## Wireless Electrophysiological Recording of Neurons by Movable Tetrodes in Freely Swimming Fish

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Dr. Ron Myers
Science Editor
Journal of Visualized Experiments,

Dear Dr. Myers,

Please find enclosed a manuscript we would like to resubmit as an article to the *Journal of Visualized Experiments*. The paper is entitled "Wireless Electrophysiological Recording of Neurons by Movable Tetrodes in Freely Swimming Fish" and is authored by Lear Cohen, Ehud Vinepinsky, and Ronen Segev.

The revised text responds to all the referees' and editor's comments.
We hope that this revised version meets your criteria for publication.

Sincerely yours,
Ehud Vinepinsky

1 **TITLE:**

2 **Wireless Electrophysiological Recording of Neurons by Movable Tetrodes in Freely Swimming**

3 **Fish**

4

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20

21 **KEYWORDS:**

22 electrophysiology, wireless technology, extracellular space, microdrive, goldfish, fish,

23 telencephalon, pallium

24

25 **SUMMARY:**

26 A novel wireless technique for recording extracellular neural signals from the brain of freely

27 swimming goldfish is presented. The recording device is composed of two tetrodes, a microdrive,

28 a neural data logger, and a waterproof case. All parts are custom-made except for the data logger

29 and its connector.

30

31 **ABSTRACT:**

32 The neural mechanisms governing fish behavior remain mostly unknown, although fish constitute

33 the majority of all vertebrates. The ability to record brain activity from freely moving fish would

34 advance research on the neural basis of fish behavior considerably. Moreover, precise control of

35 the recording location in the brain is critical to studying coordinated neural activity across regions

36 in fish brain. Here, we present a technique that records wirelessly from the brain of freely

37 swimming fish while controlling for the depth of the recording location. The system is based on

38 a neural logger associated with a novel water-compatible implant that can adjust the recording

39 location by microdrive-controlled tetrodes. The capabilities of the system are illustrated through

40 recordings from the telencephalon of goldfish.

41

42 **INTRODUCTION:**

43   Fish are the largest and most diverse group of vertebrates, and like other vertebrates they exhibit
44   complex cognitive abilities such as navigating, socializing, sleeping, hunting, etc. Nevertheless,
45   the neural mechanisms governing fish behavior remain for the most part unknown.
46
47   In the past few decades, extracellular recordings from immobilized fish have primarily been
48   implemented to investigate different aspects of the neural basis of behavior[1,2]. Although this
49   technique is appropriate for some sensory systems, investigation of the full spectrum of the
50   neural basis of behavior is difficult if not impossible in immobilized animals. The first advances
51   involved recording from the Mauthner cells of tethered swimming fish[3,4]. However, Mauthner
52   cells are disproportionately large and the recorded action potential amplitudes, which can go as
53   high as a few mV, facilitate recording. Later, Canfield et al. described a proof of concept when
54   using a tethered animal to record from the telencephalon of fish[5]. Another recent technique for
55   recording neural activity from fish is calcium imaging (see reviews by Orger and de Polavieja[6],
56   and Vanwalleghem et al.[7]). This technique was developed for use with zebrafish larvae because
57   the skin and skull are transparent during the larval stage. However, this technique cannot be used
58   to study complex behaviors in later stages of development.
59
60   Here, we present a novel technique for recording extracellular neural activity from the brains of
61   freely swimming fish. This is a modified version of the protocol described in Vinepinsky et al.[8].
62   The main innovation is the addition of a microdrive that makes it possible to control the position
63   of the electrodes after surgery. The technique is designed for recording from the telencephalon
64   of goldfish using a set of tetrodes that are connected to a neural data logger via a microdrive.
65   The whole setup is wireless and anchored to the fish's skull. The specific weight of the system is
66   equalized to the water-specific weight by adding a small float that allows the fish to swim freely.
67
68   The technique is based on the use of a neural data logger that amplifies, digitizes, and stores the
69   signal in an onboard memory device. The logger telemetry system is used to start and stop the
70   recordings, and for synchronization with the video camera. In this protocol, a 16-channel neural
71   logger is used, embedded in a waterproof box together with the microdrive.
72
73   The microdrive assembly is fabricated from two main components: the microdrive itself and the
74   microdrive housing (**Figure 1A,B**). The housing holds the microdrive and the tetrodes, and also
75   acts as the anchor between the skull and the logger box (**Figure 1C**). The PVC logger box is
76   fabricated using a machine process and is sealed using an O-ring (**Figure 1E-G**, see also
77   **Supplementary Figure 1**, **Supplementary Figure 2**, and **Supplementary Figure 3** for a three-
78   dimensional [3D] diagram). At one end, a piece of polystyrene foam is attached to the logger box
79   to compensate for the weight of the implant and provide the fish with a buoyancy-neutral
80   implant. The construction of the microdrive described in the protocol follows the procedure
81   presented by Vandecasteele et al.[9] with a modification to attach the microdrive to the housing
82   (**Figure 1A**). All major steps are presented.
83
84   The procedure described in the protocol to prepare the fish skull is similar to the one presented
85   in Vinepinsky et al.[8] and is described briefly in the protocol. One day after surgery, the fish are
86   normally fully recovered from the effects of anesthesia and are ready for the behavioral

87  experiments. Note that the tetrode location can be adjusted by turning the microdrive screw.
88  The screw has a spacing of 300 μm per full rotation and an advancement of 75 μm is
89  recommended until the target brain location is reached. An appropriate brain atlas should be
90  consulted to target the specific brain region of interest. It is advisable to test the electrode
91  impedance each time the fish is anesthetized for battery or memory card replacement.
92
93  **PROTOCOL:**
94
95  All surgery procedures must be approved by the local ethics committees on animal welfare (e.g.,
96  IACUC).
97
98  **1. Construction of the microdrive housing**
99
100 1.1. To construct the housing, cut a 1 mm wide brass plate into a 19 mm x 29 mm x 1 mm plate
101 using a saw. Cut two 5.5 mm slits on each of the long sides perpendicular to the edge, such that
102 each slit is 6.5 mm away from the narrow sides (**Figure 2A**).
103
104 1.2. Using pliers, fold the area between the slits on the long sides inward, then fold the bottom
105 part inward and the upper side outward to obtain the housing (**Figure 2B,C**).
106
107 1.3. Using a 3 mm drill bit, make holes for screws in the microdrive housing.
108
109 NOTE: These holes will be used later to attach the housing to the logger box (**Figure 2D**).
110
111 1.4. Solder the sides of the housing.
112
113 1.5. Using a fine circular file, generate a small, 1.5 mm in radius, semicircular slit at the bottom
114 of the housing (**Figure 2E**).
115
116 NOTE: This will be used later to insert the stainless steel tube to guide the electrodes.
117
118 1.6. Use a 1 mm drill bit to make a hole in the back of the housing for the tetrodes (**Figure 2F**).
119
120 NOTE: A 3D model of the housing is found in the **Supplementary housing.stl file**.
121
122 **2. Construction of the microdrive**
123
124 2.1. Using a cutter, break off a three-pin piece from a single row male pin header strip (**Figure
125 1H**). Using pliers, pull out the middle pin.
126
127 2.2. Using a cutter, cut the remaining pins to 10 mm in length (2 mm less than the screw length).
128 Another possibility is to use a longer screw (see step 2.4).
129
130 2.3. Drill a hole using a #65 drill bit through the middle pin hole. Drill a thread using a 00–99 tap.

