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Open hardware low-cost system for behavioral experiments simultaneously with electrophysiological recordings.

Leandro A. A. Aguiar * · Nivaldo A P de Vasconcelos * · Gabriela Chiuffa Tunes · Antonio J. Fontenele · Marcelo Bussotti Reyes · Romildo de Albuquerque Nogueira · Pedro V. Carelli

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Abstract A major frontier in neuroscience is to find neural correlates of perception, learning, decision making, and a variety of other types of behavior. In the last decades, modern devices have provided to simultaneously record not only the different responses but also the electrical activity of large neuronal populations. However, the commercially available instruments for behavioral recordings are expensive, and the design of low-cost operate condition chamber has emerged as an appealing alternative to resource-limited laboratories engaged in animal behavior. In this article, we provide a full description of an experimental platform for simultaneously recording behavior and electrical activity of a neural population. The programming of this platform is open source and flexible so that any behavioral task can be implemented. We show examples of behavioral experiments with freely moving rats with simultaneous electrophysiological recordings.

* LAAG and NAPV contributed equally

Leandro A. A. Aguiar
Departamento de Morfologia e Fisiologia Animal, Universidade Federal Rural de Pernambuco, Recife, PE, 52171-900, Brazil.
Physics Department, Federal University of Pernambuco, Recife, PE, 50670-901, Brazil.

Nivaldo A P de Vasconcelos
Department of Biomedical Engineering, Federal University of Pernambuco, Recife, PE, 50670-901, Brazil.
Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, 4710-057, Portugal.
ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, 4806-909, Portugal.

Gabriela Chiuffa Tunes
Center for Mathematics, Computing and Cognition, Universidade Federal do ABC, Santo André, SP, 09210-580, Brazil.

Antonio J. Fontenele
Physics Department, Federal University of Pernambuco, Recife, PE, 50670-901, Brazil.

Marcelo Bussotti Reyes
Center for Mathematics, Computing and Cognition, Universidade Federal do ABC, Santo André, SP, 09210-580, Brazil.

Romildo de Albuquerque Nogueira
Departamento de Morfologia e Fisiologia Animal, Universidade Federal Rural de Pernambuco, Recife, PE, 52171-900, Brazil.

Pedro V. Carelli
Physics Department, Federal University of Pernambuco, Recife, PE, 50670-901, Brazil.
E-mail: carelli@df.ufpe.br

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1 Introduction

To understand the neural substrate of behavioral processes is a major aim in modern neuroscience [1]. A central approach to study the neural substrate of behavior is based on changes in brain electrical properties arising from the neuronal activity along behaviors [2–5]. However, it is essential to keep in mind the notable difference among the spatiotemporal resolution in data from the neuronal activity, and data from behavior.

Thus, the recording data from the neuronal activity and the behavior usually have different requirements from a recording system. Therefore, commonly there are different recording systems for that different kind of data. Yet, this diversity of recording data requires a mechanism of synchronization (handshaking) between them.

Neuronal activity originates from the transmembrane currents in the extracellular medium [6]. The electrical potential in the extracellular medium can be measured by using an implanted electrode in the target brain area. In the 1990s, technological advances allowed the development of multi-electrode array (MEA) manufacturing [7, 8]. MEAs have enabled low-cost recording of the extracellular activity of large neuronal populations in the brain in awake animals, in both behavioral designs: freely-moving and behavioral tasks [9–12].

Since the 1930s, B. F. Skinner proposed a new experimental setup to study and change the animal behavior [13]. Among his contributions in this field, there is the operant conditioning chamber (OCC), also known as *Skinner box*. He showed that operant conditioning can modify the probability of a specific behavior by using reinforcements [13]. For example, it is possible to condition a rat to press a lever by rewarding it with a sugar pellet every time the lever is pressed. Thus, the animal tends to repeat the actions that preceded the reward, eventually associating those sequence of actions with the reward [14]. Experiments introduced by Skinner [13] inaugurated a new form of studying behavior, and formed the foundation over which an entire field of research developed. Currently, the Skinner box is a widely used device to perform behavioral studies based on animal models [1].

