Video Article

Construction of an Improved Multi-Tetrode Hyperdrive for Large-Scale Neural Recording in Behaving Rats

Li Lu[1], Briana Popeney[1], J. David Dickman[1], Dora E. Angelaki[1]

[1]Department of Neuroscience, Baylor College of Medicine

Correspondence to: Li Lu at Li.Lu@bcm.edu

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Abstract

Monitoring the activity patterns of a large population of neurons over many days in awake animals is a valuable technique in the field of systems neuroscience. One key component of this technique consists of the precise placement of multiple electrodes into desired brain regions and the maintenance of their stability. Here, we describe a protocol for the construction of a 3D-printable hyperdrive, which includes eighteen independently adjustable tetrodes, and is specifically designed for *in vivo* extracellular neural recording in freely behaving rats. The tetrodes attached to the microdrives can either be individually advanced into multiple brain regions along the track, or can be used to place an array of electrodes into a smaller area. The multiple tetrodes allow for simultaneous examination of action potentials from dozens of individual neurons, as well as local field potentials from populations of neurons in the brain during active behavior. In addition, the design provides for simpler 3D drafting software that can easily be modified for differing experimental needs.

Video Link

The video component of this article can be found at https://www.jove.com/video/57388/

Introduction

In the field of systems neuroscience, scientists study the neural correlates underlying cognitive processes such as spatial navigation, memory, and decision-making. For these types of studies, it is critical to monitor the activity of many individual neurons during animal behavior. Over the past decades, two important advances have been made to meet the experimental needs for extracellular neural recording in small animals[1,2,3]. First was the development of the tetrode, a bundle of four microwires used to record neural activity of neurons simultaneously[1,2,4]. The differential signal amplitudes of activity across the four channels of a tetrode allows for the isolation of individual neuron activity from many simultaneously recorded cells[5]. In addition, the flexible nature of the microwires allows greater stability of the tetrode minimizing the relative displacement between the tetrode and the target cell population. Tetrodes are now widely used instead of a single electrode for many brain studies in various species, including rodents[1,2,6], primates[7], and insects[8]. Second was the development of a hyperdrive carrying multiple independently movable tetrodes, which allows for the simultaneous monitoring of neural activity from larger populations of neurons from multiple recording locations[3,9,10,11,12].

The availability of a reliable and affordable multi-tetrode recording device for small animals is limited. The classic hyperdrive, initially developed by Bruce McNaughton[13], has been successfully used for neural recordings in freely behaving rats in many labs in the past two decades[9,10,14,15]. However, for technical reasons, the original components needed to build the McNaughton drive are now very difficult to obtain and are not compatible with recently improved data acquisition interfaces. The other well accepted design of hyperdrive requires the microdrives to be individually handcrafted, which could yield inconsistent results and consume substantial time[12]. In order to record neural activity from various brain regions in behaving rats, we developed a new hyperdrive using stereolithographic technology. We sought to satisfy the following requirements: (1) the new hyperdrive must allow precise displacement of tetrodes in the brain and provide stable recording from multiple target regions; (2) the new hyperdrive must be compatible with the magnetic quickclip system recently developed to allow easy connection; and (3) the new hyperdrive can be accurately reproduced with materials easily available. Here, we provide a technique for building the 3D-printable hyperdrive containing eighteen independently movable tetrodes, based upon the McNaughton design. In the protocol, we describe the details of the fabrication process of the new hyperdrive, which we have used successfully to record single-neuron action potentials and local field potentials from the postrhinal and medial entorhinal cortices over weeks in a freely behaving rat during natural foraging tasks.

Protocol

1. Stereolithography of 3D Models

1. Use stereolithographic techniques to print the hyperdrive parts and accessories. Each hyperdrive is comprised of eighteen shuttles, eighteen shuttle bolts, and one each of all other plastic pieces (**Figure 1**).

NOTE: The accessories are not part of the hyperdrive but are necessary for hyperdrive construction.

2. Preparation of Accessories (Figure 2).

1. **Preparation of the microdrive rack** (**Figure 2C**).
    1. Clean and expand the smaller through-holes and the larger blind-holes in the rack with a ø 0.71 mm (0.028") drill bit and a ø 0.84 mm (0.033") drill bit, respectively.
    2. Cut a ø 0.89 mm (0.035") welding rod into 17 mm long segments, round both ends, and insert each guide rod into the ø 0.84 mm (0.033") holes on the rack, leaving 11.5 mm outside (flush with the threaded rods).
    3. Fully insert six 0-80 threaded, 15.88 mm (5/8") long flat head screws down into the slots in the rack. Ensure that the guide rods and threaded rods are straight and parallel to one another. Fill the remaining space in the slots with dilute dental cement. Air dry on a benchtop for 15 min.
    4. Glue the welding rods and screws into the rack with thin super glue and allow to air dry for 15 min.

2. **Preparation of the core station** (**Figure 2E**) .
    1. Thread the four holes with a 2-56 tap, and use 2-56, 4.76 mm (3/16") long nylon screws to secure the core in the station, if necessary.