131

2.4. Assemble the microdrive and the brass plates (7.5 mm x 2.5 mm x 0.6 mm, see **Supplementary Figure 4**) such that the brass plates are touching the pins. Insert a screw (#00-90 round head, 12 mm, brass) through the first brass plate, then through the pin header thread and the second brass plate. Finally, place a nut on the screw and gently tighten the assembled microdrive.

2.5. Solder the pins together with the brass plates, and the nut with the tip of the screw.

2.6. Solder the microdrive into the microdrive housing at four points on the sides of the microdrive brass plates.

2.7. Cut one stainless steel tube 6 mm long with an inner diameter of 1.5 mm and another stainless steel tube 3 mm long with an inner diameter of 1.2 mm. Polish the ends of the tubes to avoid sharp ends.

2.8. Glue the 6 mm long tube to the small semicircular slit at the bottom of the microdrive housing using epoxy. Glue the 3 mm long stainless steel to the pin header, lined up with the 6 mm long tube on the housing.

2.9. Cut two 5 cm long silicone tube segments and one 5 cm long polyimide tube.

2.10. Insert the three tubes into the two stainless steel tubes. Glue the tubes to the stainless steel tube attached to the pin header using cyanoacrylate glue. Screw the microdrive all the way up and cut off the excess tubing from the top and bottom of the two steel tubes.

NOTE: The microdrive with the housing is now ready for use (**Figure 1C**).

**3. Preparing the tetrode array**

3.1. To fabricate a two-tetrode implant with four electrodes on each tetrode, prepare eight wires, each 12 cm long, Formvar insulated, out of 25 µm diameter tungsten wire.

NOTE: The same design can accommodate four tetrodes.

3.2. Place a holder for a 16-channel electrode interface board (EIB-16) PCB (see **Table of Materials**) under the microscope.

3.3. Using a soft tipped tweezer and a lighter, remove the coating off each of the eight wires on one side using the flame.

NOTE: This is to ensure that the wire will be properly connected to the PCB connector later on.

174     3.4. Push a wire into one of the holes in the EIB-16 with the coated side in the hole. Place a pin
175     and press it using pliers. Check the connectivity by measuring the resistance between the pin and
176     the uncoated side of the wire.
177
178     NOTE: The resistance is on the order of tens of Ohms.
179
180     3.5. Repeat step 3.4 with all eight wires.
181
182     3.6. Tape two groups of four wires together using duct tape at the end of each wire.
183
184     NOTE: Each group will be glued together later to form a tetrode.
185
186     3.7. Cut one piece of tungsten wire 12 cm long with a 50 µm diameter. Connect it to one of the
187     EIB-16 connections.
188
189     NOTE: This wire will serve as the reference electrode.
190
191     3.8. Cut two bare silver wires 12 cm long with a 75 µm diameter that will serve as grounds for
192     the recording logger. Solder the two wires to the ground connection in the EIB-16.
193
194     3.9. Hold the EIB-16 above a motorized turning device and place the duct tape end of one group
195     of four wires on the motorized tuning device. Apply 130 rounds clockwise followed by 20 counter-
196     clockwise rotations. Apply cyanoacrylate glue to cover the tetrode.
197
198     3.10. Wait for the glue to cure. Cut the tetrode close to the duct tape.
199
200     3.11. Repeat steps 3.9 and 3.10 with the second tetrode.
201
202     NOTE: This produces the finished two-tetrode array (**Figure 1D**).
203
204     **4. Assembling the implant**
205
206     4.1. Screw the microdrive all the way down.
207
208     4.2. Using 1 x 3M Phillips round head screws, attach the EIB-16 to the PVC plate.
209
210     4.3. Using soft end tweezers, pull all the tetrodes and wires through the hole in the front of the
211     logger box cover.
212
213     4.4. Using the 2 x 6M Phillips flat head screws, attach the PVC plate to the logger box cover. Keep
214     the EIB-16 connector in the correct orientation so that the logger can be mounted on the EIB-16.
215     Make sure the EIB-16 is fixed in place to avoid motion artifacts in the recorded signal.
216

4.5. Seal the wires to the box using epoxy. Apply as little as possible because the primary sealing will be done by room temperature vulcanizing (RTV) later on.

4.6. Attach the microdrive housing to the logger box cover using 2 mm screws.

4.7. Thread the tetrodes and all wires through the hole at the back of the microdrive housing. Thread the tetrodes through the two silicone tubes in the microdrive. Thread the 50 µm tungsten wire through the polyimide tube in the microdrive.

4.8. Glue the tetrodes and wires to their tubes by applying cyanoacrylate glue to the top end of the tubes, to ensure the movement is consistent with the microdrive. Screw the microdrive all the way to the top.

4.9. Apply soft petroleum (see **Table of Materials**) on the exposed tetrode and wires inside the microdrive housing to prevent motion.

4.10. Cut a 12 mm x 14.5 mm Petri dish bottom window using a heated razor blade. Attach the window to the front of the microdrive housing with epoxy. Keep the ground wires outside the window.

4.11. Apply RTV coating to the exposed tetrodes and wires between the logger box cover and the microdrive housing.

4.12. After the RTV is cured, close the box with a small weight inside, and submerge in water overnight to ensure there is no water leakage into the box.

4.13. Cut the tetrodes and reference wire to the desired length using sharp scissors.

4.14. Attach the marked extruded polystyrene foam (see **Table of Materials**) to the box. Adjust its size so its buoyancy is balanced when submerged in a water bath.

4.15. Dip the tetrode tips in platinum black solution and use a direct current (-0.2 µA) to coat the electrodes and set the electrodes' impedance as desired. Use a multielectrode impedance tester (see **Table of Materials**) for coating and impedance measurements.

NOTE: In the goldfish pallium, a value of 40 kOhm is best. Depending on the application, the electrode impedance can be adjusted by modifying the platinum black coating[10, 11].

**5. Anesthesia preparation— 1% MS-222 stock solution**

CAUTION: Anesthesia preparation includes the use of powdered MS-222, a carcinogen. Hence, steps 5.2 and 5.3 must be done in a chemical hood using gloves.

5.1. Add 100 mL of water to a tube that can contain more than 100 mL.

261

262 5.2. In a chemical hood, place a disposable weighting plate on a scale. Add 1 g of MS-222 powder
263 using a spatula, then add the powder to the tube.

264

265 5.3. Shake the tube well.

266

267 NOTE: In liquid form, MS-222 can be used outside the chemical hood wearing gloves but does
268 not require a mask.

269

270 5.4. Place a disposable weighting plate on a scale. Add 2 g of sodium bicarbonate using a spatula,
271 then add the powder to the tube. Shake the tube well.

