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Short communication

# A versatile and modular tetrode-based device for single-unit recordings in rodent *ex vivo* and *in vivo* acute preparations

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ABSTRACT

*Background:* The demand for affordable tools for recording extracellular activity and successfully isolating single units from different brain preparations has pushed researchers and companies to invest in developing and fabricating new recording devices. However, depending on the brain region of interest, experimental question or type of preparation, different devices are required thus adding substantial financial burden to laboratories.
*New method:* We have developed a simple and affordable tetrode-based device that allows interchangeable extracellular recordings of neuronal activity between *in vivo* and *ex vivo* preparations and can be easily implemented in all wet-bench laboratories.
*Results:* Spontaneous activity from several putative single neurons could be easily recorded and isolated by lowering the device into *ex vivo* cerebellum brain slices. The same device was also used *in vivo*, lowered into primary auditory cortex of adult anesthetized transgenic mice expressing channelrhodopsin in cortical neurons. Acoustic stimulation of the contralateral ear or direct laser optogenetic stimulation successfully evoked cortical activity at the recording site. Several isolated putative single neurons presented time-locked activity response to the different stimuli.
*Comparison with existing methods:* Besides low fabrication cost, our device uses an omnetics connector compatible with the majority of headstages already available at most electrophysiology laboratories. The device allows custom tetrode configuration arrays and extensions for optogenetics and pharmacology, providing experimental flexibility not available in commercial off-the-shelf microelectrode arrays and silicon probes.
*Conclusions:* We developed an affordable, versatile and modular device to facilitate tetrode extracellular recordings interchangeably between *in vivo* anaesthetized animals and *ex vivo* brain slice recordings.

## 1. Introduction

Extracellular neuronal activity has been recorded and analyzed in neuroscience research for decades, providing insight into cell- and circuit-level brain computations (Henze et al., 2000; Barthó et al., 2004). The ability to perform spike sorting on extracellular activity recordings has been particularly useful since it allows the isolation of putative single neurons and the analysis of their activity/responses individually (Rey et al., 2015). However, spike sorting is less commonly used in *ex vivo* brain slice recordings because typical single-wire pipette extracellular recordings are not ideal for this purpose and commercially available options are usually costly, namely multi-electrode arrays (MEA) (Meister et al., 1991; Heuschkel et al., 2002) and especially designed silicon probes (Aivar et al., 2014). Tetrodes are a simple and cost-effective approach that has been routinely used for chronic recordings of extracellular activity in freely-moving rodents (Wilson and McNaughton, 1993). Their long lasting success in neuroscience relies not only on their cost and easy implementation but also on their ability to facilitate spike sorting (Wilson and McNaughton, 1993; Gray et al., 1995). Surprisingly, despite their success in freely-moving electrophysiology, tetrodes have been less frequently used in acute preparations including *in vivo* anesthetized animals and especially *ex vivo* slice preparations. One main factor limiting the use of tetrodes for the recording of extracellular spikes and subsequent unit isolation in acute preparations is the non-existence of commercially available devices that can simultaneously work as tetrode interface boards and structurally support/guide tetrodes into slices or whole-brains in anaesthetized animals.

Here, we propose a versatile and modular tetrode-based device that takes inspiration from probe design and freely-moving rodent tetrode

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microdrives, working for both *ex vivo* brain slices and *in vivo* anaesthetized recordings. The device parts can be easily produced by affordable and widespread technologies, many times in-lab, such as printed circuit board (PCB) manufacturing and 3D printing. Additionally, the device can be: quickly assembled; integrated with any amplifier/recording system already available at the lab for extracellular recordings; and used unlimited times, including inter-changeably between *ex vivo* and *in vivo* experiments, with multiple tetrodes and configurations. For *in vivo* preparations it can also be easily extended to be coupled with optical fiber or cannulas for optogenetic and pharmacological experiments. Using this device, we recorded spontaneous and opto- and sound-evoked neuronal activity in transgenic mice, both *in vivo* and *ex vivo*. In both configurations it was possible to reliably isolate several good quality single units from short recordings and analyze individual units' responses to light and sound modulation. This device will facilitate a more widespread use of tetrodes for acute preparations in neuroscience experiments.