3. **Preparation of the turning tool** (**Figure 2F**) .
    1. Thread the hole on the handle with a 4-40 tap. Insert the machined tip into the slot in the handle and secure with a 4-40, 4.76 mm (3/16") long cup screw.

4. **Preparation of the hyperdrive holder** (**Figure 2G**) .
    1. Thread the screw hole with an 8-32 tap. Use an 8-32, 9.52 mm (3/8") long nylon thumb screw to secure the hyperdrive when in use.

5. **Preparation of the rod positioning complex** (**Figure 2H**).
    1. Thread the stem from the side with the larger hole (top) with an 8-32 tap to a depth of about 7 mm. Thread the smaller holes (six in the top, eighteen in the bottom) with a 0-80 tap. Expand the central hole in the top with a ø 4.76 mm (3/16") drill bit, if necessary.
    2. Assemble the stem to the top, using an 8-32, ø 4.76 mm (3/16"), 6.35 mm (1/4") long shoulder screw. Secure the bottom to the top with 0-80, 6.35 mm (1/4") long screws when in use.

3. Preparation of the Hyperdrive Components (Figure 3).

1. **Preparation of the hyperdrive nut** (**Figure 3A**).
    1. Using the nut holder (**Figure 2D**), thread the nut with a 3/8-24 bottoming tap until smooth.

2. **Assembly of the hyperdrive core** (**Figure 3B**).
    1. Clean and expand the holes in the core using different sized drill bits (twelve ground wire through-holes (inner ring):ø 0.61 mm (0.024"); the eighteen tetrode through-holes (middle ring): ø 0.66 mm (0.026") first, and then ø 0.71 mm (0.028"); the eighteen guide rod blind-holes (outer ring): ø 0.84 mm (0.033")).
    2. Thread the two through-holes on top of the core and the remaining eight blind-holes (four on the side, four near the bottom) with a 0-80 tap. Use a bottoming tap for the blind-holes.
    3. Create external threads at the base of the core using a 3/8-24 die. Adjust the die properly so the hyperdrive nut will fit over the new threads.
    4. Depending on the number of ground wires desired, insert multiple 6 mm long segments of 23-gauge metal tubing (cannulas) into the ground wire holes in the core, gluing them if necessary. File the ends of the ground wire cannulas until flush with the outside of the core, and clean the cannulas with a ø 0.30 mm (0.012") steel wire.
    5. Fully insert eighteen 0-80, 15.88 mm (5/8") long flat head screws head down into the slots in the core. Do not bend the screws or damage the threads during this process.
    6. Using the rod positioning complex and the core station, position eighteen 17 mm segments of ø 0.89 mm (0.035") welding rod over the guide rod holes in the core and hammer them down to be flush with the screws (about 5 mm).
    7. Correct the positions of the welding rods and screws if necessary, then tighten the central shoulder screw and the surrounding six screws in the rod positioning complex to secure the outward directions of the rods in the core. Screw the nut onto the core (with the rod positioning complex) and fit the core into the hyperdrive holder to allow easier positioning under a stereoscope.
    8. Fill the slots with dilute dental cement to secure the screws to the core and allow air drying for 15 min. Fill 2-3 slots at a time before the dental cement gets too thick. Scrape away any excess dental cement on the core to maintain a proper fit with the shield.
    9. Glue the screws and rods into the core with thin super glue, allow air drying for 15 min.

3. **Assembly of the microdrive** (**Figure 3C**) .
    1. Clean and expand the two outer holes in the shuttle with drill bits (smaller hole: ø 0.61 mm (0.024") drill bit; larger hole: ø 0.89 mm (0.035") drill bit).
    2. Insert the shuttle bolt into the bolt holder base. Pay attention to the orientation. Close the bolt holder lid, hold tightly, and thread slowly through the hole in the lid with a 0-80 tap. Tap 2-3 times until smooth.
    3. Insert the shuttle bolt into the shuttle from the side with the smaller opening. Place the shuttle-shuttle bolt complex upside-down in the microdrive assembly station base.
    4. Cut a 15 mm segment of 23 gauge metal tubing and smooth both ends, then position the tubing over the ø 0.61 mm (0.024") hole, guided by the slot on the station Lid. Hammer the cannula into the hole until the upper end is flush with the station Lid.
    5. Remove the outer half of the upper tip of the cannula with a sanding wheel. Clean the cannula with a ø 0.30 mm (0.012") metal wire. Glue the cannula onto the shuttle using thin super glue, making sure not to glue the shuttle bolt to the shuttle, and air dry for 15 min.

6. Prepare at least eighteen microdrives, test the microdrive on the microdrive rack. Make sure that the shuttle bolt can rotate smoothly in the shuttle and that the entire microdrive moves freely along the length of the threaded rod.

4. **Preparation of the central column** (**Figure 3D**).
   1. Sand the top and bottom of the central column until flat, if needed. Thread the two holes in the central column with a 0-80 tap. Insert a 0-80 hex nut (3.18 mm (1/8") wide, 1.19 mm (3/64") high) into each slot.

5. **Preparation of the hyperdrive cap** (**Figure 3E**).
   1. Using non-magnetic forceps, glue four magnets (3 mm in diameter, 1 mm thick) into the four wells, matching them to the N and S poles on the electrode interface board.