272

273 **6. Preparing the fish skull**

274

275 NOTE: At this stage, the fish is ready for implant surgery. Prior to surgery, make sure that all
276 components and supplies have been sterilized by the appropriate procedures. For this step, an
277 out of water U-shaped fish holder is needed. In this protocol, an aluminum holder that fits a 15
278 cm head to tail long goldfish is used. This system holds the fish out of water while perfusing the
279 gills with oxygenated water. For details see Vinepinsky et al.[8].

280

281 6.1. Place the fish in a 0.02% MS-222 water bath for 20 min until the fish is asleep.

282

283 6.2. Using sterile gloves, take the fish out of the water and place it in the holder.

284

285 NOTE: The oxygenated water perfusing the fish contains MS-222 at a concentration of 0.02%, so
286 that the fish remains anesthetized during surgery.

287

288 6.3. Using a sterile spatula, apply lidocaine 5% paste on the skin above the designated place for
289 surgery for 10 min, then remove the lidocaine.

290

291 NOTE: Consult an appropriate brain atlas to target the specific brain region.

292

293 6.4. Using a sterile 15 blade scalpel, remove the skin above the skull in the region of implant.

294

295 6.5. Using a dental drill with 0.7 mm drill bits, drill 4 holes in the skull. Insert a 1 mm screw (3 mm
296 long) into each hole and apply cyanoacrylate glue on the holes right before inserting the screw.

297

298 6.6. Using a dental burnisher, apply dental cement on the screws and on the periphery of the
299 exposed skull.

300

301 6.7. Using the dental drill, make a 5 mm diameter hole in the skull above the brain region of
302 interest. Remove the fatty tissue between the skull and the brain and expose the brain region
303 target using fine tweezers and soft tissue paper. Be careful not to damage the large blood vessels
304 underneath the skull.

305

306  NOTE: By the end of this stage, the fish is prepared to implant the probe. Only the main steps
307  specific to this protocol are described here. Several postoperative procedures (such as detailed
308  documentation on the animal's health and sterilization of the surgery tools and area) are not
309  presented or discussed because they are applicable to all surgeries with fish or small animals.

310

311  **7. Implanting the probe**

312

313  NOTE: To complete the final step in the protocol, a manipulator that can hold the implant in place
314  while it is inserted into the brain is needed.

315

316  7.1. Use the manipulator to hold the logger box cover with the tetrodes pointing down toward
317  the fish brain.

318

319  7.2. Bend the reference electrode such that when the tetrodes are lowered into the brain, the
320  reference stays outside the brain.

321

322  7.3. Cut the grounds such that they fit into the skull. Optionally, connect one ground wire to one
323  of the skull screws.

324

325  7.4. Lower the implant such that the electrodes are inserted into the brain while the bottom part
326  of the microdrive housing is near the skull.

327

328  7.5. Start attaching the implant to the skull by applying a small quantity of dental cement
329  between the housing and the nearest skull screw.

330

331  7.6. After the first part of the dental cement is cured, apply dental cement and close the hole
332  above the skull and the entire exposed skull.

333

334  NOTE: Usually several rounds of dental cement applications are needed in order to cover the
335  entire exposed skull.

336

337  7.7. Install the logger and the battery in the box and seal the box with all the screws.

338

339  7.8. Apply antibiotics and local painkillers according to the type of fish used for the experiments.

340

341  7.9. Flush the fish's gills with fresh water until the fish starts to wake up. Remove the fish from
342  the holder and place it back in its home tank.

343

344  NOTE: The fish is fully recovered within 60 min after surgery.

345

346  7.10. Make sure the fish is able to swim freely with the implant (**Figure 3**, **Supplementary Video
347  1**). If needed, readjust the size of the extruded polystyrene foam above the logger box such that
348  the fish can balance easily.

349
350 **REPRESENTATIVE RESULTS:**
351 During a recording session the goldfish swam freely in a square water tank while the neural
352 activity in its telencephalon was recorded. The goal of these experiments was to study how the
353 neural activity of single cells determines the fish's behavior. To do so, spiking activity needed to
354 be identified in the recorded data. The brain activity, while being recorded, was digitized at
355 31,250 Hz and high-pass filtered at 300 Hz by the data logger. Then, offline, a band-pass filter
356 (300–5,000 Hz) was applied to the signals, and the presorted raw data were separated into each
357 tetrode's channels and the reference channel (**Figure 4A**). Next, common spike sorting
358 algorithms[12] were used to characterize single cell activity. First, each channel was manually
359 filtered by the minimal spike amplitude threshold (relative to the noise levels of each channel).
360 Then, because the tetrodes' tips are not in the same site, and the reference electrode was outside
361 the brain, spikes that appeared in more than one tetrode or in the reference channel were also
362 filtered. The filtered data were then manually clustered and filtered by shape, length, inter-spike
363 interval (the time between subsequent action potentials must adhere to the refractory period of
364 neurons), and by principal component analysis (PCA). Examples of single cell clustering vs.
365 multiunit and noise clusters are presented in **Figure 4**.
366
367 **FIGURE CAPTIONS:**
368 **Figure 1: Implant assembly.** (**A**) Microdrive, made out of a pin header, brass plates, and a screw.
369 (**B**) Microdrive housing, made from a single brass plate by folding. (**C**) Microdrive assembly made
370 with the microdrive (**A**) and the housing (**B**). (**D**) The tetrode array was made using EIB-16, two
371 tetrodes, a reference electrode, and grounds connected to a connector (see **Table of Materials**).
372 (**E**) and (**F**) The microdrive implant assembly is connected to the waterproof logger box cover.
373 The tetrode assembly connector is located inside the box and the tetrodes are glued to the
374 microdrive. (**G**) The logger box base where the logger and the battery are located. The O ring
375 around the base is used for sealing. (**H**) Single row male pin headers strip.
376
377 **Figure 2: Microdrive housing folding technique.** (**A**) Start with a 1 mm wide brass plate and make
378 four slits. (**B**) Fold the middle part of the side inward. (**C**) Fold the upper part backward and the
379 bottom part inward. (**D**) Drill three 3 mm holes in the top. (**E**) Engrave a 1 mm semicircle on the
380 bottom. (**F**) Drill a 1 mm hole in the middle of the upper side.
381
382 **Figure 3: Recording from a freely behaving goldfish.** (**A**) The tetrodes are implanted in the fish
383 brain and the assembly is connected to the fish's skull. (**B**) The box is sealed with the logger inside.
384 (**C**–**E**) A fish swimming freely with the assembly after surgery.
385
386 **Figure 4: Representative results.** (**A**) Recording 0.5 s long from a freely swimming fish 24 h after
387 surgery. The signal is filtered using a band-pass filter (300–10,000 Hz). There is no high amplitude
388 noise in the reference electrode, indicating a lack of motion artifacts. There are no action
389 potentials in the second tetrode (green channels). The first electrode data are shown in the
390 brown channels. Blue and red stars indicate spikes from blue and red clusters shown in panels B
391 and C, respectively. (**B**) Spike shapes of two different clusters of single neurons, recorded from
392 tetrode 1. (**C**) Projection on the first three principal components of the data from the first tetrode