## 2. Materials and methods

### 2.1. Tetrode fabrication

Nichrome tetrodes (NiCr, 12.5 µm diameter, RO-800 Hard PAC, Sandvik) were fabricated with standard methods, described elsewhere (Nguyen et al., 2009). Briefly, a 50 cm long strand of insulated NiCr wire was folded and twisted by a motorized tetrode spinner (Tetrode Spinner 2.0, Neuralynx) while being tensioned. After the twisting procedure, the wires' insulation was fused at 420 °C with the aid of a heat gun and then carefully cut at the top and bottom. Tetrodes were then stored for later use.

### 2.2. Electrode interface board and tetrode guide

The proposed device consists of two parts: an electrode interface board (EIB) and a tetrode guide (Fig. 1A).

The EIB is a printed circuit board (PCB) that allows the electrical connection of tetrode wires to gold coated vias (0.3 mm diameter) in the board by means of gold pins (small EIB pins, Neuralynx). These vias are routed to a female omnetics connector (A79024-001, Omnetics Connector Corporation) that mates with an appropriate headstage with male omnetics connector (which are used by many popular electrophysiology companies). There are 32 electrode vias (for 8 tetrodes) plus 2 ground and 2 reference vias. The EIB is a 2-layer PCB (10 mil trace/space, 1.6 mm thickness) and was manufactured with electroless nickel immersion gold (ENIG) surface finishing.

The tetrode guide physically holds and guides the tetrodes into the tissue. This structure was 3D-printed in plastic (PA12 multijet fusion plastic; 70 × 10 × 1.5 mm) and has a single groove track that, depending on dimensions, can hold up to 8 of tetrodes tightly packed and lined up in a single row (the track used in the experiments described is 0.6 mm wide and 0.6 mm deep and holds eight 50 µm tetrodes lined in a row). The guide can be printed in any plastic material by any 3D printer with sufficient precision to print the tetrode track. The number, size and position of the tracks can also be easily adjusted by simple changes to the 3D part design file (Github tetrode device, 2020). For optogenetic experiments, a different tetrode guide (which includes an additional track 0.3 mm wide and 0.8 mm deep), can be used for positioning a 200 µm optic fiber close to the tetrode tips (Fig. 1A – bottom).

### 2.3. Device assembly and tetrode loading

The omnetics connector was soldered to the board by hot air soldering with solder paste (Leaded, No Clean Solder Paste, MG Chemicals). A 40 cm long 4-gauge isolated copper wire was looped through two drill holes on the PCB, designed for the purpose, and soldered to the ground vias. The EIB and the tetrode guide were connected by means of two 2 mm diameter screws. There are two square holes on both parts that line up and allow passing of tetrode wires from one side of the board to the other, if necessary.

Tetrodes were placed in the guide track with the aid of ceramic coated tweezers (11252-50, Fine Science Tools) under a microscope (SZ51, Olympus) and glued in place with 5-min epoxy (Devcon). Each tetrode wire was then routed to an electrode *via* in the EIB and secured with a gold pin. Tightly pressing the gold pin against the electrode wire inside the *via* removes the electrode's insulation and allows electrical connection. Fig. 1A shows how the different assembly parts are connected and Fig. 1B shows a fully assembled device with 8 NiCr tetrodes loaded and connected to the EIB.

The EIB with the omnetics connector can be used unlimited times. The tetrode guide can be reused or changed/disposed according to the experimental needs. The same device with the same tetrode guide and tetrodes, *i.e.,* without any change of parts or configuration, can be used several times between experiments, including interchangeably between *ex vivo* and *in vivo*, as long as the tetrode tips are cut fresh and electroplated (see below) and the tetrodes are of sufficient length to reach the desired target.

