw, and roll directions using optical computer mice through an acquisition board.

2.3 Dispense water rewards (5µL/reward) through a water spout operated by an electronically controlled solenoid valve, mounted on a post a few mm away from the mice's mouths.

2.4 Monitor tongue spout contacts (a proxy for licking) by a capacitive touch circuit connected to the spout and confirmed with live video taken from a camera positioned to capture orofacial movements.

Pavlovian Conditioning Phase

3 Approximately three weeks post-implantation, start training mice on a dual-cue delay Pavlovian conditioning task.

4 In each session (one session/day), present mice with two different cues (light or tone) in a pseudorandom order (20 presentations of each; total 40 presentations).

4.1 Present light cues via a LED calibrated to deliver light at 7 mW intensity and mounted on a post holder ~ 20 cm away from the mouse, positioned 45 degrees contralateral to the implanted side.

4.2 Present tone cues (12 kHz, 80 dB) via a USB speaker placed ~ 30 cm from the mouse.

5 Present cue (light or tone) one at a time for 6s and pair with a water reward (5µL) after a fixed 3s delay from the cue onset (100% probability)(**see Figure 1**).

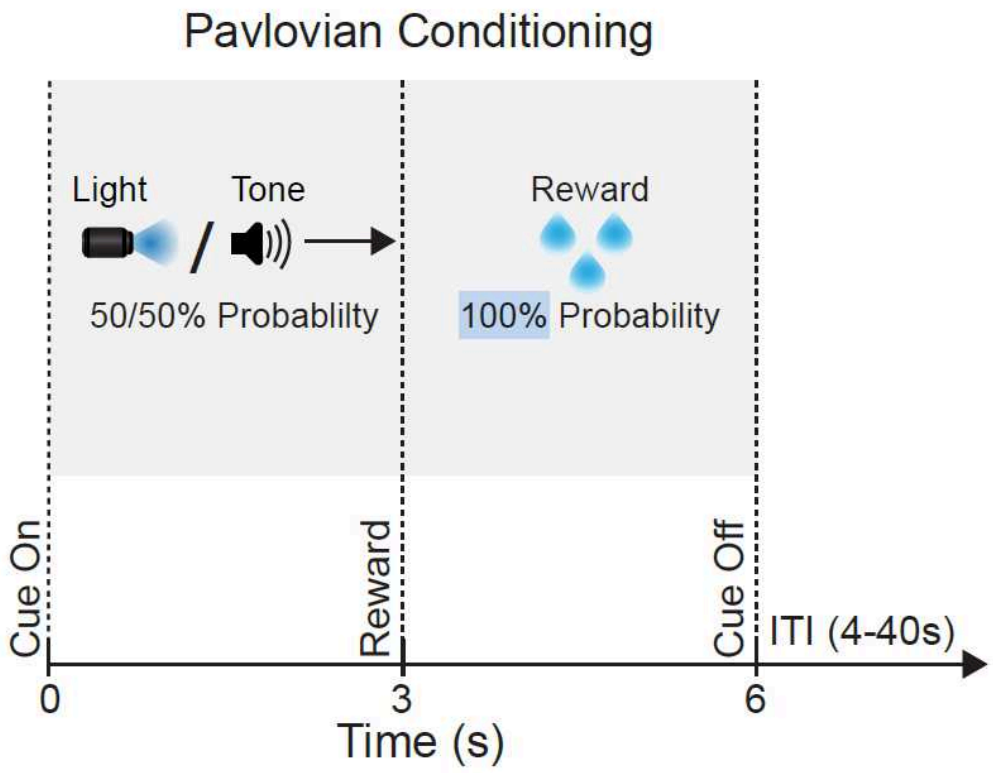

Figure 1 - Schematic of dual-cue delay Pavlovian conditioning task.

6 Randomly draw an inter-trial interval (ITI; time between separate trials) from a uniform distribution of 4-40s.

Note

In our study, we delivered a total of eight random non-contingent rewards per session during the inter-trial interval (ITI; time between separate trials) periods.

7 Train mice for 7-12 consecutive days until they learn that both light and tone cues are associated with the delivery of a water reward (as measured by the lick index).