6. **Assembly of the guide cannulas into a bundle** (**Figure 3F**).
   1. Place eighteen 30 gauge, thin wall cannulas (ID 0.19 mm,0.0075") into ø 2.29 mm (0.09") heat-shrink tubes (3-5 mm long, spaced apart along the bundle by 5-10 mm). Make all cannulas flush with one another on one end of the bundle.
   2. Shrink the heat-shrink tubes using a heat gun until the bundle is tight. Squeeze the bundle gently to shape it as desired (round or oval). Confirm that all cannulas are in the correct positions with no twisting, crossing, or bending.
   3. Mark the area(s) for soldering on the cannulas. The unsoldered portion should be 26 mm in length, while the soldered portion should be 5-10 mm. Move the shrinking tubes to the soldering marks to prevent spreading.
   4. Apply flux to one soldering area and solder while rotating the bundle. Cool at room temperature for at least 1 min. Repeat this step to solder the same area two more times. Smooth out the soldered portion by soldering without applying flux and filler material. Cool at room temperature for at least 1 min.
   5. Cut the bundle to the proper length with a diamond wheel at the highest speed, polish both ends to adjust the length (unsoldered part: 26 mm, soldered part: 5-10 mm as desired). Clean the guide cannulas with a ø 0.18 mm (0.007") metal wire under a stereoscope.

7. **Preparing the tetrodes. Similar procedures have been described**[8,16,17] .
   1. Adjust the height of the horizontal T bar and the position of the magnetic stirrer, so that the horizontal arm at the cross of the T bar is directly above the center of the magnetic stirrer. Hook one end of an S-hook to the center of a small magnetic stir bar, then glue them together. Clean the tetrode making space with compressed air and ethanol wipes.
   2. Circle the two ends of a piece of single tetrode wire around 40 cm in length together, then secure with a piece of copper tape.
   3. Lift the wire circle by holding the copper tape. Place the end opposite to the copper tape onto the horizontal arm of the T bar. Lower the copper tape gently (while the other end is still on the T bar), twist once, and place the copper tape onto the T bar. The tetrode circle is now in a figure eight ("∞") configuration with the copper tape sitting on top of the cross of the horizontal bar.
   4. Hold the copper tape on the T bar with one hand gently. With your other hand, hook the free end of the S-hook (with a magnetic stir attached to the other end) through the bottom of the tetrode wire circle, release the S-hook gently and let it straighten the four wires by the weight of the S hook.
   5. Adjust the height of the horizontal bar until the bottom of the S-hook is about 1 cm above the center of the magnetic stirrer plate.
   6. Bend the edge of the copper tape down to secure it to the horizontal bar. Examine the four straight tetrode wires by eye, then remove any debris.
   7. Turn on the stirrer twisting the four wires at a speed around 60 rpm, until the angle between the two opposite untwisted wires is about 60°.
   8. Set the heat gun to 210 °C, and heat the twisted wires by sweeping the gun along the straight length of the wires from different angles for 2 min to fuse them together by melting the VG bond coat.
   9. Lift the S-hook with stir gently and cut the lower end of the tetrode with fine scissors.
   10. Hold the copper tape on the horizontal bar with a finger, cut the wires from both edges of the copper tape with scissors, and remove the copper tape. Cut the remaining wire on the horizontal bar to release the tetrode.
   11. Place the completed tetrode in a dust-free box for storage. Prepare at least twenty-five tetrodes.

4. Assembly of the hyperdrive (Figure 4).

1. Inserting the guide cannulas into the hyperdrive core (**Figure 4A**).
   1. Remove the heat-shrink tubes and slide a 4 mm segment of silicon tubing (ID 1.02 mm (0.04"), OD 2.16 mm (0.085")) along the bundle to the soldered/unsoldered border. Wedge the slit in the hyperdrive spacer to widen the central hole, allowing the spacer to slip around the silicon tube. Remove the wedge when the spacer sits at the center of the silicon tube.
   2. Organize the positions of the guide cannulas in the bundle by placing long segments (10 cm) of the ø 0.18mm (0.007") metal wire through each cannula into a specific tetrode hole in the hyperdrive core, preventing any crossover of the wires or cannulas in the process. Bend the ends of the wires to hold them in place.
   3. Push the cannulas through their respective holes in the core, being careful to avoid bending or crossing between them, until the free end of each cannula is at least 2 mm outside the upper end of the tetrode hole. Secure the spacer by screwing the nut onto the core, being careful to prevent the spacer from rotating. Apply a drop of very dilute dental cement from the top of the core onto the junction of the cannulas to secure their relative positions.
   4. Cut the guide wires from the soldered end of the bundle, and remove them from the cannulas by retracting from the free end.