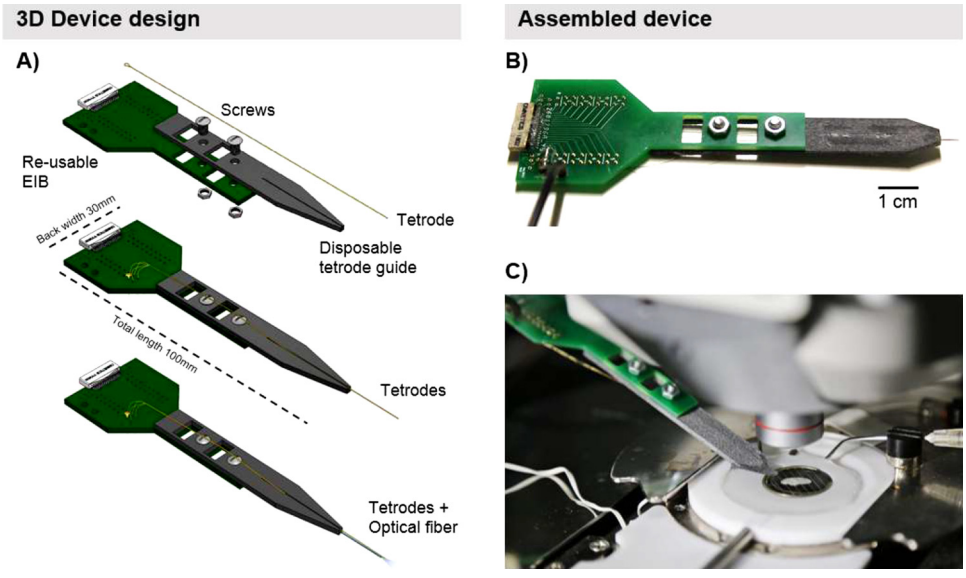


**Fig. 1.** Tetrode device design overview. (A) 3D rendering of the device's parts, including the electrode interface board (EIB) and the tetrode guide, separately (top), fully assembled (middle) and with optical fiber for depth optogenetic experiments *in vivo* (bottom). (B) Photo of assembled device with loaded NiCr tetrodes and ground wire. (C) *Ex vivo* recording with device being held by micromanipulator and tetrodes guided into the slice inside the recording chamber.

### 2.4. Tetrode electrodeposition

After gluing the tetrodes to the guide structure and physically connecting each tetrode wire to the electrode vias in the EIB, tetrodes' tips were cut with sharp stainless-steel scissors (14568-09, Fine Science Tools) and electroplated. The plating solution consisted of 5% gold non-cyanide solution (Sifco 5355 Gold Plating Solution, Neuralynx) with 1% Poly(ethylene glycol) (PEG) addictive (BioUltra, 8,000, Sigma-Aldrich) mixed in a 75 % v/v PEG to gold ratio. This solution has been shown to promote a large surface area with low impedance (Ferguson et al., 2009). Electrode impedance was lowered to 150–200 kOhm in successive steps of electrodeposition at -0.05 µA with the aid of NanoZ (White Matter LLC).

### 2.5. Ex vivo and in vivo electrophysiological recordings

#### 2.5.1. Animals

*Emx1-Cre:Ai27D* or *Pvalb-Cre:Ai27D* male mice were originally purchase from JAX (stocks #008069, #005628, #012567) (Gorski et al., 2002; Hippenmeyer et al., 2005; Madisen et al., 2012). All experiments were conducted in accordance with European Union Directive 2016/63/EU and the Portuguese regulations and laws on the protection of animals used for scientific purposes (DL Nº 113/2013). This study was approved by the Ethics Subcommittee for Life and Health Sciences of University of Minho (ID: SECVS 01/18) and the Portuguese Veterinary General Direction (ID: DGAV 8519).