8 Train mice for an additional 2-6 days.

Partial Extinction Phase

9 Submit mice to a partial extinction phase in which the reward probability associated with one of the two cues is downshifted to 20% and the other cue to 80% (**see**

Figure 2).

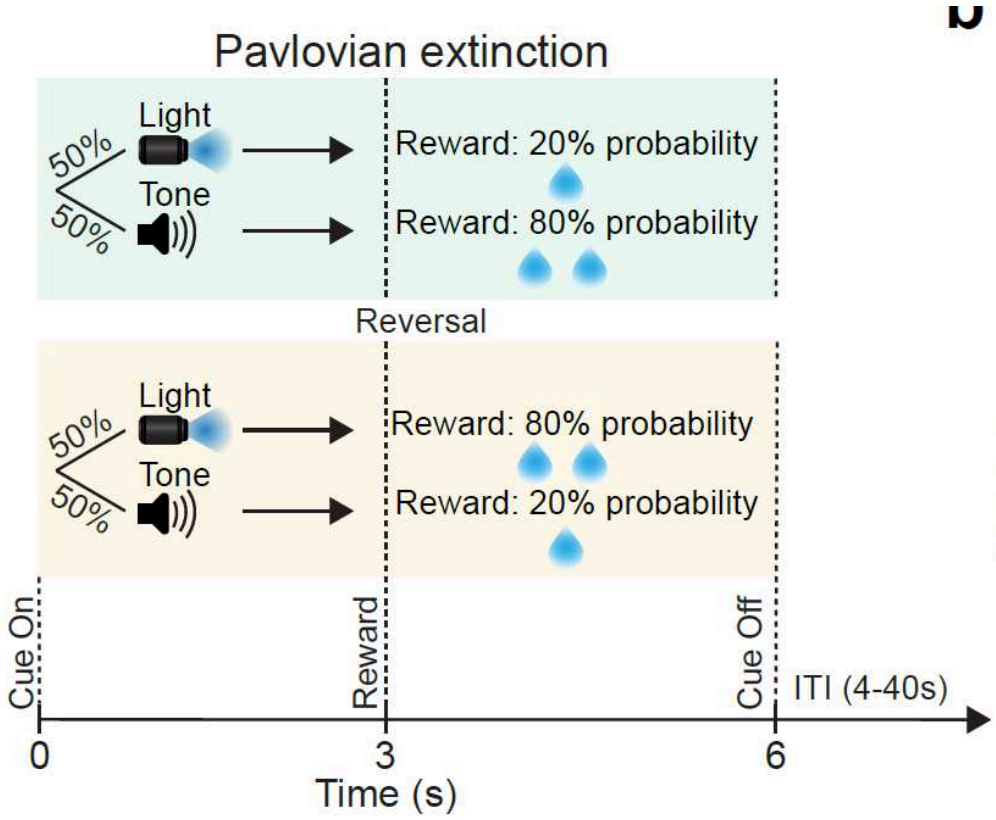

Figure 2 - Schematic of the partial extinction paradigm.

10 In each session (one session/day), present mice with two different cues (light or tone) in a pseudorandom order (30 presentations of each; total 60 presentations).

11 Continue training until mice show significantly diminish pre-licking for the 20% cue relative to the 80% for 4-7 sessions, then reverse cue probabilities.

Note

In our study, we counterbalance the order of light and tone cue probabilities across mice.

For TelC experiments, we trained TelC and control mice for a maximum of 8 sessions for each extinction phase.

Microscope Setup for Multi-Fiber Photometry

12 As mice perform the dual-cue delay Pavlovian conditioning (**steps 3 to 8**) or partial extinction (**steps 9 to 11**) tasks, conduct fluorescence measurements from the multi-

fiber arrays using a custom built microscope (**see Figure 3**) mounted on a 4′ W x 8′ L x 12′ thick vibration isolation table.