393    of all spike candidates that crossed the threshold. Blue and red clusters correspond to blue and
394    red spike shapes from panel B. Gray dots represent neural noise or multiunit activity.
395
396    **Supplementary Video 1: Swimming patterns: example of goldfish a day before implantation**
397    **surgery (left) and a day after (right).** Video shows similar swimming patterns, indicating that the
398    fish is not impeded by surgery. Video speed is x1.8.
399
400    **Supplementary Figure 1: Diagram of the logger box main chamber.**
401
402    **Supplementary Figure 2: Diagram of the logger box cover.**
403
404    **Supplementary Figure 3: Diagram of the EIB-16 chamber cover.**
405
406    **Supplementary Figure 4: Diagram of the brass plate used for the microdrive.**
407
408    **DISCUSSION:**
409    This protocol details the steps involved in implanting a tetrode array into the telencephalon of
410    freely swimming goldfish. This technique implements a neural logger that amplifies and records
411    the signals acquired from up to 16 channels along with a microdrive that can adjust the tetrode
412    position in the brain. The microdrive makes it possible to adjust the position in the brain to
413    optimize the recording.
414
415    This protocol can easily be modified for recording from other brain regions (see Vinepinsky et al.[8]
416    for recording from the optic tectum using a similar technique) or any other aquatic animal 15 cm
417    long or larger (approximately equal to a goldfish head to tail, ~100 grams weight). In addition,
418    the protocol can be modified to work with any data logger as long as it communicates at a
419    frequency that can penetrate water. The logger used here communicates using a radio frequency
420    of 900 MHz and can communicate through about 20 cm of water. A radio frequency of 2.4 GHz
421    can also penetrate through ~15 cm of fresh water. Lower frequencies and other alternatives
422    might give even better results[13-15]. The protocol presented here used a two-tetrode array with
423    eight recording channels. In addition, the protocol can be modified to incorporate other probe
424    geometries such as a wire array[16] or silicone probes[9].
425
426    There are several advantages to using a data logger over a full telemetry recording system or a
427    tethered system. First, wireless communication adds noise to the recording. Therefore, full
428    transmission of the data will reduce the signal quality. In addition, logging the data ensures no
429    data are lost if communication fails. Furthermore, wireless systems allow the fish to swim freely,
430    unlike in tethered animals. Finally, this protocol was developed to record action potentials but
431    can also be used to record local field potentials by setting the logger analog high-pass filter to 1
432    Hz instead of 300 Hz. One disadvantage of the logger is the need to physically download the data
433    and replace the battery when it runs down.
434
435    The microdrive suggested in the protocol significantly increases the likelihood of recording single
436    cell activity. Without the microdrive device, the implanted tetrodes are placed approximately in