#### 2.5.2. Ex vivo cerebellar electrophysiological recordings

Briefly, animals were deeply anaesthetized with avertin (0.5 mg/g body weight; tribromoethanol; 20 mg/mL; Sigma–Aldrich) by intraperitoneal injection and decapitated. The brain was quickly removed to prepare 300 µm cerebellum parasagittal slices according to the protocol of (Huang and Uusisaari, 2013). All recordings were performed at 34 °C in artificial cerebrospinal fluid gassed with 5 % $CO_2$/95 % $O_2$.

The tetrode device containing 8 NiCr electroplated tetrodes was secured to a 3-axis micromanipulator (PatchStar, Scientifica) and connected to a 32-channel headstage (RHD2132, Intan). The tetrodes were then guided into the slice with the micromanipulator under visual inspection of a microscope (Fig. 1C) and placed close to the Purkinje cell layer at cerebellum lobule IV. Extracellular activity was recorded with an Open Ephys acquisition system (Open Ephys) (Siegle et al., 2017) connected to the headstage by serial peripheral interface (SPI) cables (Intan). Signals were acquired at 30 kS/s.

#### 2.5.3. In vivo auditory cortex electrophysiological recordings

*2.5.3.1. Surgical procedure and in vivo extracellular recordings*. Mice were anesthetized by intraperitoneal injection mix of ketamine (75 mg/kg) and medetomidine (1 mg/kg) and positioned in a stereotaxic frame (Stoelting). Dorsal skull was exposed and the animal was rotated 90° degrees to facilitate removal of the temporal muscle and access to the primary auditory cortex (A1). A 1.5 × 1.5 mm area above A1 centered at −2.5 mm AP and 4.0 mm ML from bregma (Franklin and Paxinos, 2007) was opened and the dura removed under a microscope (S6, Leica Mycrosystems). The tetrode device containing 8 NiCr electroplated tetrodes was attached to a micrometric stereotaxic arm (1760, Kopf Instruments) and connected to an headstage (RHD2132, Intan). Tetrodes were lowered into the brain, through the skull opening, to -0.6 mm (DV) from the brain surface. A stainless-steel screw electrode (E363-20-2.4-03, Plastics One) secured in a burr hole at the back of the skull was connected to the device's ground wire. The surgical procedure and the following stimulation experiments were conducted on a custom-made anti-vibration table inside a double-walled sound-proof chamber. Extracellular signals were acquired with an Open Ephys acquisition system at 30 kS/s.

*2.5.3.2. Evoked auditory response*. To evoke responses from A1 neurons to sound stimuli, tones of variable frequency (8, 12, 20, 24, 28 and 32 kHz, 25 ms duration, 975 ms intertrial interval) and variable sound pressure level (50, 60, 70 and 80 dB SPL) were pseudo-randomly delivered from a free field electrostatic speaker (ES1, TDT) positioned 10 cm away from the mouse's contralateral ear. The speaker was driven by a speaker driver (ED1, TDT) and tones were generated by an high-sampling rate sound card (HARP, Champalimaud Foundation) controlled by Bonsai (Lopes et al., 2015).

*2.5.3.3. Optogenetic stimulation*. To evoke neuronal activity from A1 neurons to light stimuli, the surface of the exposed brain was illuminated with blue light pulses (473 nm wavelength, 10 Hz with 30 ms pulse width, 5 s On, 55 s Off). Light was delivered by an optical fiber (200 µm diameter) connected to a fiber-coupled DPSS laser source (CNI). Light pulses were generated by a waveform function generator (DG1022, Rigol) connected to the laser source. The optical fiber was held by 3-axis micromanipulator (LBM-2025-00, Scientifica) and placed 5 mm above brain surface.