Commercial OCC systems may be costly. This can be a problem to resource-limited researchers [15]. High-cost creates financial obstacles to the scientific development as a whole. Thus, the design of low-cost OCCs has emerged as an appealing alternative to resource-limited laboratories run behavioral studies based on animal models. Some research groups have developed low-cost OCC [15, 16]. However, none of them provided an efficient system able to send information to the electrophysiology recording system regarding the behavioral events produced in the OCC.

Arduino is a user friendly and very flexible open electronic prototyping platform [17]. It is able to monitor and analyze input and output signals through digital and analog ports, as well as sending and receiving information through Transistor-Transistor Logic (TTL). It is based on 16MHz micro-controller clock, more than enough to accurately describe behavioral or electrophysiological activity. Further, it has a serial communication port for communication with the computer via Universal Serial Bus (USB) [15]. This fine temporal resolution makes the Arduino a feasable low-cost device to synchronize behavioral and electrophysiological data [18].

In this paper, we developed a low-cost OCC, able to perform different behavioral tests, simultaneously sending synchronization pulses through an optic fiber to be recorded by the electrophysiological system. Thus both behavioral and electrophysiological data are stored together, without electric noise generation when the reward mechanism is triggered.

2 Material and methods

We assembled an operant conditioning chamber 25 × 25 × 30 cm, with walls based on 6–4 mm recycled acrylic plates, such as is shown on Fig. 1a (top). Encompassing this acrylic parallelogram were associated mechanical and electronic devices: food dispenser disk, an Arduino Uno microcontroller, an auxiliary electronic board, a stepper motor, and a device for optical coupling with the recording system (Fig. 1a, bottom). All mechanical and electronic materials were low-cost, easily purchased from electronics stores.

For each behavioral protocol, the program used by the microcontroller must be able to autonomously control the entire behavioral experiment: monitoring the lever, generating the sensory stimuli, evaluating the animal response, and the corresponding reward management. All controlling and informing signals were based on TTL pulses. Please, refer to Figs. 1b and 1c for description and results of two examples of behavioral protocols used with the box.

2.1 Hardware

2.1.1 Food pellet dispenser disk

A food pellet dispenser disk was developed to release sugar pellets (50 mg), which may be used as reward in behavioral tasks. The dispenser disk was cut from a 4 mm polymethyl methacrylate plate and was coupled to the stepper motor. The disk has two aligned hole circumferences. The outer, composed by the smaller holes (0.05 mm in diameter), is used by the infrared-light (IR) sensor to check the disk position, avoiding cumulative calibration errors of the pitch motor positioning. The inner, composed by larger holes (0.50 mm in diameter) that serves to hold the sugar pellets and The technical description is available in [19].

2.1.2 Controller

We set up an auxiliary electronic circuit (Fig. 2) to power the Arduino, generate the visual stimuli, monitor the lever and IR sensor, and run the stepper motor coils.