2. Assembly of the microdrives onto the hyperdrive Core (**Figure 4B**). A detailed spatial arrangement of the microdrives in the hyperdrive has been previously described[11,13].
   1. Load the microdrives slowly and carefully onto each threaded rod of the core. Confirm that (1) the 23 gauge microdrive cannula goes into the tetrode hole smoothly, (2) the 30 gauge guide cannula goes into the 23 gauge microdrive cannula smoothly, and (3) the shuttle bolt turns smoothly along the threaded rod. Screw the microdrives down to 1.0-1.5 mm above the lower end of the threaded rods.
   2. Cut eighteen pieces of polyimide tubing (ID 0.11 mm (0.0045"), OD 0.14 mm (0.0055")) into 38-43 mm segments (length of the guide cannula bundle plus 7 mm). Clean each tube with a ø 0.08 mm (0.003") steel wire.

3. Invert the core, insert the polyimide tubes carefully into the guide cannulas from the soldered end, and push them all the way in under a stereoscope. Flip the core upright and glue the upper end of the polyimide tube onto the microdrive cannula with thick super glue. Place the core upside-down and let the glue dry for 15 min.
4. Cut the extra polyimide tubing at the upper end, leaving 0.5-1.0 mm outside of the microdrive cannula.

3. **Assembly of the ground wires** (**Figure 4C**).
   1. Cut the number of ground wires necessary to lengths of 25-30 mm from coated steel wire (coated ø 0.20 mm (0.008"), bare ø 0.13 mm (0.005")). Strip 2 mm of the plastic insulation from both tips of the wires and insert one end of each into the ends of 6-8 mm long 30 gauge cannulas. Flatten the ends of the cannulas to secure the connection to their respective wires.
   2. Use a Dremel tool to cut the cannulas in half to create two complete ground wires from each.
   3. Insert the round end of the 30 gauge cannula into the upper end of the ground wire cannula in the core and press to make the insertion tight.

4. **Assembly of the electrode interface board** (**Figure 4D**).
   1. Insert the central column into the core and secure with two 0-80, 7.94 mm (5/16") long socket head screws. Glue if necessary to make the central column steady in the core.
   2. Expand the portions of the slots in the EIB-72-QC-Large board that correspond to the two tapped holes in the central column with a ø 1.2 mm tap. Attach the electrode interface board to the central column with two 0-80, 3.97 mm (5/32") long pan head screws. Make sure the board is situated in the center and is secure.

5. **Connecting the ground wires** (**Figure 4E**).
   1. Route each ground wire around the central column and connect the exposed free end to the electrode interface board with a gold pin at the designated ground hole.

6. **Loading the tetrodes into the hyperdrive, as previously described**[16,17] .
   1. Load each tetrode carefully into the polyimide tubes of the microdrives, being careful not to bend them during the process.
   2. Gently feed the free end wires into their designated holes in the electrode interface board and electrically connect them using gold pins.
   3. Cut the tetrodes individually to a proper length. Confirm that the portion of tetrodes protruding from the lower ends of the polyimide tubes after cutting is straight, otherwise replace the entire tetrode and recut.

7. **Attaching the shield.**
   1. Attach the shield to the core using four 0-80, 3.97 mm (5/32") pan head screws. The numbers on the shield should match with the numbers on the electrode interface board.

8. **Plating the tetrode tips.**
   1. Plate the tips of the tetrodes using the NanoZ plating device equipped with an ADPT-NZ-EIB-36 connector and an ADPT-EIB-72-QC-HS-36 adaptor[17]. Alternatively, plate them manually one by one as described elsewhere[16]. Plate the tetrode tips prior to use (*e.g.*, one day before implantation), as impedance will gradually increase over time after plating. Replace the tetrodes that are shorted or obstructed during the process of plating, cut them to a proper length, and re-plate.

9. **Finalizing the hyperdrive** (**Figure 4F**).
   1. Glue the tetrodes to their polyimide tubes as previously described[16]. Retract all of them back into their guide cannulas so the plated tips are not exposed.
   2. Screw four 0-80, 6.35 mm (1/4") long socket head screws into the four holes near the bottom of the hyperdrive core.
   3. Using a stereoscope, lower each tetrode slowly until the tip of the tetrode is just above the edge of the guide cannula. Meanwhile, locate the position of each tetrode in the guide cannula bundle. The map of the tetrode's position is critical for reconstruction of recording sites.
   4. Attach the cap to the drive and store the hyperdrive properly for implantation.

Representative Results

We used a newly built hyperdrive to obtain trial results. The drive was equipped with tetrodes constructed from ø 17 µm (0.0007"), polyimide-coated platinum-iridium (90%-10%) wire. The tips of the tetrodes were plated in platinum black solution to reduce electrode impedances to between 100 and 200 kΩ at 1 kHz. The hyperdrive was implanted 4.6 mm left of the midline and 0.5 mm anterior to the transverse sinus on the skull of a 550 g, male Long-Evans rat. Additional ground wires were connected to skull screws over the cerebellum. All procedures were performed as approved by the Institutional Animal Care and Use Committee (IACUC) of Baylor College of Medicine and were similar to those previously described[18]. Immediately following the surgical implantation, the tetrodes were advanced 1 mm into the brain. On subsequent days, smaller advanced increments of no more than 80 µm were used. The tetrodes were allowed to stabilize after each advancement for at least 20 h before neural recordings were performed.

