Appendix 1 : Miniature motorized microdrive construction protocol

The motorized microdrive was designed primarily by Michale Fee. My contributions to the design of the instrument include work on the split connector that allows the motor assembly to be removed from the microdrive barrel, the lateral positioner that provides limited xy positioning of the electrodes, and some of the electrical modifications to the Sutter MP-285 that is used to remotely position the electrodes. I also made numerous contributions to the motordrive construction protocol described below. I built by hand each of the drives used in the LMAN project. The work described in this appendix is excerpted from: Fee, M.S. and Leonardo, A. Miniature motorized microdrive and commutator system for chronic neural recording in small animals. J. Neuroscience Methods. 112, 83-94 (2001).

The use of chronically implanted electrodes for neural recordings in small, freely behaving animals poses several unique technical challenges. Because of the need for an extremely lightweight apparatus, chronic recording technology has been limited to manually operated microdrives, despite the advantage of motorized manipulators for positioning electrodes. Here we describe a motorized, miniature chronically implantable microdrive for independently positioning three electrodes in the brain. The electrodes are controlled remotely, avoiding the need to disturb the animal during electrode positioning. The microdrive is approximately 6 mm in diameter, 17 mm high and weighs only 1.5 grams, including the headstage preamplifier. Use of the motorized microdrive has produced a tenfold increase in our data yield compared to those experiments done using a manually operated drive. In addition, we are able to record from multiple single neurons in the behaving animal with signal quality comparable to that seen in a head-fixed anesthetized animal.

The motorized microdrive described in this appendix was used to record from neurons in nucleus RA and nucleus LMAN in the song control system of the zebra finch. These small birds tolerated the microdrive very well, exhibited all of their normal activities

in the aviary, and were able to fly freely with the microdrive implanted. Singing behavior was unaffected by the microdrive, and the temporal and spectral pattern of syllables retained the hallmark stability seen in normal adult zebra finches. As we discuss in more detail below, the number of useful neural recordings obtained concurrently with singing behavior was dramatically larger in birds implanted with the motorized microdrive rather than with a traditional manually operated microdrive.

Background

Prior to the development of the motorized microdrive, recordings were attempted in four birds with a non-motorized version of this microdrive (similar to that described in (Venkatachalam et al., 1999)). In these four animals, a total of ten cells were recorded during singing behavior, four of these were in one bird and six in another bird. In only one instance were two neurons recorded simultaneously during singing, and only two song motifs were recorded while holding this pair. This is in sharp contrast to the 87 single units, 40 pairs and 5 triplets recorded with the motorized drive in nucleus RA (chapter 2).

Two central difficulties were encountered in the experiments with the manual microdrive, both of which were a result of the need to catch, restrain, and then release the animal in order to adjust the electrodes. Although it was often straightforward to isolate single neurons while manually manipulating the electrodes, it was extremely difficult to release the animal without losing the cell. Furthermore, with the manually operated drive it was not possible to 'tweak' a slightly degraded signal from an electrode, since attempts to catch the bird inevitably resulted in losing the cell. Efforts to record two cells simultaneously on two electrodes were confounded by the same problem. Once a cell was successfully recorded during singing, attempts to catch the bird and isolate another cell on a different electrode inevitably failed.

The second fundamental difficulty was that handling the birds to manipulate the electrodes had the effect of suppressing singing behavior. The zebra finches used in these experiments could normally be reliably induced to sing by placing a caged female zebra finch nearby. The effectiveness of this stimulus was greatly reduced, often for several hours, by the process of capturing and restraining the bird. On many occasions, with a manually

operated microdrive, single-unit signals were obtained and held for tens of minutes, during which the bird could not be induced to sing.

Using the motorized microdrive largely eliminated these difficulties. The greater controllability afforded by the motorized control made the process of getting high-quality signals much simpler that with the manual microdrive. Isolating single neurons was done in the same manner as in acute recording experiments, simply advancing and retracting the electrode to find the optimum signal. Simultaneous recordings could often be isolated by dialing in a single-unit on one electrode and then dialing in a single-unit on another electrode. In addition, electrodes whose signal quality had degraded after some time could usually be improved by adjusting the electrode position. Furthermore, the singing behavior seemed unaffected by the manipulation of the electrodes; the birds showed no response to the operation of the motors.

Neural recordings obtained in singing zebra finches with the motorized microdrive were of the same quality as those seen in head-fixed anesthetized animals. Because of the thread size used to drive the shuttles, and the computer-control of the MP-285 (described below), we were able to move the electrodes with a positional resolution of less than one micron. This allowed us to obtain signal-to-noise ratios that are particularly high compared to those normally seen in chronic neural recordings with microwires. Typical peak-to-peak amplitudes for cells across the entire population were 1-4 mV; excellent signals could exceed 10 mV in amplitude. Good single-unit isolations with the motorized microdrive were often found to be stable for over an hour, and degradation in signal quality could be compensated for by readjustment of the electrode position. A quantitative measure of the effectiveness of the motorized microdrive system is that the yield of cells recorded during the singing behavior was roughly ten times higher than without the motorized microdrive. This dramatic increase in data yield is mirrored by a corresponding increase in data quality; the acquisition of many simultaneous pairs and triplets of cells is virtually unobtainable with a manually operated microdrive in singing zebra finches.