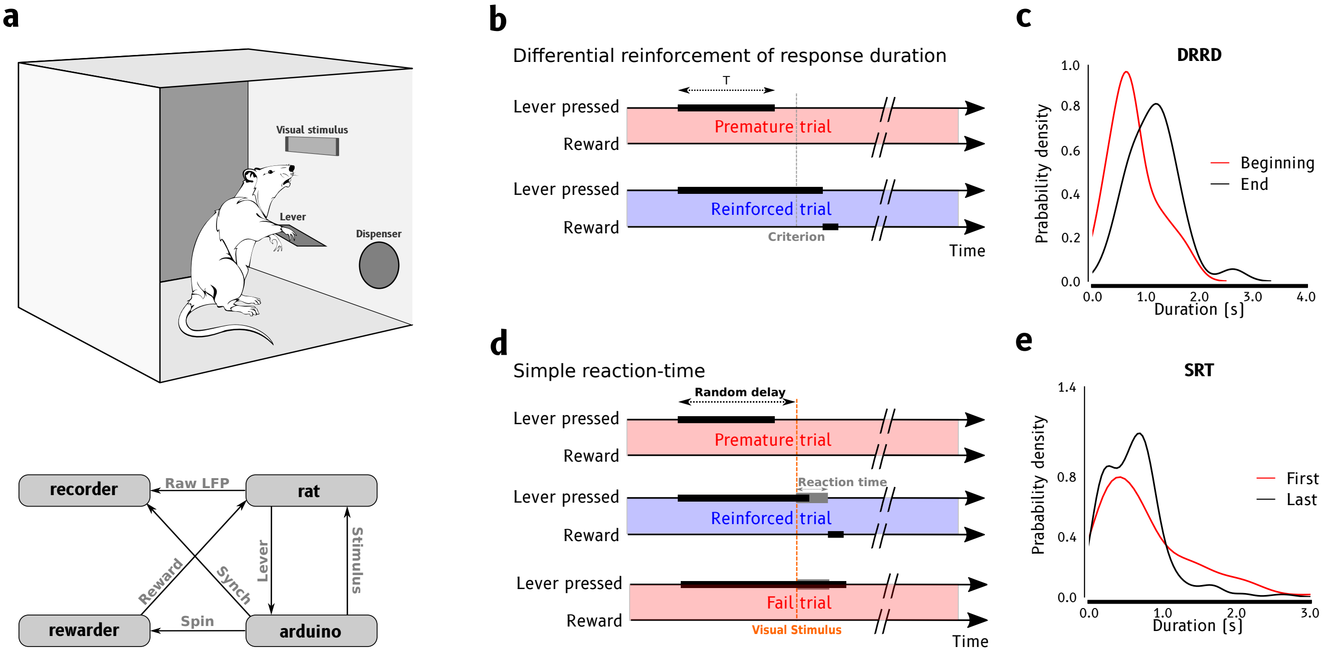

Fig. 1 Overview of the experimental behavioral box. (**a**) **top**: operant conditioning chamber containing the LED (for providing visual stimuli), lever, pellet dispenser, and underlying electronic components. **bottom**: Diagram of main components, and its interactions, in the current proposal: the recording system (recorder), which records both behavioral and wideband LFP synchronized data; the animal (rat); the reward device (rewarder), which is a pellet dispenser, controlled by the microcontroller board (arduino) delivers the reward to animal; and the microcontroller board (arduino), a Arduino Uno that controls the reward device and informs the animal behavior along the time. (**b**) description for differential reinforcement of response duration (DRRD) behavioral tasks. (**c**)Probability density functions of response duration at the beginning (red) and at the end (black) of the DRRD session. (d) description for simple reaction-time (SRT) behavioral tasks. (**c**)Probability density functions of response duration at the first (red) and at the last (black) of the SRT behavioral tasks.