To record neural activity, the hyperdrive was connected to a headstage pre-amplifier (Neuralynx, HS-72-QC), and the latter was connected to a data acquisition system with programmable amplifiers (Neuralynx, Digital Lynx SX). Local field potentials were referenced to the ground wire, sampled at 2 kHz, and band-pass filtered at 0.1—500 Hz. Unit activity was referenced to a tetrode with no observable activity located 500 µm from the brain surface, sampled at 32 kHz, and band-pass filtered at 600 Hz-6 kHz. Only spike waveforms above a threshold of 50 µV were recorded.

Figure 5A illustrates neural activity recorded from a tetrode located in the postrhinal cortex (2.1 mm below the brain surface), while the animal was freely foraging inside a 1.5 m open box three weeks after implantation. The recording session lasted approximately 30 min and the units recorded remained stable across the entire session (demonstrated by the small variation in spike waveforms). **Figure 5B** shows local field potentials recorded simultaneously from four different tetrodes located in the medial entorhinal cortex (3.4-3.7 mm deep) while the same animal was actively exploring the open arena seven weeks after implantation. Clear field potential activity in the theta frequency range (6-10 Hz) was present. Individual neuron spike data was isolated using the sorting software MClust (A.D. Redish), and local field potential data was visualized by custom-written Matlab scripts. Examples of low quality tetrode recordings, possibly resulting from a poorly prepared drive, have been shown previously[17].

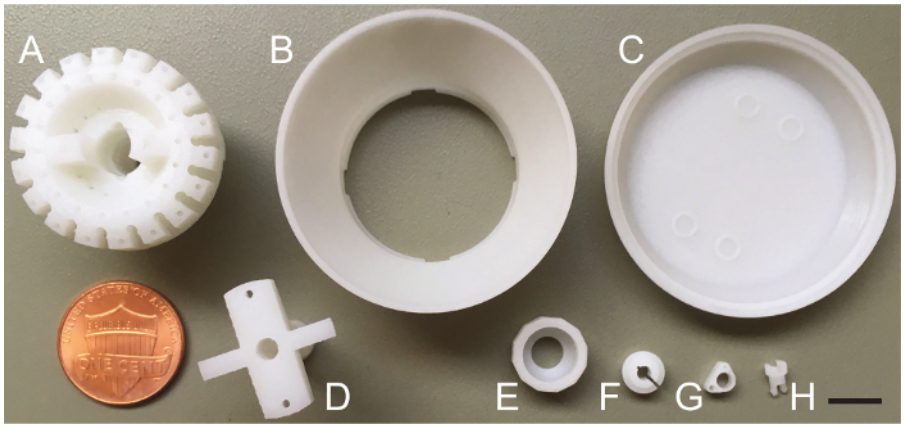

Figure 1: Hyperdrive components created by stereolithographic technology. Image of the 3D-printable hyperdrive components (1¢ coin for size comparison). (**A**) the hyperdrive core; (**B**) the protective shield; (**C**) the protective cap; (**D**) the central column; (**E**) the nut; (**F**) the spacer; (**G**) the shuttle; (**H**) the shuttle bolt. Scale bar: 1 cm. These components were created by a UnionTech RSPro450 printer using the plastic material Somos EvoLVe 128. Please click here to view a larger version of this figure.

