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Optrode Construction

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We use this protocol and it's working

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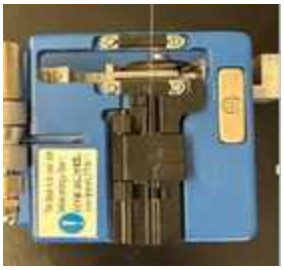
Abstract

We constructed optrodes for use in optogenetic experiments, including the manipulation of auditory brainstem nuclei expressing excitatory and inhibitory opsins. Here, we provide a step-by-step description of the construction of these optrodes, including the construction of optical cannulas and the process used to join them with tungsten electrodes. This was accomplished by aligning cannulas with electrodes under a microscope and adhering them to each other using UV cure dental cement.

1 Fiber Preparation

1.1 Strip approximately 3 cm of fiber (FG105UCA, Thorlabs) using a fiber stripper (TO6S13, Thorlabs)

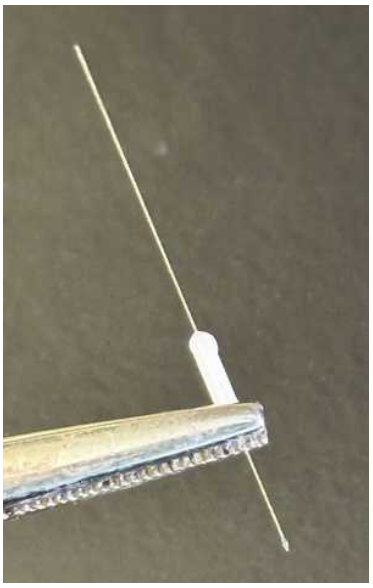
1.2 Cleave fiber using a fiber cleaver (FC-6S, Sumitomo Electric)



2 Cannula Construction

2.1 Prepare 5 minute epoxy such as Dap Tank Bond

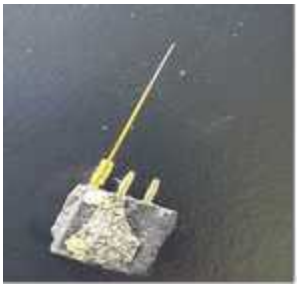
2.2 Place a dab of mixed epoxy on the concave end of ceramic ferule (MM-FER2007C-1270, Precision Fiber Products) and push the cleaved fiber through it so that 1.9 cm still protrudes from the concave end. The cleaved end of the fiber should be on the side that protrudes from the concave end. Allow epoxy to cure.



2.3 Cut the fiber flush to the convex end of ferrule using a fiber scribe (S90C, Thorlabs). This should be accomplished by gently scoring the fiber with the scribe around three times. The fiber should then break off with a very light touch. This completes the cannula. It is recommended to test each cannula for coupling efficiency and good light transmission before using it for an optrode. Only cannulas that efficiently transmit laser light without excessive loss of laser power should be carried over to the next step. Use of index matching gel is recommended for this purpose (G608N3, Thorlabs).

3 Optrode Assembly

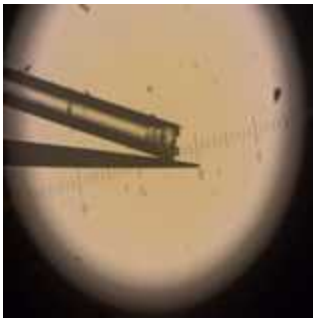
3.1 Remove the rubber insulating sleeve from the gold pin connector at the base of a tungsten electrode (WE(25mm)PT34.0B10, Microprobes for Life Science) using a razor blade, and insert the gold pin into a pin header connector.



3.2 Place pin header connector with electrode under a microscope. This can be accomplished by cementing an alligator clip head or similar clip to a glass microscope slide and placing the pin header connector into the clip. A cannula made in the previous step should then be placed in a ceramic split sleeve (SM-CS125S, Precision Fiber Products), which is then secured onto a manipulator stage (423 series, Newport Corporation).

3.3 Align electrode tip and cannula tip under the microscope such that both are in the same plane of focus, with the tip of the fiber contacting the electrode 100 microns behind the

electrode tip, as indicated by an eyepiece micrometer.



3.4 Using a spare piece of optical fiber, gently apply UV cure dental cement (Flow-It ALC, NV11VXA, Pentron) to affix the tip of the fiber to the tip of the electrode, taking care not to cover the tip of either. Ensure that the electrode and the optrode are still in contact and in the same plane of focus. Cure dental cement. Gradually add more dental cement, working from the tip of the optrode toward the base, gently pressing the fiber toward the electrode so that the completed optrode will not be excessively wide. Continue curing dental cement as you proceed. As the base of the optrode is approached, very gently bend the remaining uncovered fiber away from the electrode, so that the ferrule and the gold pin are not so close together that attaching them to cables or couplers will be difficult. Cover the rest of the optrode in dental cement and cure it such that only the gold pin and the end of the ferrule remain uncovered. Enough of the ferrule should remain uncovered that a ceramic split sleeve can easily be attached.

3.5 Connect completed optrode to a laser and ensure good light transmission, again using index matching gel (G608N3, Thorlabs).