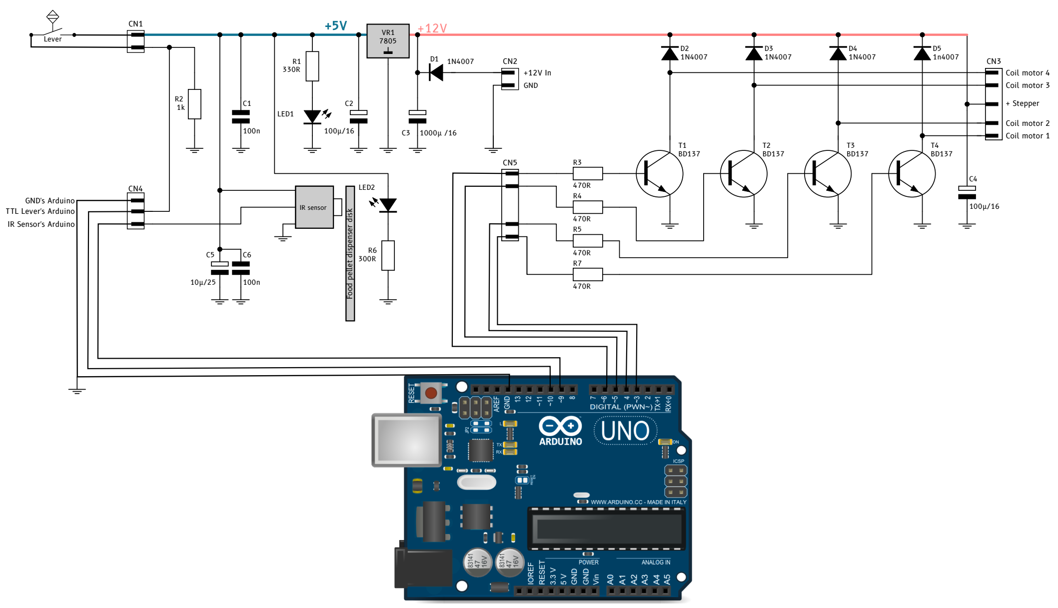

Fig. 2 Circuit diagram of auxiliary electronic. Arduino Uno sends and receives TTL controlling signals. Based on the +12V battery, a voltage regulator (VR 7805) is the supplier of two power lines for the electronic components: +5V and +12V. The +5V (in blue) supplies to two LEDs, an infrared receiver (TSOP 1838), and the TTL level to the switch (CN1) used on the lever inside the box. The LED1 is the power-on light, and the LED2 is the emitter to the infrared sensor (IR sensor) under the Food pellet dispenser disk. The +12V (in light red) is the supplier for sequence to the transistors (T1–T4, BD137), which mediate the controlling signals to stepper motor (CN3) from output digital ports in Arduino.

Such as is shown in Fig. 2, the auxiliary electronic circuit works supplied by two power supplies: +12 V and +5 V.

The +12 V line is used to power the Arduino (Arduino Uno) and the stepper motor (24BYJ48, with four coils). Each motor coil is connected to the collector pin of a pnp bipolar junction transistor (BD137, as shown in Fig. 2). Each transistor, at the collector pin, provides a +12 V TTL signal, controlled by a +5 V TTL signal in its base, from a Arduino I/O pin.

The +5 V line was created through a voltage regulator 7805. This line powered the circuit held for the lever press detection, and the visual stimuli. The early generates a TTL pulse whenever the lever is pressed, and the last is driven by the Arduino. Further, this line also supplies the infrared (IR) sensor.

The Arduino acts over the setup in two functions: present the visual stimuli, and run the stepper motor. In both functions it uses its digital I/O ports: 13 to present the visual stimuli, and 3,4,5,6 to run the stepper motor. Upon the moment of the reward, the Arduino sequentially activates the stepper motor rotating it until the dispenser releases a sugar pellet in the conduit to the food dispenser. An IR light sensor (Tsop1838) detects the passage of the pellet through the conduit, and sends a TTL signal to the port 11 Arduino digital port, which in turn stops the disk rotation.

2.1.3 Optical communication

The recording of the behavioral activity, can be done either through the serial port of a computer connected to the Arduino, or through the TTL signals generated by the Arduino for the acquisition system (Figure 3).

The recording made by the serial port is useful when doing behavioral experiments without electrophysiology. A code for each behavioral event has been set combining the time stamp plus the event code. For example, if the rat pressed the lever (code 001) 800 ms after the session beginning, the serial port of the Arduino sends the following string "800.001".

When the electrophysiology data was recorded the Arduino's serial port was not used for recording behavioral activity. The objective was to reduce unnecessary sources of electrical noise that the computer could insert into the system. Our implementation made use an optical fiber to send the synchronization pulses from the Arduino to the Ephys. The TTL signal from the Arduino was converted to a red light pulse by a fiber optic module (RFT-4112SS). In a second step, the light pulse was converted to a TTL pulse to be acquired by the acquisition system. This conversion was made by a phototransistor. The Ephys has 8 ADCs in its front panel that acquire the synchronization pulses in parallel with the electrophysiological data.