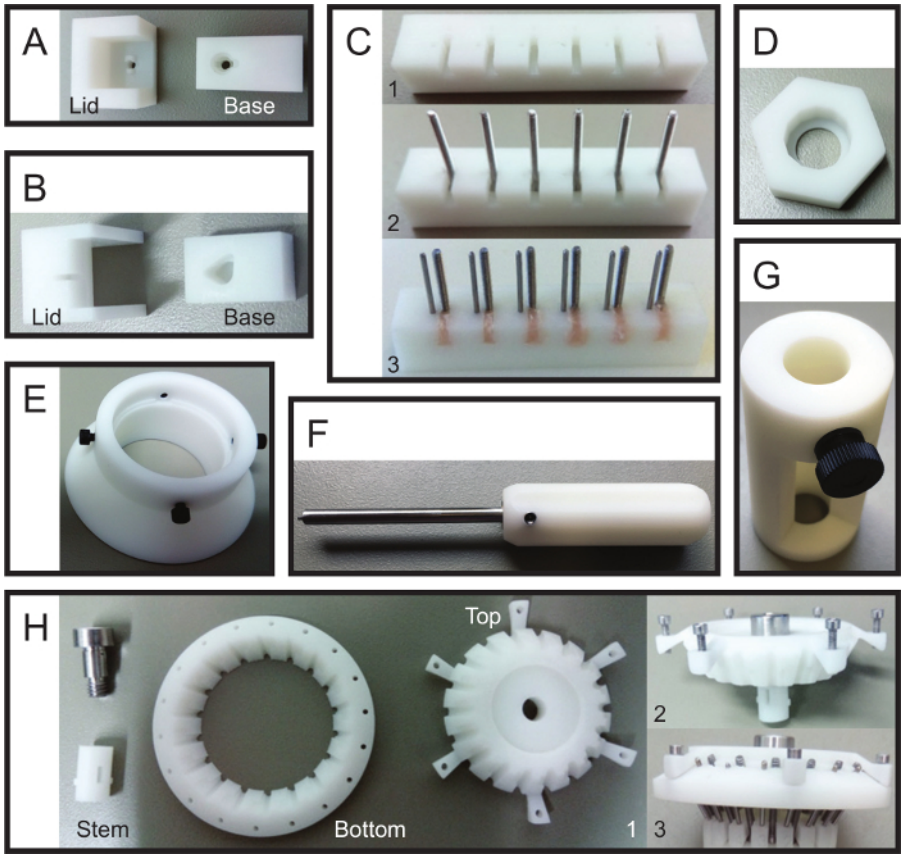

Figure 2: Custom-designed accessories for hyperdrive construction. These accessories were designed specifically to aid in the preparation of the hyperdrive. Their main components were created by stereolithographic printing. (**A**) The shuttle bolt holder, which secures the shuttle bolt while tapping the threads. (**B**) The microdrive assembly station, which guides the cannula insertion into the shuttle. (**C**) The microdrive rack, which helps to test the assembled microdrives and holds them in place while gluing the cannulas. 1: a microdrive rack base; 2: a microdrive rack with screws fully inserted in the slots; 3: a microdrive rack ready for use. (**D**) The nut holder, which holds the hyperdrive nut when threading the hole. (**E**) The hyperdrive core station, which secures the core while hammering the guide rods. (**F**) The turning tool, which drives the shuttle bolt to rotate in the shuttle. (**G**) The hyperdrive holder, which helps to place the hyperdrive under a stereoscope. The holder also protects the tetrodes after they have been loaded into the hyperdrive. (**H**) The rod positioning complex, which helps to position the threaded rods and guide rods in the hyperdrive core. 1: major components of the complex; 2: the upper portion of the complex after assembly; 3: a rod positioning complex in use.
Please click here to view a larger version of this figure.

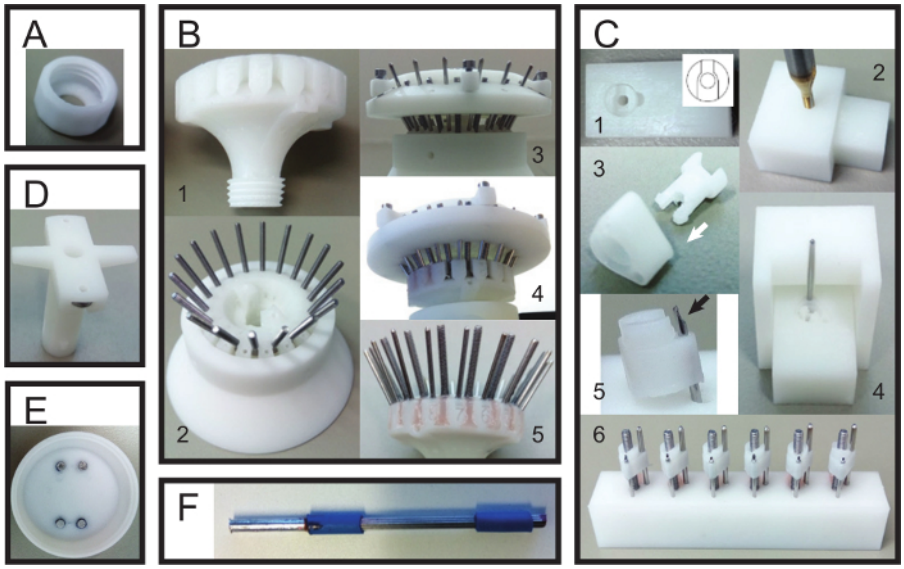

Figure 3: Preparation of the hyperdrive parts before assembly. Images showing the preparation process of the hyperdrive core and the microdrive, as well as other prepared hyperdrive parts. (**A**) A threaded hyperdrive nut. (**B**) Preparation of the hyperdrive core. 1: the core with external threads created for the nut; 2: the core placed in a core station with screws fully inserted in the slots; 3: guide rods positioned by the rod positioning complex, ready to be hammered into the core; 4: filling the remaining space in the slots with dilute dental cement; 5: the upper portion of a prepared hyperdrive core. (**C**) Preparation of the microdrive. 1: a shuttle bolt placed in a shuttle bolt holder base, note the smaller opening is facing away from the experimenter; 2: threading the threads inside the shuttle bolt; 3: insertion of the shuttle bolt into the shuttle; 4: a microdrive placed in the microdrive assembly station base with the cannula guided by the station lid, ready to be inserted; 5: a microdrive with the outer half of the upper cannula tip removed (indicated by arrow); 6: assembled microdrives tested on the microdrive rack. (**D**) A central column with threaded holes and inserted screw nuts. (**E**) A hyperdrive cap with four magnets glued in the wells. (**F**) A 36 mm long guide cannula bundle, with the soldered portion on the left. Please click here to view a larger version of this figure.