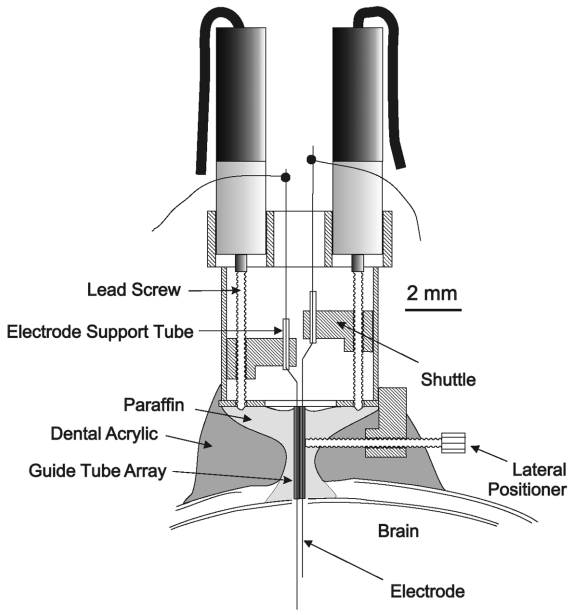

Figure A.1. Overview of motorized microdrive. Each electrode is held by a moveable shuttle that can be advanced and retracted by rotating a threaded lead screw. The shuttle moves in a cylindrical channel within the microdrive body. The lead screw is rotated by a small brushless DC motor that weighs ~100 mg. The device described here has three motors and shuttles arranged in a circle. Limited lateral positioning of the electrodes is accomplished with a threaded rod placed against the electrode bundle.

Methods

The basic design of the microdrive is derived from one described previously (Venkatachalam et al., 1999). Electrodes are held by threaded shuttles that travel along small threaded rods (Figure A.1). The shuttles for multiple electrodes are arranged concentrically to permit a compact arrangement of bundles of electrodes. In contrast to previous designs in which the threaded rods are rotated manually, each threaded rod is mounted to the output shaft of miniature synchronous motor. The motors are 1.9 mm in diameter and weigh approximately 100 mg. The motorized microdrive consists of three main subassemblies. The microdrive/connector assembly is constructed first, followed by the motor assembly, and finally, the electrode assembly. (See Figure A.2 b, c and d). In the following subsections we describe the construction of each of these components in detail.

Microdrive/Connector assembly

The bottom plate is attached to the microdrive body. A double loop of 0.016” diameter solid hook-up wire is wrapped around the base of the microdrive to make the ground connections between the two connectors and to the microdrive body, and also serves to hold the connectors in place prior to gluing. The ground lead of the main connector (Omnetics Connector Co., #A7255-001 and A7732-001) is soldered to the hook-up wire and the