Fig. 3 Optical communication scheme. A TTL signal comes out of a digital Arduino port is amplified and converted into light through a LED. The light is transmitted through an optical fiber to the terminal where it will be converted into a TTL signal.

2.2 Software and Behavioral Protocols

The Arduino has an integrated development environment (IDE). The software was written in a C language and loaded directly into the Arduino board. Here we exemplify with three behavioral protocols we implemented and tested.

2.2.1 Autoshape sessions

Before initiating the experimental protocols of DRRD and SRT the rat performed autoshape sessions, also referred to as fixed-ratio 1 (FR1) protocol. In these sessions the animal learned to associate the lever with the delivery of a sugar pellet (50 mg sucrose), which was used as reinforcement. During the session the stimulus was kept on for the entire duration (60 minutes). The trial began when the animal pressed the lever. Only when the lever was released – and despite the duration of the lever press – the Arduino board delivered a sugar pellet. Animals were considered trained when they received 100 sugar pellets within a single session.

During the FR1 sessions, the Arduino board monitors a digital port that receives information from the lever, stores it and generates TTL signal according to the state of the lever. It also sends out a signal that rotates of the disk (carousel) that delivers the sugar pellets.

2.2.2 Differential Reinforcement of Response Duration

The differential reinforcement of response duration (DRRD) task requires that the rat sustains a behavioral response for a minimum amount of time (criterion) [20]. The trial was initiated when the animal pressed the lever. The animals should push the levers down for more than 1500 ms to receive the reward. Trials in which the animal releases the lever before this minimum time are considered premature and are not reinforced.

During DRRD sessions, for each lever press, the Arduino board monitored for how long the rat kept the lever pressed. If it kept the bar pressed for a period longer than the criteria, the Arduino released the reward initiating the stepper motor that rotates the carousel until a sugar pellet drops. Further description of the task can be seen in [21].

2.2.3 Simple reaction-time

The Simple reaction-time (SRT) task requires that the animal hold the lever pressed for a minimum time to characterize engagement (500 ms). When the waiting period is over, the system presents a stimulus preceded by a delay – randomly presented and uniformly distributed from 0 to 200 ms.

The animal receives a reward it releases the lever within a period of reaction time (300 ms in this example) after the stimulus presentation (reinforced trials). Trials are not reinforced either if the rat releases the bar before the visual stimulus (premature trial) nor if the rat releases the bar after the reaction time (Fail trial).

In addition to monitoring the lever status and the reward, this software also controlled the stimulus appearance. We used a modified version of the protocol used by Narayanan and coleagues [22].

We used four TTL signals to encode for the events in the SRT experiments: 1) Bar state; 2) Stimulus on/off; 3) Food delivery/Success trial; 4: Fail trial.

2.2.4 GO-NOGO task

We have also implemented a GO-NOGO task. This task also requires the animal to hold the lever down for a minimum time to characterize engagement (500 ms). After this waiting period, the visual stimulus is presented (randomly sampled between GO and NOGO stimuli). For the visual stimulus, we used arrays of vertical or horizontal LED patterns. If the stimulus conveys a GO trials, animals must release the lever within 500 ms after the stimulus onset to be rewarded with a sugar pellet. If the stimulus indicates a NOGO trial, the animal must hold the lever for more than 500 ms after the stimulus onset to be rewarded. If the rat releases the bar before the visual stimulus, the trial is not rewarded (premature trial).

We used five TTL signals to code the events in the SRT experiments: 1) Bar state; 2) stimulus GO on/off; 3) Stimulus NOGO on/off 4) Food delivery/Success trial; 5) Failed trial.