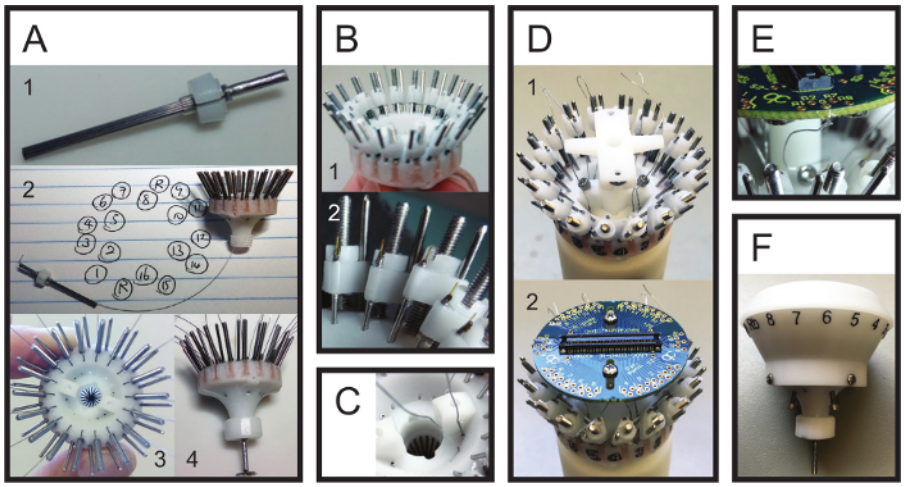

Figure 4: Assembly of the hyperdrive. Images showing stages of the hyperdrive assembly. (**A**) Insertion of the guide cannulas into the core. 1: the guide cannula bundle slid into the silicon tube and the spacer; 2: one guide cannula being placed in its designated hole in the core. Hand writing shows the organization of the guide cannulas; 3: guide cannulas pushed into the core; 4: the core with the guide cannulas inserted and secured by the nut. (**B**) Assembly of the microdrives to the core. 1: the core with microdrives loaded; 2: the microdrives with polyimide tubes inserted in the cannulas. (**C**) Insertion of the ground wires into the core. (**D**) Attachment of the electrode interface board. 1: the hyperdrive with the central column inserted; 2: the hyperdrive with the electrode interface board attached to the central column. (**E**) Connection of the ground wire to the designated hole in the electrode interface board. (**F**) A finalized hyperdrive ready for implantation (total weight of 20 g). Please click here to view a larger version of this figure.

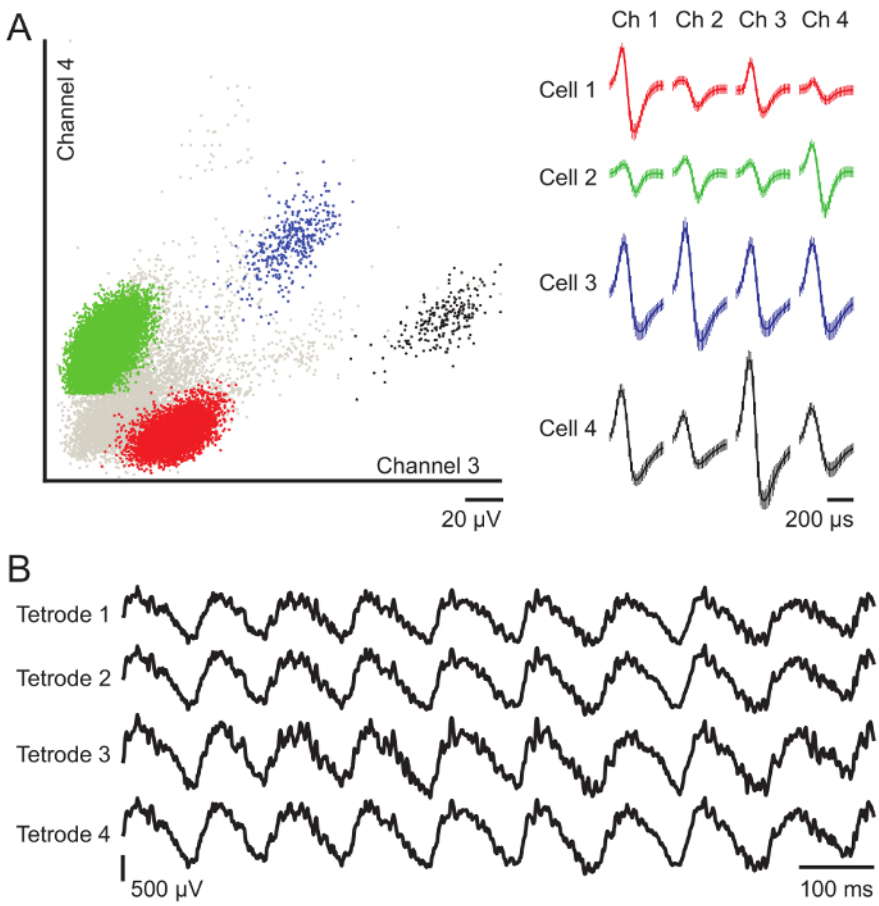

Figure 5: Neural signals recorded by the hyperdrive. Representative recordings showing unit neural activity and local field potential in a behaving rat's brain. (**A**) Two-dimensional cluster diagrams illustrating individual spikes from simultaneously recorded neurons by a tetrode located in the postrhinal cortex (depth: 2.1 mm). Left: scatter plot showing relationship between peak-to-peak amplitudes of spikes recorded from two electrodes of the tetrode. Each dot corresponds to one spike. Clusters of spikes are likely to originate from the same cell. Four clusters are color coded. Scale bar: 20 µV. Right: spike waveforms (means ± S.D.) of the color-coded cells shown on the left. Note the small variation of the waveforms. Scale bar: 200 µs. (**B**) Traces of local field potential in the theta frequency range recorded simultaneously from four different tetrodes located in the medial entorhinal cortex (depth: 3.4-3.7 mm) when the rat was freely foraging. Scale bar at bottom left: 500 µV; scale bar at bottom right: 100 ms. Please click here to view a larger version of this figure.