#### 2.5.4. Signal processing and spike sorting

Signals were analyzed with custom-written Matlab (Mathworks) code. Both *ex vivo* and *in vivo* recordings were filtered between 0.6 and 6 kHz. Spikes were detected using a variable amplitude threshold that was a multiple (4–6 times) of the median from an estimate of the background noise's standard deviation as in (Quiroga et al., 2004). Spike sorting was achieved by performing weighted principal component analysis (wPCA) on the waveforms of detected spikes followed by Gaussian Mixture Model (GMM) unsupervised clustering of the first principal components of pairs of electrodes from the same tetrode (Fig. 2B) (Souza et al., 2019). In a final step, clusters were manually split or merged based on visual inspection of cluster waveforms and their inter-spike interval histograms and auto-correlograms.

## 3. Results

Device fabrication required EIB and tetrode guide printing, as well as soldering of the omnetics connector and the ground wire to its respective pads in the EIB. The soldering takes approximately 1 h and it's a one-time only step as the EIB can then be reused unlimited times.

Assembling the device for the experiments required connecting the EIB to the printed tetrode guide with two screws, fabricating, loading and electroplating the tetrodes. These steps were completed in under 2 h. The same device with the same guide and tetrodes were used in both the *ex vivo* and *in vivo* experiments. The tetrode tips were cut fresh and electroplated between the *ex vivo* and *in vivo* experiments which took under 1 h.

In the *ex vivo* brain slice experiment (Fig. 2A–C) we isolated 9 putative single neurons from eight tetrodes positioned at the Purkinje cell layer (cerebellum lobule IV) from a 30 min recording of spontaneous activity. Fig. 2C shows an example of 4 units isolated from a single tetrode wire.

In the *in vivo* experiment (Fig. 2D–H) 12 putative neurons were isolated from six tetrodes from a 30 min recording from the primary auditory cortex (A1) in an anesthetized mouse. By identifying putative neurons through spike sorting, we were able to analyze single-units' responses to specific light and sound stimuli. Fig. 2F shows examples of 2 units responding to light stimulation and Fig. 2G-H shows an example of frequency tuning of 1 unit to sound stimulation.

The initial cost for building the device was approximately 80 USD, including 10 USD for PCB printing, 60 USD for the omnetics connector, and 10 USD for 32 gold pins. After that, the only recurring costs are 5 USD for each guide printing (guides can also be printed in-house for a fraction of the cost and either be re-used or changed/disposed according to the experimental needs) and less than 1 USD for 8 tetrodes' fabrication and deposition for each experiment.