2.3 Subjects

For the experiments described here, we used six rat Long-Evans *Rattus norvegicus*. They were obtained from the animal house from the Laboratory of Computational and Systems Neuroscience, Department of Physics, Universidade Federal de Pernambuco (Recife, Brazil). They weighed 300 g - 370 g and thew were 12 to 15 weeks old. The rats were housed in cages and maintained in the light / dark cycle of 12 h and had free access to water and commercial feed Presence (Neovia - Paulínica, Brazil) until the beginning of the training. The animals were kept under food restriction to keep them at 90 % of the *ad libitum* weight . The experimental protocol was approved by the Ethics Committee on Animal Use (CEUA) of UFPE (CEUA: 24/2016), in accordance with the basic principles for research animals established by the National Council for the Control of Animal Experimentation (CONCEA). The rats were divided into two groups according to the behavioral task they were trained: *SRT* (n=3) *DRRD* (n=3).

2.4 Surgical procedures and Recordings

The multielectrode arrays were built with 50 μm tungsten wires, coated with Teflon (California Fine Wire Company) soldered in a printed circuit board and connected to a miniature connector (Omnetics Connector Corporation, Minneapolis, MN). The microelectrode arrays were designed for five different cortical regions: PFC, S1, M1, PPC and V1. Table 1 shows the target stereotaxic coordinates of each region according to atlas from Paxinos and Watson (2007). The arrays were placed in rats under deep ketamine (150 mg/kg i.p.) and xylazine (5 mg/kg i.p.) anesthesia, using a stereotaxic head holder. through a surgical procedure described by Wiest et al. [23]. Rats were allowed seven days to recover from the surgery and start the behavioral tasks.

	PFC	**M1**	**S1**	**PPC**	**V1**
AP (mm)	+4.00	2.00	0.84	-4.36	-7.20
ML (mm)	0.50	2.50	5.00	4.20	3.50
DV (mm)	-3.70	-1.50	-2.00	-1.80	-1.50

Table 1 Steriotactic coordinates and arrangement of arrays used in the DRRD task.

Electrophysiological data was recorded simultaneously from five regions, along with the behavioral activity, at a sampling rate of 24414 Hz, and with a bandwidth of 0.1 to 8 kHz. A ZIF-Clip® connector attached the rats to the PZ2 aplifier via a commutator (AC32, Tucker-Davis Technologies, Alachua, FL), which allows the rats to freely move during the recordings. Besides, PZ2 amplifier system (TDT) was responsible to record the local field potential (LFP). Behavioral activity was recorded as digital inputs together with the electrophysiology.

2.5 Data analysis

We used the Matlab toolbox Wave_Clus [24] to do spike sorting in the raw data. Both off-line raw extracellular recordings and behavioral data were stored locally and in the cloud [25] for later processing, including automatic and manual spike sorting.

To evaluate LFP noise we calculated the spectrogram and coherence matrix. The short-time spectral analysis around the time t was calculated by using the short-time Fourier transform (SFFT). Thus, we used the Eq. 1, to calculate the spectrogram, $S_i(t, \omega)$, of i-th LFP channel, x_i [26, 27].

$$S_i(t, \omega) = |X_i(t, \omega)|^2 = \left| \int_{\infty}^{\infty} w(\tau) x_i(t + \tau) e^{-j\omega t} d\tau \right|^2 \quad (1)$$

where $w(\tau)$ is a T-width time window. Given a pair of time-series of LFP channels, $x_i(t)$ and $x_j(t)$ the coherence between in them is given by the Eq. 2:

$$C_{i,j}(f) = \frac{|G_{i,j}(f)|^2}{G_{i,i}(f) G_{j,j}(f)} \quad (2)$$

where, $G_{i,j}(f)$ is the cross-spectral density between those time-series x_i and x_j; whereas $G_{k,k}$ is the auto-spectral density of the k-th time-series [26]. The element $a_{i,j}$ in the coherence matrix, such as is shown in Fig 4c, was calculated as the average coherence in a frequency range above the low frequencies of the LFP, where the spikes power spectral begins (300–500Hz). All LFP and spike data was analysed based custom made Python routines, and open packages: Numpy (1.16.4), Scipy (1.3.0) and Matplotlib (3.1.0), Pandas (0.25.0) and Seaborn (0.9.0). We calculated the response distribution by convolving the histogram of the response duration in 200-ms bins with a Gaussian kernel.