Supplementary Files: The supplementary files include 20 files in the .stl format detailing the hyperdrive components and accessories ready for stereolithographic printing (units in mm), and 1 file in .pdf format which is the blueprint of the turning tool tip ready for machining. The original 3D model files were created with software AutoCAD in .dwg format, which will be available upon request. Please click here to download this file.

Discussion

Here, we describe the process of constructing a newly developed hyperdrive comprised of eighteen independently movable tetrodes. The drive can be constructed from affordable parts purchased at many available hardware stores, combined with components created by stereolithographic printing. The hyperdrive can be chronically implanted onto a rat's skull using standard surgical procedures and is capable of recording extracellular neural activity while the animal performs various behavioral tasks.

The hyperdrive retains many of the desirable features of the original McNaughton hyperdrive, including the tripod microdrives that are oriented outward by 30 degrees from the drive center[13], which provides reliable support for the tetrodes. Once implanted, the hyperdrive affords the execution of small movements of the tetrodes within the brain of an awake animal with considerable precision. One full turn of a shuttle on the threaded rod corresponds to a linear displacement of 317.5 µm. With proper training, an experimenter can advance a shuttle in 1/16 turn steps (20 µm). We designed the hyperdrive for use in adult rats, but the drive could easily be used in any animal with a body size of 350 g or greater (limited by head size). One limitation of the device may be noted in the restricted depth of recording, as the maximum travel distance of the tetrodes along the threaded rods is about 7 mm, which could fall short of deeper structures in some animals' brains.

Stereolithographic printing provides for sufficient resolution to create plastic components in great detail with high fidelity, and has been previously used in hyperdrive fabrication[12,19,20]. In this case, an industrial printer commonly available through third party production facilities was used. There all the hyperdrive components were printed precisely, including the hyperdrive core, despite its complex geometry, and the small structures such as the ø 0.6 mm through holes and the 0.3 mm thin walls. This precision makes stereolithography an ideal choice for manufacturing hyperdrive components. Based on prior experience, less expensive, desktop 3D printers are less likely to have the precision necessary for

reliable reproduction of the hyperdrive components needed. Still, stereolithographic technology has its limitations. First, it has a limited selection of materials. The plastic we chose for the hyperdrive was the most durable of those we have tested, yet it is still not optimal for the manufacture of very small pieces. The shuttles and the shuttle bolts need to be handled with extra caution as they can break during preparation. The plastic components are not autoclavable, as the heat deflection temperature of the material is around 50 °C. In addition, the printing material used is not acetone resistant. These issues could be resolved when new stereolithography materials are developed and tested. Yet, considering the relatively low-cost of stereolithography, the advantages of the technique and cost far exceed the defects. Second, due to the nature of stereolithography, during which photopolymers are photochemically solidified by a UV laser to form a single layer of the desired 3D model[21], the objects created by stereolithographic printing are vulnerable to UV light. Consequently, exposing them to strong UV (*e.g.*, direct sunlight) for many hours will irreversibly reduce their physical strength (based on personal communication with the print shop). Considering the environmental UV in the laboratory space (*e.g.*, from the fluorescent lights), it is best to store the stereolithographic components in a dark box when not in use, which will retain components' physical strength for years. Moreover, it is important to use other methods aside from UV light to disinfect the hyperdrive surface before surgery. This test hyperdrive has remained implanted on the rat in good condition in an ordinary laboratory environment over the course of four months, without any indication of a reduction in physical strength or performance.

The 3D printable nature of this hyperdrive also allows rapid modifications and flexible redesign. For example, the hyperdrive can be easily modified to target separated multiple brain regions[11]. Moreover, this drive could be adjusted to allow simultaneous monitoring of neural activity and local brain manipulation. Incorporation of a microdialysis probe with the array of tetrodes allows for pharmacological activation and deactivation of neurons by the infusion of various drugs during neural recording[22]. Furthermore, neurons engineered to express light-sensitive channels can be activated or deactivated by the incorporation of an optical fiber in the tetrode bundle and optogenetic technique[19]. In addition, the drive can be easily rescaled with a fewer number of tetrodes for animals with smaller head sizes, such as mice or juvenile rats.

In summary, the easy mutability coupled with the simpler, more affordable method of constructing an effective neural recording implant that can be reliably and accurately reproduced, makes this hyperdrive a powerful tool in the field.

Disclosures

The authors have nothing to disclose.

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