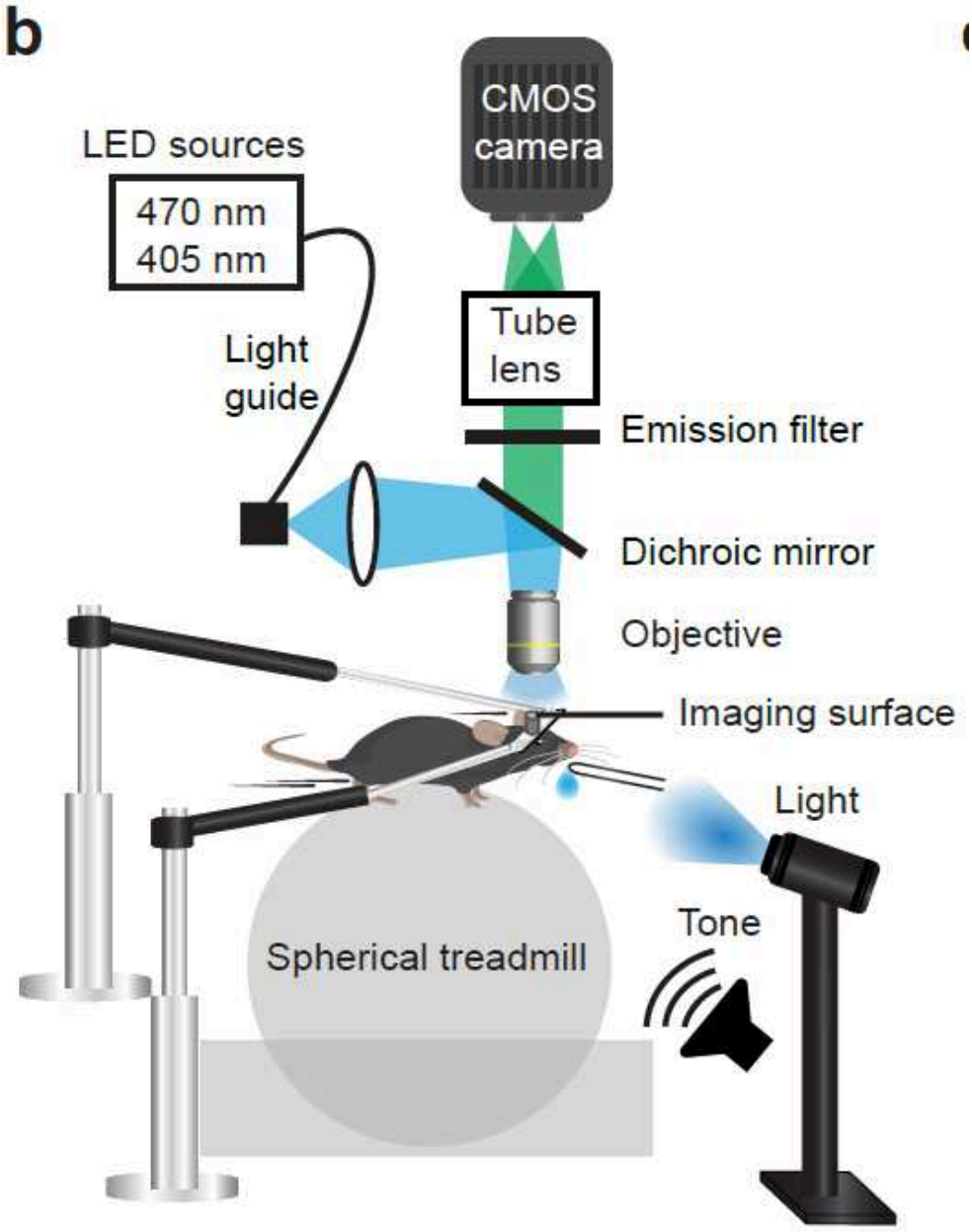

Figure 3 - Spherical treadmill and imaging set up for head-fixed mice.
Details of the microscope are described in Vu et al., 2024.

13 Filter and focus emission light using a bandpass filter and a tube lens onto the CMOS sensor of the Hamamatsu camera, creating an image of the fiber bundle (**see Figure 4**).