3 Results

The duration of the lever pressing in a trial is named response duration. The distribution of response duration along the session is a description of the behavior across trials in a session. A behavioral experiment is operating conditioning when it shapes the distribution of response duration along sessions, driven by reward [13].

The Fig. 1c is shown probability density functions of response duration at the beginning (red) and at the end (black) of the DRRD session. There was a overall statistical difference ($p < 0.001$, Mann-Whitney) among the distributions of response duration, as demonstration of effective operating conditioning. Where we found larger means for response duration during the beginning session (0.77 second) when compared with the end sessions (1.14 seconds). The proportion of short responses decreases over responses around the criterion (1.5 seconds). Our data show that a small number of training days elicited a significant change in behavior.

We found similar differences among session in data from SRT task, shown on the Fig. 1e ($p = 0.020$, Mann-Whitney), where we found larger means for response duration during the last session (0.81 second) when compared with the first sessions (0.61 second). These results show that rats learned the task because the proportion of premature responses decreases while the proportion of responses after the stimulus (SRT trials) increases. After the sessions of training, most of the responses concentrate around the reaction time.

The data show that the training elicited a significant change in behavior along with the sessions, in both behavioral tasks.

An essential advantage of the OCC presented here is that it does not induce noise in the electrophysiological recordings. In Fig. 4, we show the raw signals (recorded from PFC, M1, S1, PPC and V1 cortical areas) during an FR1 task (panel b) and the spikes extracted from the raw data (through a spike sorting process) confirming the quality of the recordings (panel d). Such very low voltage (few hundreds of micro-volts) recordings are quite sensitive to electromagnetic artifacts, and two events are critical: the lever press (black line) and the activation of the stepper motor (gray line). If the electric circuits are not properly isolated, such events typically create electrical artifacts in the recordings due to static energy discharge when the animal touches the lever, sudden changes in potential upon lever press (switches), or to inductive charges from the stepper motor coils. Our recording remained unaltered by these events shown in panel (Fig. 4b). The spectrograms of the LFP signals were also free of artifacts (Fig. 4e) – no increase in the fundamental (60 Hz), harmonics (120 and 180 Hz) levels, nor in higher frequencies were observed.

The coherence map (Fig. 4c) also tests the quality in the recordings. It is possible to identify four broad clusters of high coherence (warmer colors). It is expected that the coherence was high for electrodes in the same cortical region, but low for electrodes more distant apart. The first cluster corresponds to the electrodes inserted in PFC, the second in M1, the third in S1 and PPC (highly synchronized) and the fourth in