437 the same recording site in the brain for the whole time the fish is being tested. This physically
438 limits the likelihood of recording multiple single neurons from the same fish, and therefore
439 curtails the recording yield per fish. The fact that the specific recording site in the brain remains
440 unknown until after surgery strengthens the need for a movable device that makes it possible to
441 move the electrodes in the brain after fixation as well.
442
443 An important feature of this protocol that was omitted for clarity is the determination of the
444 electrode impedance. The electrode impedance can be adjusted by the selection of the wire
445 diameter (i.e., a higher diameter leads to lower impedance), wire composition (e.g., tungsten or
446 nichrome), and electrode coating (e.g., platinum black for tungsten and gold for nichrome) which
447 yields wires with lower diameters and lower impedance. Because all these parameters are critical
448 to the success of neuronal recordings, the reader is strongly encouraged to consult the vast
449 literature on this subject, including Harris et al.[17].
450
451 Note the importance of the reference electrode in detecting possible external noise sources in
452 the system. The reference electrode is a relatively low impedance electrode that is inserted into
453 the skull but is outside the brain. Because it does not come into contact with brain tissue, it
454 records the signal's signature, which is composed of thermal noise, motion artifacts, and external
455 noise. The major noise sources in this system are motion artifact and communication noises that
456 can be controlled and timed by the logger. These noises can easily be detected by the signature
457 they impose on the signal of the reference electrode.
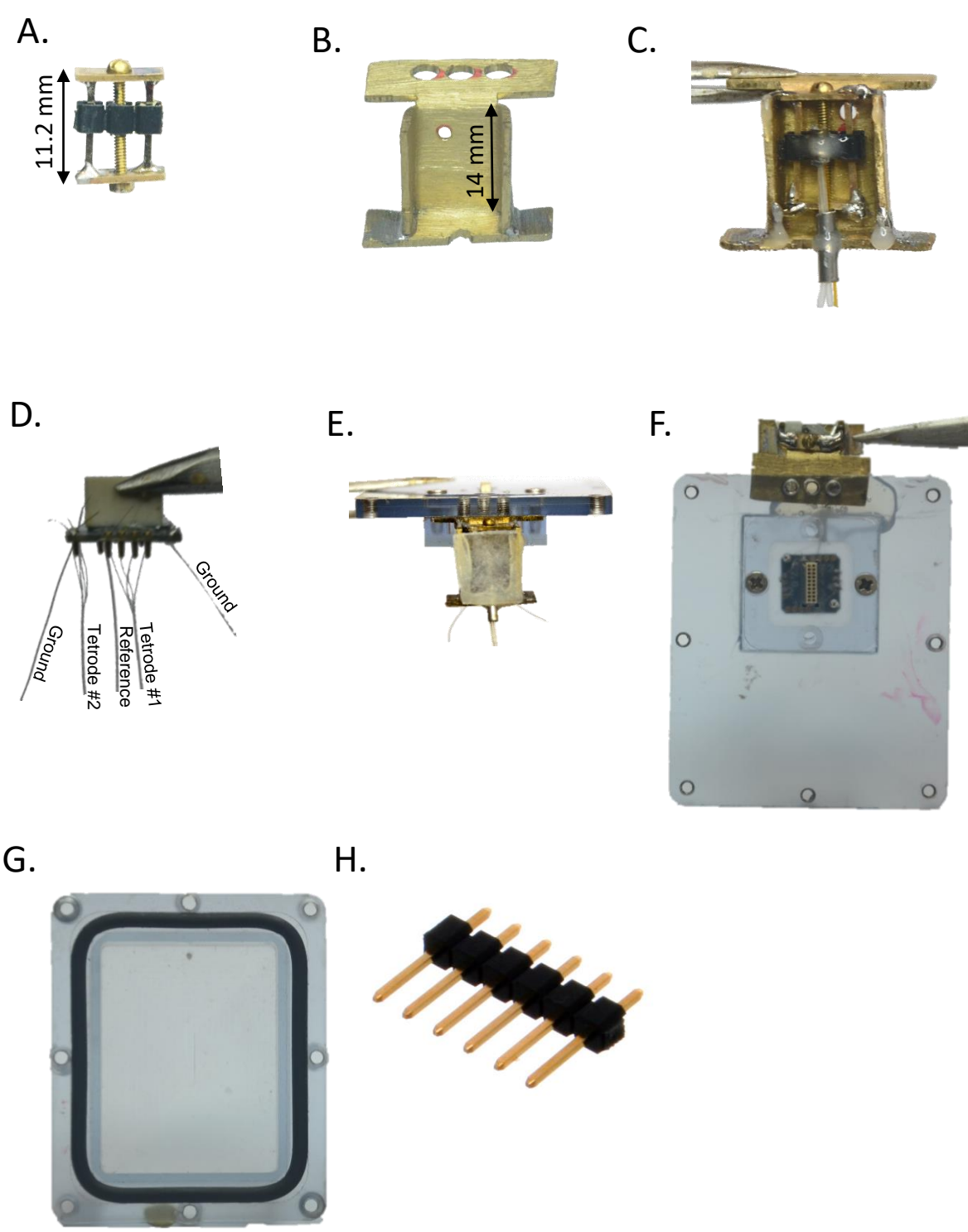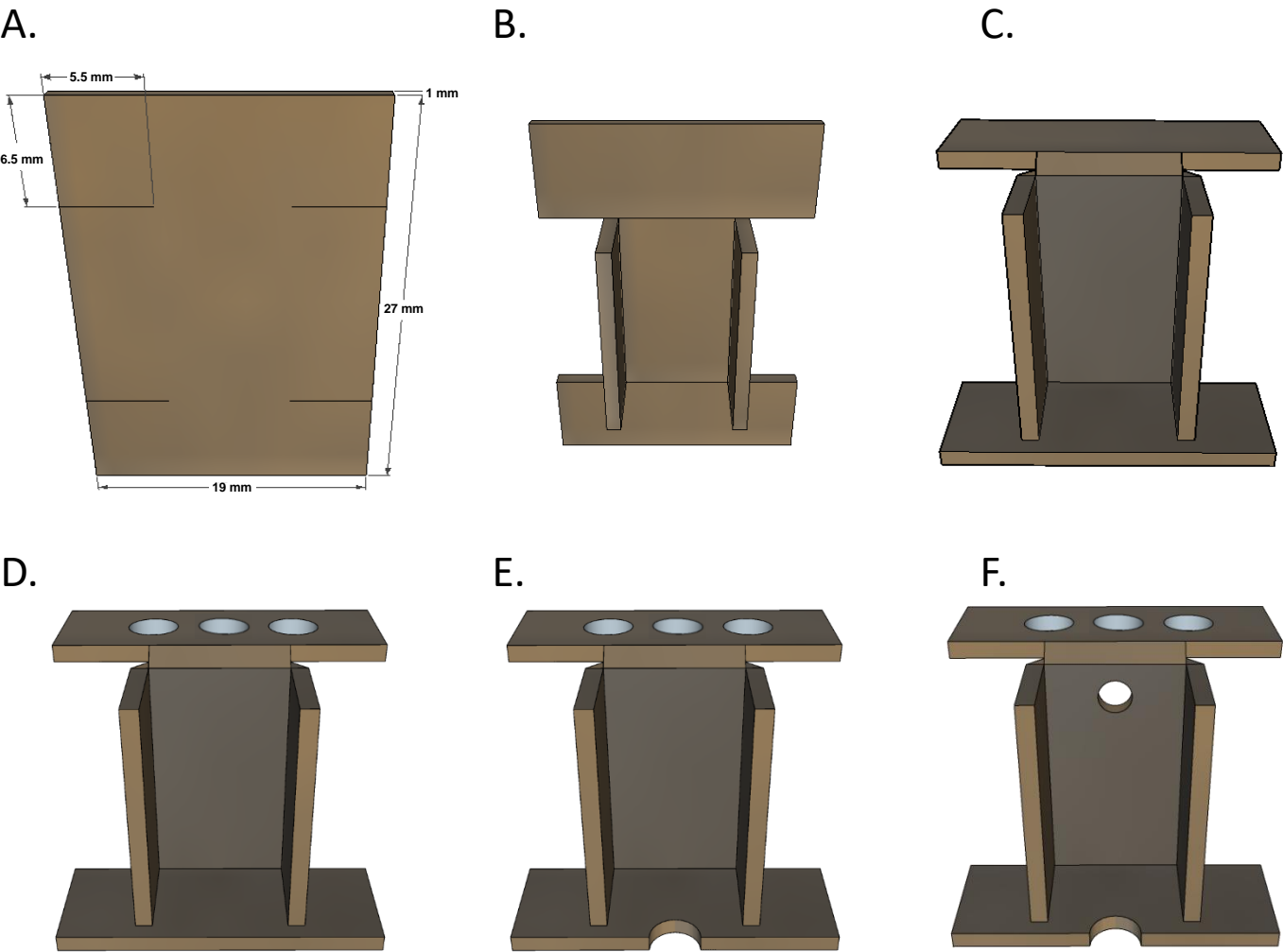458

466 **DISCLOSURES:**
467 The authors have nothing to disclose.
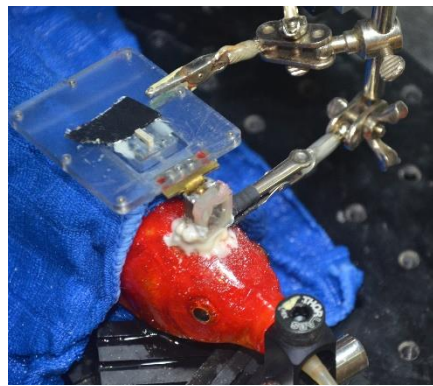468