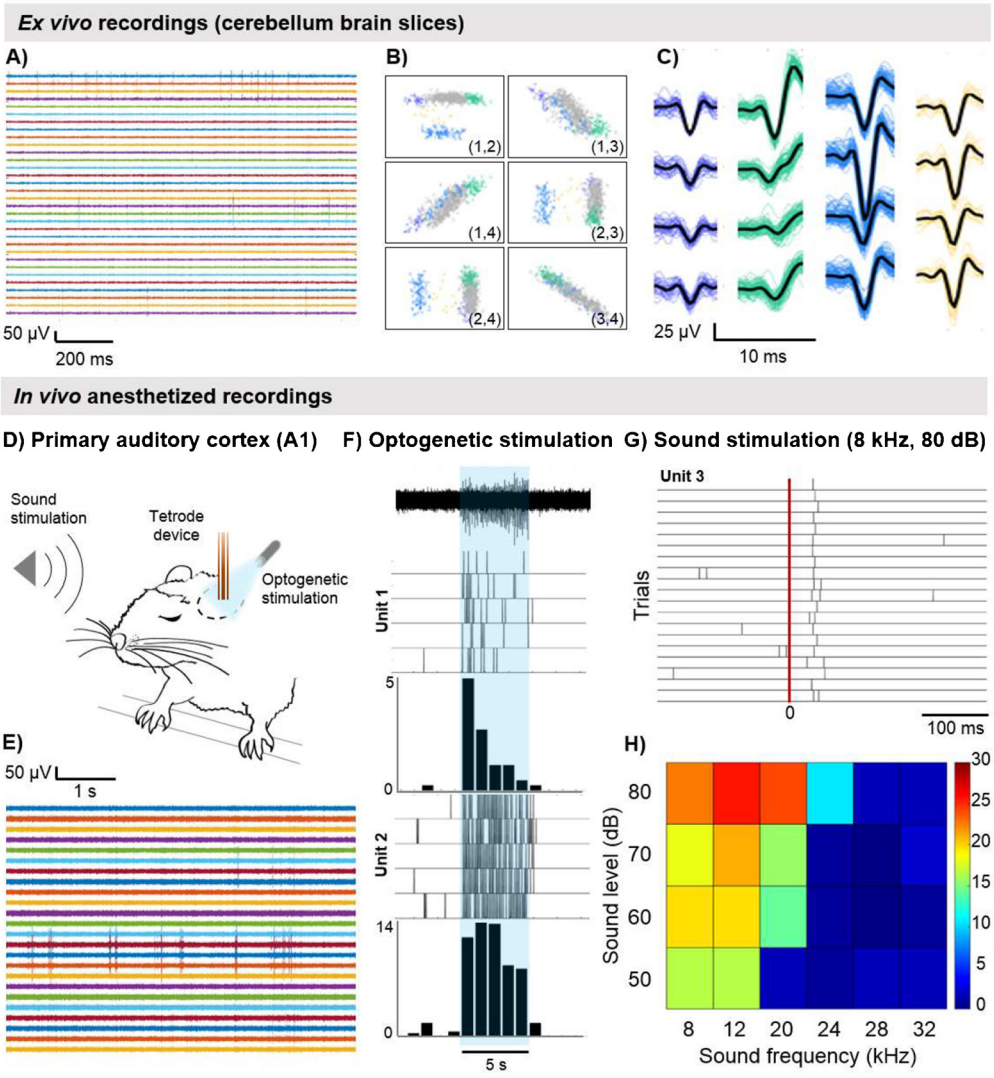


**Fig. 2.** *Ex vivo* and *in vivo* electrophysiological recordings. (A) Extracellular signals recorded on 8 tetrodes from a mouse cerebellum brain slice (signals band-passed filtered between 0.6 and 6 kHz). (B) Cluster isolation for one tetrode using wPCA and GMM based spike sorting. Each scatterplot shows the projections of two pairs of tetrode's channel waveforms onto the first principal component. Colored points correspond to isolated single units (respective waveforms on right with matching colors). Grey points correspond to multi-unit activity which was not possible to isolate into single units. (C) Sample waveforms of four isolated single units from one tetrode (average waveform in black). (D) Representative drawing of the *in vivo* experiment combining tetrode recordings with optogenetic and sound stimulation in primary auditory cortex (A1) of an anesthetized mouse. (E) Extracellular signals recorded on 6 tetrodes from A1 (signals band-passed filtered between 0.6 and 6 kHz). (F) Evoked activity of isolated single units in A1 by optogenetic stimulation. Extracellular activity recorded during one trial of light stimulation (light stimulation period in blue, corresponding to 5 s) (top); Raster plots and average peri-stimulus time histograms of two isolated single units over five trials. (G–H) Evoked activity of one isolated single unit in A1 by sound stimulation. (G) Raster plot of one isolated single unit responding to 8 kHz tone stimuli over 20 presentations (red line marks tone presentation). (H) Tuning heatmap of the same unit showing average firing rate across the different sound frequencies and sound levels presented during the evoked auditory response protocol.