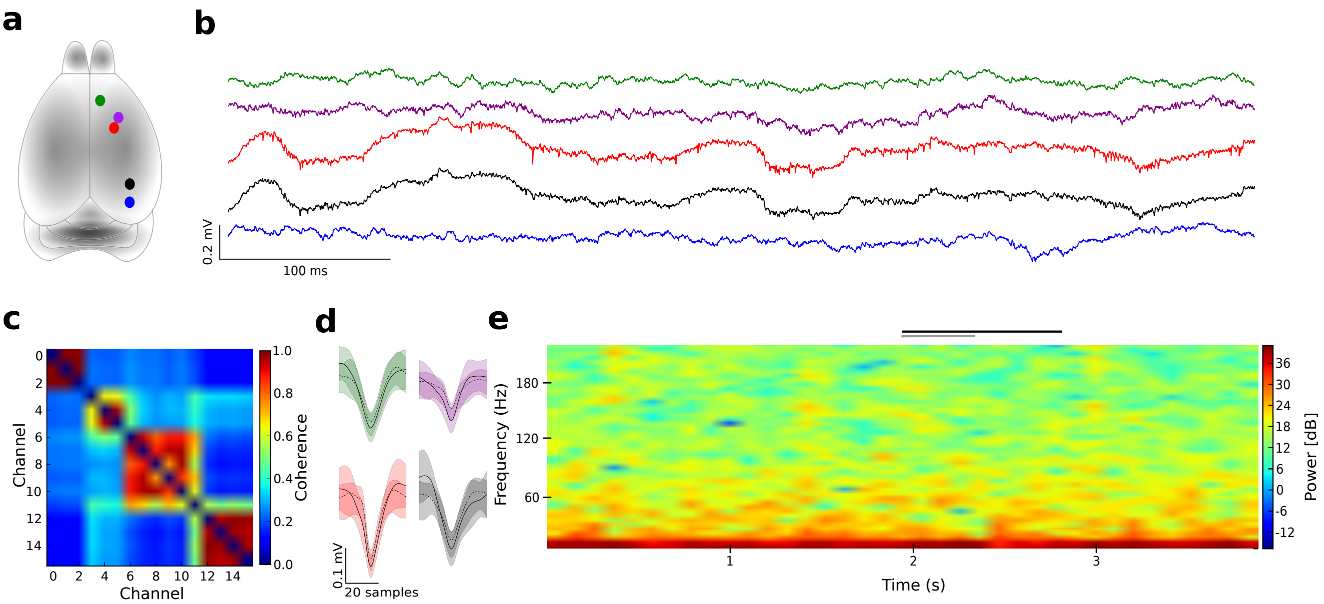

Fig. 4 Samples of electrophysiological recordings in a behaving animal. (**a**) Whole rat brain using a color code along the implanted areas: prefrontal cortex (PFC, green), primary motor cortex (M1, purple), primary somatosensory cortex (S1, red), posterior parietal cortex (PPC, black), and primary visual cortex (V1, blue). (**b**) Sample of simultaneous raw wideband LFP signals, according the color code in (a), while the rat performed autoshape task. The color scheme is the same described in **a**. (**c**) Coherence matrix between the raw LFP across channels, at low frequencies (0-50 Hz). (**d**) Samples of two spike waveforms found in four areas, using color shades based on the color code in (a); within each the cluster shadow represents the amplitude range (mean ± standard deviation), and filled lines represents the average amplitude, along the waveform samples. (**e**) Sample of spectrogram for low frequencies (up to 200 Hz) of a raw extracellular potential in the PPC, along 4s; upper horizontal black bar represents the period for which the animal kept the lever pressed, while the nearby gray line represents the period in which the reward device was powered on. Local powerline works at 60 Hz.

V1, confirming that the high coherence was only observed in adjacent electrodes.

4 Discussion

The Arduino-based systems have been successful used to record simultaneously neural activity and behavior [16, 28–30].

Among the recent studies, the most similar system to ours is composed by a behavioral system integrated into two-photon imaging experiments, during a GoNoGo task [31]. In that system, they used water as reward, and the Arduino was held for controlling the water dispenser. However, the synchronization between the two-photon imaging, and the behavioral system was done by the serial port, which can be a source of noise for electrophysiological experiments. We use sugar pellets as a reward, released by a food pellet dispenser disk. It requires a more sophisticated releasing mechanism, when compared with water dispenser control [30, 31] used in used water-based reward mechanism.

In this article we developed and tested a low-cost behavioral system using an Arduino microcontroller, that can synchronize behavioral experiments and electrophysiological recordings, also able to work under low-cost silicon probe designs [32]. Also, we have used an optical link to relay the signal from the operant conditioning chamber to the acquisition system, avoiding noise contamination in the recordings. The system is also free hardware, free software and easily adaptable to any behavioral task desired. The main contribution of our systems is to provide an affordable and flexible system to investigate the neural correlates of behavior.

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