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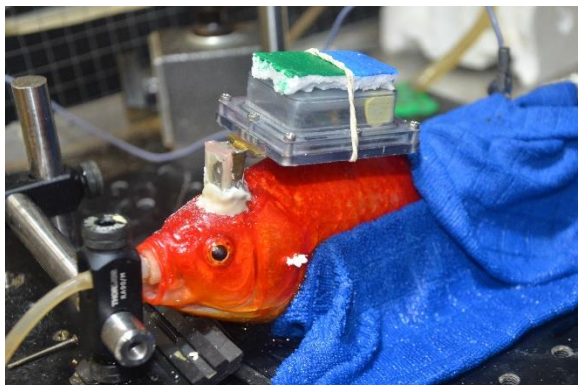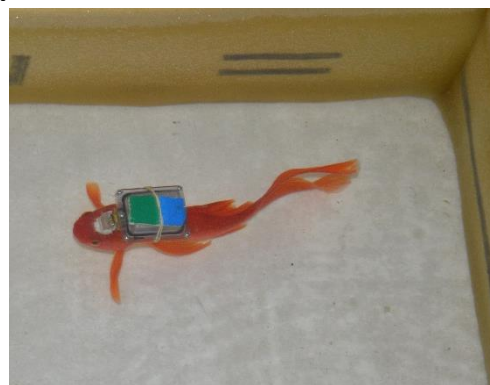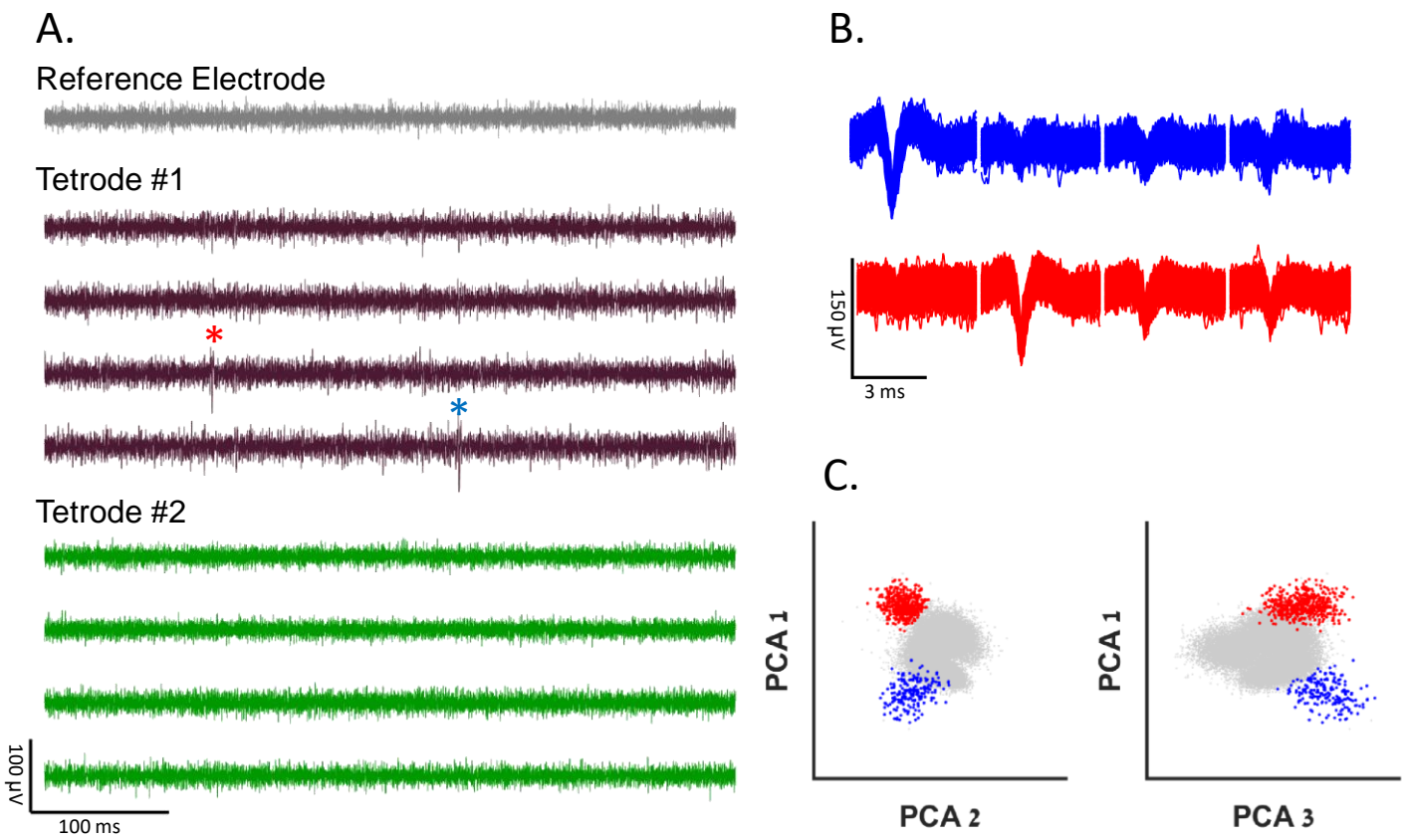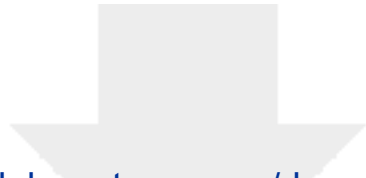507

Figure1

# Cohen & Vinepinsky et al – Figure 1

A.

11.2 mm

B.

14 mm

C.

D.

Ground

Ground
Tetrode #2
Reference
Tetrode #1

E.

F.

G.

H.

Figure2 Cohen & Vinepinsky et al – Figure 2

A.

5.5 mm
1 mm
6.5 mm
27 mm
19 mm

B.

C.

D.

E.

F.

Figure3

Cohen & Vinepinsky et al – Figure 3

A.

B.

C.

D.

E.

Figure4

# Cohen & Vinepinsky et al – Figure 4

**A.**

Reference Electrode

Tetrode #1

Tetrode #2

100 µV

100 ms

**B.**

150 µV

3 ms

**C.**

PCA 1

PCA 2

PCA 1

PCA 3

Supplementary video

Click here to access/download
**Video or Animated Figure**
SupplementaryVideo1V5.mp4

Supplementary housing.stl file

| Name of Material/ Equipment | Company |
| --- | --- |
| 0.7 mm round drill bits | |
| 15-blade Scalpel | Sigma-Aldrich |
| 16 channel PCB board | Neurlynx |
| 1X3M phillips flat head screws | |
| 1X3M phillips round head screws | |
| 27 cm X 19 cm X 1 mm brass plate | |
| 2X6M phillips flat head screws | |
| 3140 RTV coating | Dow Crowning |
| 75 μm Silver wire | A-M Systems |
| Brass machine screws #00-90 | |
| Brass plates 7.5mm X 2.5mm X 0.6mm | |
| Coated Tungsten wire 25μm | California Fine Wire Company |
| Coated Tungsten wire 50μm | A-M Systems |
| Cyanoacrilic glue | |
| Dental Burnisher | ComDent UK |
| Dental cement - GC Fuji PLUS | GC |
| Dental drill or nail polish drill | |
| Drill bit #65 | |
| Fast curing epoxy | |
| Logger box with O-ring sealing | |

| | |
|---|---|
| Motorized turning device | |
| Mouselog-16 Neural logger | Deuteron Technologies Ltd |
| MS-222 | Sigma Aldrich |
| Nano-Z plating | White Matter LLC |
| PCB pins | Neurlynx |
| Polymide tubing  250µm | A-M Systems |
| Rechargable battery | |
| Silicone tubing  0.64 mm | A-M Systems |
| Stainless steel 1.5 mm | A-M Systems |
| Sudium Bicarbonate | Sigma Aldrich |
| Tap #00-90 | |
| Vaseline | |

**Catalog Number**

EIB-16

2767996

947-1006

5000160

795500

431011

947-65

E10521


Neuralynx EIB Pins

822000


806100

846000
S9625
947-1301

# Comments/Description

Compatible with the drill.

Stainless steel. Any type.

Stainless steel. Any type.

See Figure 2

Stainless steel. Any type.

A 3D drawing is provided. See supplementary 1

Depending on the appication the tetrodes can be fabricated from any type of wire. Popular wires are nicrome wires that can be found with lower diameters

Can be replaced with any other wire with low impedance

Any small sterille stainless-still tool will do.
Other dental cements would probably will work as well although we have never tried any other.

Dental drills are expensive, a nail polish drill can be a cheap replacement.

Any 5 minutes curing epoxy can be used here.