## 4. Discussion

This manuscript describes the design and implementation of an affordable, versatile and modular tetrode-based device that allows extracellular recordings in both *ex vivo* and *in vivo* preparations. The same device can be used in both preparations without any changes in configuration or parts, as performed here. Its probe-like design allows easy positioning in micromanipulators for *ex vivo* slice recordings and stereotaxic frames for *in vivo* recordings. The proposed device is mostly based on parts that can be easily produced in-lab or sourced for a low cost. The only fixed costs are for PCB manufacturing and omnetics connector purchasing (approx. 70 USD). Recurring costs include the tetrode guide (approx. 5 USD, which can, nevertheless, be reused if desired and printed in-house for a significantly lower cost) and the tetrode wire (which can also be reused a few times, as described here). The cost of the device and its versatility are especially important when compared with other alternatives (Meister et al., 1991; Heuschkel et al., 2002; Aivar et al., 2014; Lévesque et al., 2016; Li-Yuan Chen et al., 2018). Although MEAs and silicon probes can be used several consecutive times, their initial cost is ten to twenty times higher. Comparably to MEAs and silicon probes, our device presented good mechanical stability and was able to record the same neuronal units for at least 30 min. Moreover, depending on the brain region, experimental question or type of preparation, different non-interchangeable devices may be required thus adding substantial financial burden to the laboratories. Our approach further allows unlimited cost-effective modifications for different experimental needs, including electrode positioning and extensions for optogenetics.

The EIB was inspired by EIBs from microdrive systems for tetrode positioning in freely-moving animals (Voigts et al., 2013; Kloosterman et al., 2009; Chang et al., 2013) where the easiness of assembly must be combined with quick and non-damaging part recovery. PCB manufacturing is currently a widespread service accessible at a low cost. The present EIB was designed to match headstages with male omnetics connectors which are used by many popular electrophysiology companies.

Similarly, 3D printing has also become an affordable technology and most labs now have access to it in-house. The tetrode guide design was inspired by silicon probe tip's design and can be printed in virtually any commercial or in-house 3D printer, contingent to printer's precision and the desired tetrode track's dimensions. Since 3D printing allows easy customization, the proposed design for the guide structure can be easily changed by simple alterations to 3D part design files (Github tetrode device, 2020) to accommodate several configurations, from single

tetrode tracks to multiple tracks in the same or different locations. The possibility of changing the track dimensions and position allows versatile tetrode positioning - for example, for targeting different brain structures simultaneously – and the use of tetrode wires of different diameters for different experimental demands. The guide design presented here can also be modified to accommodate additional tracks for optical fiber for optogenetic experiments or cannula for pharmacological experiments. Despite this versatility, and although tetrodes can be cut with precision scissors and lined up in a row under a microscope, when compared with MEAs and silicon probes, their real geometry is not fixed/known which requires, for example, additional modeling for current source density analysis studies (Pettersen et al., 2012).

The whole device was designed for constant reuse with minimal cost and minimal damage to the parts upon recovery. In fact, the same tetrodes glued to the guide structure and connected to the EIB can be used for several sequential recordings across different days as long as tetrode tips are cut fresh and electroplated between experiments (and as long as the tetrodes are of sufficient length for the intended target). In our experience, for both setups, we have been able to use the same tetrodes up to 6 times, *i.e.*, in 6 different recording sessions (including interchangeably between *in vivo* and *ex vivo* as we describe here). When necessary, the whole device can be quickly recovered for a new experiment. The EIB can be reused by removing the gold pins from the vias and discarding the tetrodes. The same gold pins can then be reused for the next application. The tetrode guide can be recovered by immersion in 70 % alcohol solution overnight to remove the epoxy, or it can also be discarded between experiments given that it can be printed effortlessly.

The affordability and easiness of manufacturing and assembly of the proposed device will allow a more widespread use of tetrodes for acute extracellular recordings and unit isolation in *ex vivo* and *in vivo* preparations.

## Conflicts of interest

None.

## CRediT authorship contribution statement

**Francisca Machado:** Investigation, Formal analysis, Software, Data curation, Writing - original draft. **Nuno Sousa:** Conceptualization, Funding acquisition. **Patricia Monteiro:** Investigation, Conceptualization, Methodology, Validation, Supervision, Resources, Funding acquisition, Writing - review & editing. **Luis Jacinto:** Investigation, Conceptualization, Methodology, Software, Validation, Supervision, Writing - review & editing.

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