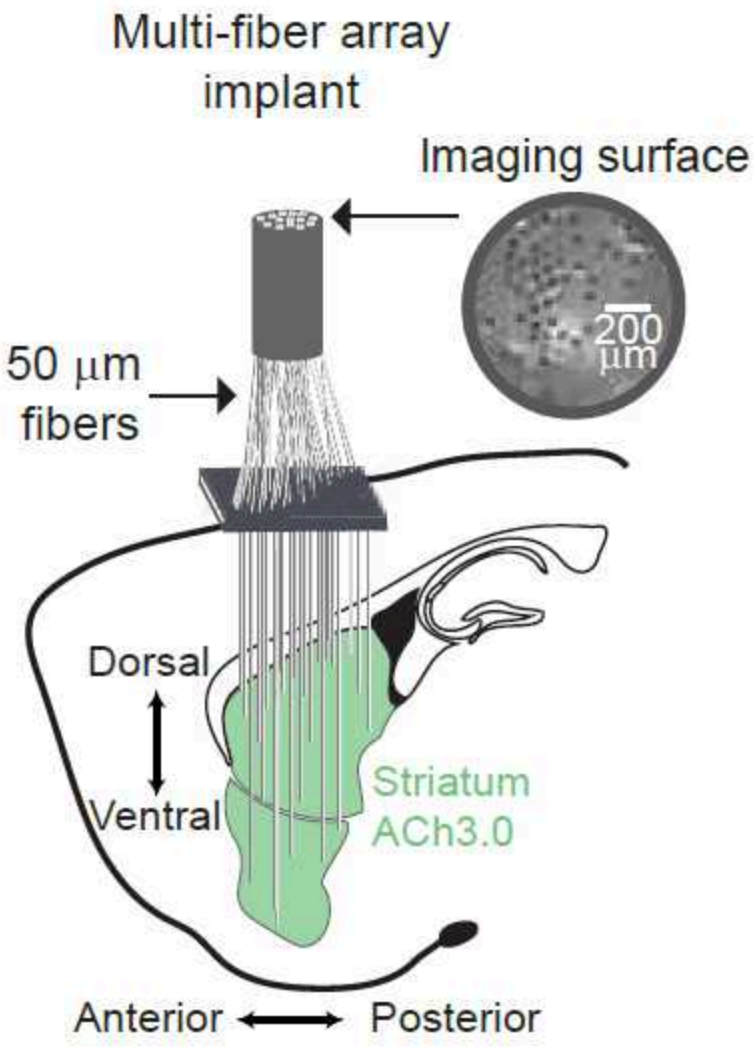

Figure 4 - Schematic of the fiber array approach for measuring ACh release across the striatum.

14 To enable precise manual focusing, connect microscope to a micromanipulator and mount on a rotatable arm extending over the head-fixation setup to facilitate positioning of the objective above the imaging surface over the mouse head.

Multi-Fiber Photometry Image Acquisition

15 Open the basic imaging application HCImage Live that is included with the Hamamatsu camera.

16 Carry out single wavelength excitation with continuous imaging at 30Hz (33.33ms exposure time), via internal triggering.

17 Perform dual wavelength excitation in a quasi-simultaneous externally triggered imaging mode, where the two LEDs are alternated and synchronized with imaging acquisition via 5V digital TTL pulses.

18 Alternate 70 nm excitation with 405 nm excitation at either 36Hz (20ms exposure time) or 22Hz (33.33ms exposure time) to achieve a frame rate of 18 Hz or 11 Hz for each excitation wavelength, respectively.

19 Acquire recordings at different sampling rates were down-sampled or up-sampled using a 1-D interpolation with MATLAB interp1 function using the spline method.

20 Control the timing and duration of TTL pulses through a custom MATLAB software and a programmable digital acquisition card.

21 Transmit voltage pulses to the NIDAQ from the camera following the exposure of each frame to confirm proper camera triggering and to synchronize imaging data with behaviour data.

Protocol references

Dombeck, D., Harvey, C., Tian, L. *et al.* **Functional imaging of hippocampal place cells at cellular resolution during virtual navigation.** *Nat Neurosci* **13**, 1433–1440 (2010).

Vu, M.-A.T., Brown, E.H., Wen, M.J., Noggle, C.A., Zhang, Z., Monk, K.J., Bouabid, S., Mroz, L., Graham, B.M., Zhuo, Y., *et al.* **Targeted micro-fiber arrays for measuring and manipulating localized multi-scale neural dynamics over large, deep brain volumes during behavior.** *Neuron* **112** , 909-923.e9 (2024).