A 3D drawing is provided. See supplementary 1-3. The box should be machine fabricated (do not use 3D printers). Use transperant material, to be able to see

Custom made as described in "open ephys" website. Can also be purchusaed from neurolynx ("Tetrode Spinner 2.0") or bulit by other means.
There are several neural loggers available on the market, including: SpikeGadget (UH32 32channels) and Neurologger 2/2A/2B of Alexei Vyssotski.

Ethtl 3-aminobenzoate methanesulfonate 98%

The nano-Z can be bought from several supllieres. Any impedance meter can be used, e.g.  IMP-1 / 6662 / 2788, BAK Electronics.

3.7 Lipo battery, 370 mAh. Holds about 6 hours of recording. Smaller or larger battries can be used to reduce the weight or extend recording time.

Any type of soft petroleum  skin protectant can be used here.

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1. Please note that the editor has formatted the manuscript to match the journal's style. Please retain the same. The updated manuscript is attached and please use this version to incorporate the changes that are requested.
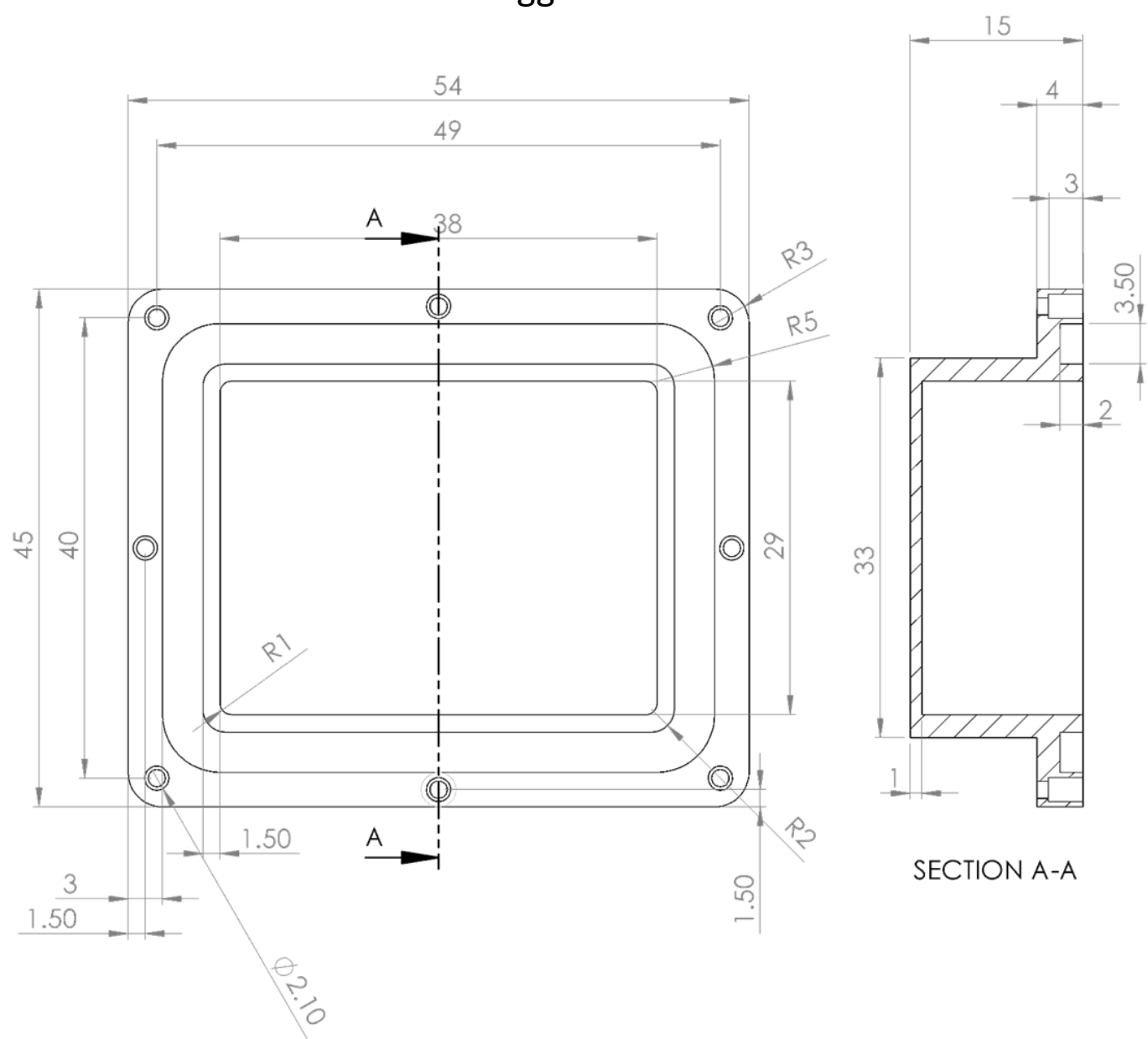
The format sent is used for the revised manuscript.

2. Please review the highlighted content of the protocol and shorten it to no more than 3 pages (currently it is more than 3 pages, see the attached highlighting only file). Please note that the editor has made some minor changes (e.g., combined some shorter steps and added steps for continuity). Note that the highlighted content should contain essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next.

The highlighted text was shortened to less than 3 pages. We've noted the editorial changes and we thank the editor for it.

3. Please address specific comments marked in the attached manuscript. Please turn on Track Changes to keep track of the changes you make to the manuscript.
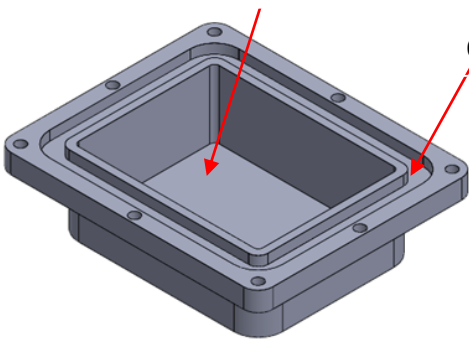
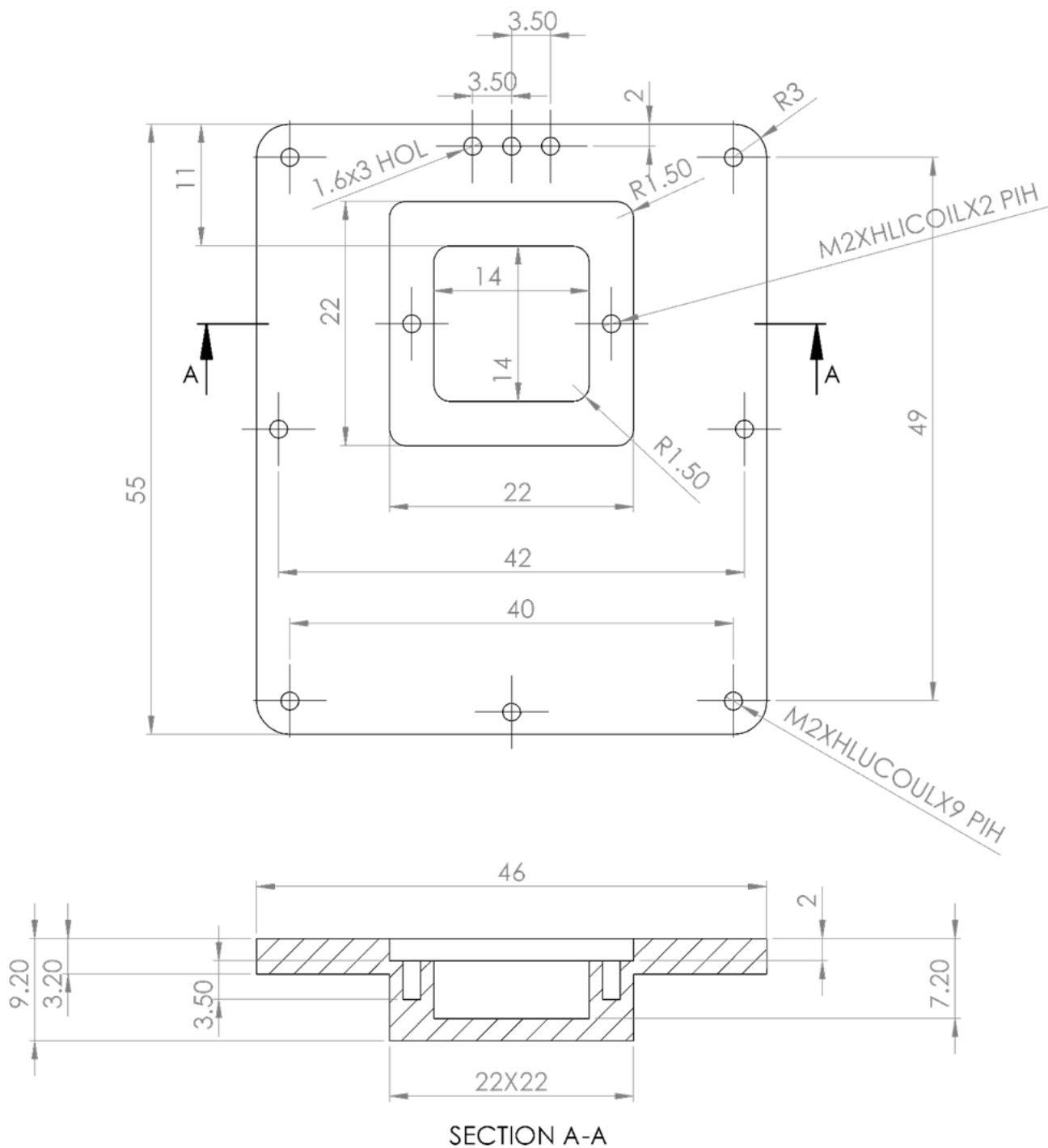All comments were addressed, and changes are trackable in the revised manuscript.

# Cohen & Vinepinsky et al. – Supplementary 1

## Logger Box



Main chamber:
Data logger and battery

O-ring slot

Units: mm

# Cohen & Vinepinsky et al. – Supplementary 2

## Logger Box Cover



SECTION A-A

EIB chamber

Units: mm

# Cohen & Vinepinsky et al. – Supplementary 3

## EIB chamber cover



SECTION B-B

Units: mm

# Cohen & Vinepinsky et al.– Supplementary 4
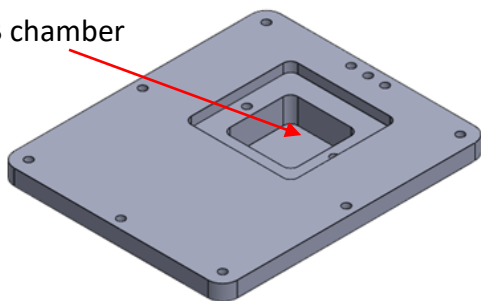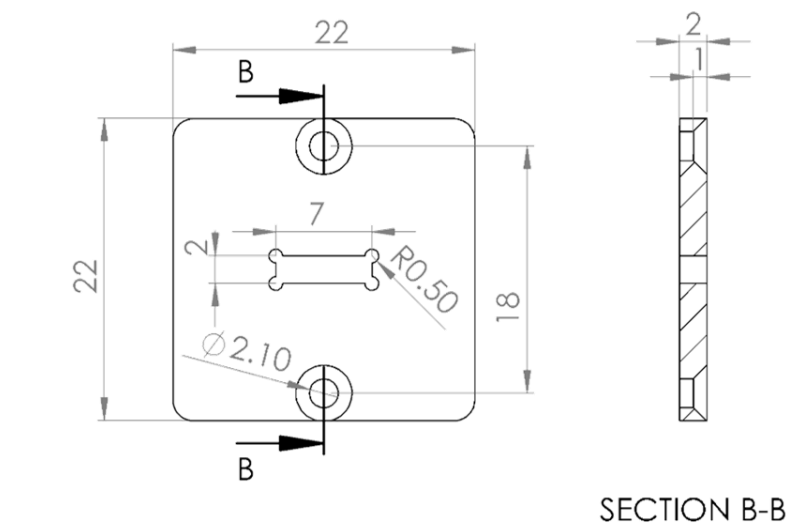
## Brass Plate for the Microdrive



Units: mm

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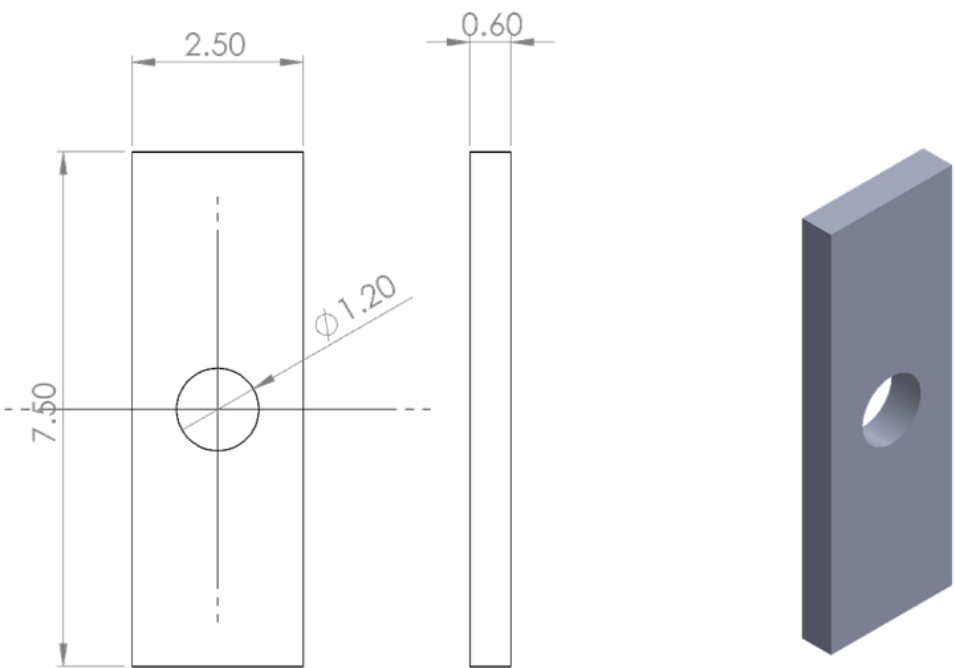
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