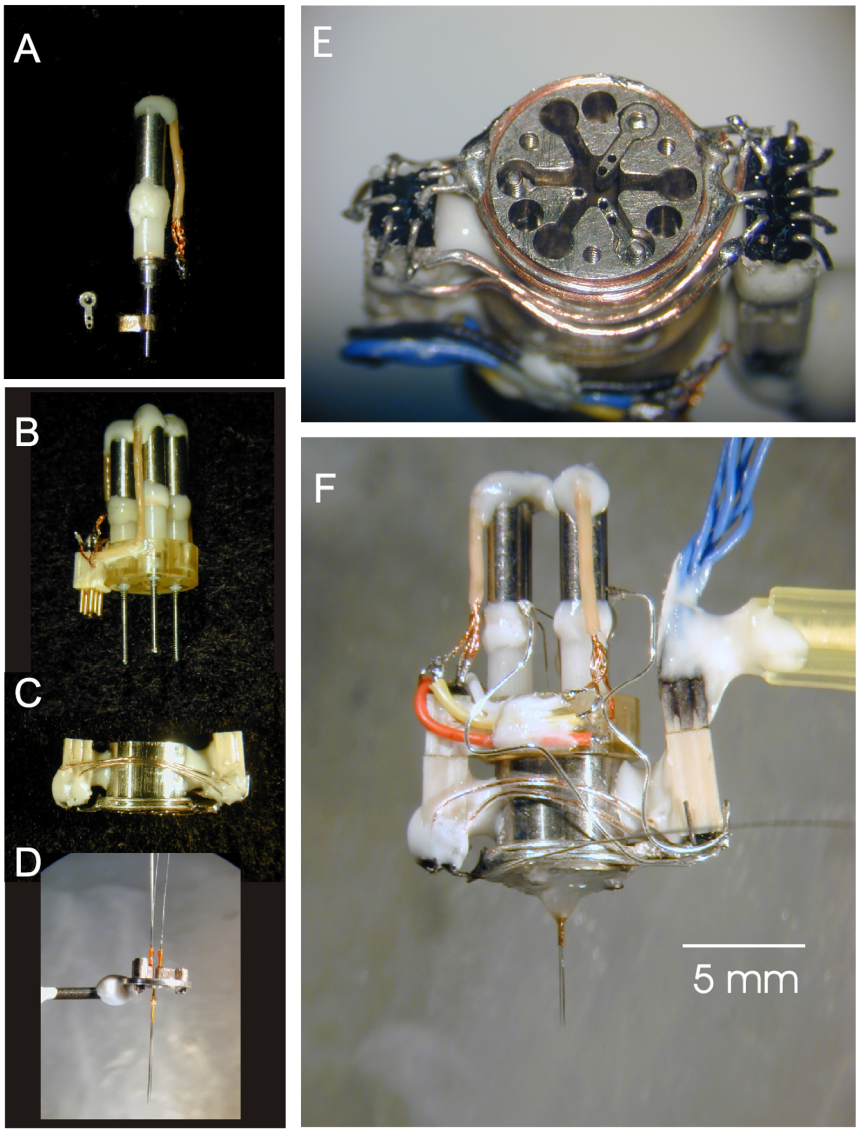

Figure A.2. Photographs of microdrive subassemblies and completed microdrive. A) A single micromotor with attached lead screw and shuttle. Rotations of the motor shaft produce a translation of the shuttle. To the left is a top view of an individual shuttle. B) The motor assembly: Individual motors are pressed into the motor mounting plate. Electrical connections are made from the motors to the connector glued to the side of the motor mounting plate. C) The microdrive body showing the attached miniature electrical connectors (Omnetics, Inc.). On the right is the main connector through which all signals are routed to and from the commutator. The motor control signals are further routed to the motor connector on the left. When the motor assembly is attached to the microdrive body, the motor connectors are mated. D) The electrode assembly can be constructed by temporarily screwing the shuttles to a ring in the proper orientation. The electrodes are inserted and polyamide guide tubes are placed over the electrodes and arranged into an array. E) Bottom view of the microdrive (bottom plate removed) showing the shuttles threaded onto the lead screws. F) Side view of the completed microdrive, loaded with electrodes and ready for implantation. A thin coating of paraffin has been applied to the bottom of the drive to keep the electrodes and the interior of the microdrive free of the acrylic used to attach the microdrive onto the cranium.

connector is positioned as desired. The ground lead of the motor connector is soldered to the hook-up wire on the opposite side and positioned parallel to the microdrive body. Both connectors are then glued in place with Torr Seal (Varian Vacuum Products, Inc.). The motor control signals from the main connector are soldered to the appropriate pins on the motor connector (Cooner Wire, Inc., #CZ-1187, teflon-coated copper stranded wire).

Motor assembly

The motors were purchased from Micro Mo Electronics (www.micromo.com, Part #0206A0.5B+02/1 47:1). The attachment of the motor to the planetary gearhead is extremely fragile and must be reinforced (Figure 3A). A small drop of cyanoacrylate glue is applied to the junction of the motor and planetary gearhead. Once this has hardened, a ring of Torr Seal epoxy (Varian Vacuum Products, Inc.) is applied around the junction and slightly heated with a heat gun (100 C) to facilitate flowing, and to speed the hardening of the epoxy.

The threaded rod is attached to the output shaft of the planetary gearhead. The output shaft is cut with small diagonal cutters to a length of 0.5 mm (± 0.1 mm). The shaft is brittle and can be trimmed with further clipping. A 5.5 mm length of #0000-160 threaded rod is cut from stock with diagonal cutters and the ends cleaned up with a small sharpening stone to remove burrs. The motor is mounted vertically in a holder under a dissecting microscope with the output shaft facing upward. The output shaft (and rotating plate) is covered with a small amount of Torr Seal and the shaft coupler is placed over the output shaft. One end of the threaded rod is heated with the heat gun and the tip is dipped in Torr Seal. The threaded rod is inserted into the shaft coupler. The motor is electrically connected to the motor controller (SC-1900, Minimotor Inc.) and started at a slow speed (~60 RPM). The threaded rod is centered with forceps so that no wobble is visible at the top or bottom as it rotates. Care must be taken since excess epoxy between the coupler and the threaded rod can come in contact with the microdrive body and the resulting friction will impede shuttle movement.

The motor is connected to the controller (SC-1900) to test for proper functioning. Then the connector is displaced to make connection with only a single pair of contacts at a time. Motor rotation is observed for each pair of contacts. There is usually one pair of contacts for which there is the least amount of motor rotation. This pair is chosen for the motor ground and motor common connections (this usually corresponds to the green and

unmarked bundles in the motor cable). The motor cable is bent back down the side of the motor by heating (with a soldering iron) the plastic cable covering at the back end of the motor. With the cable secured along the side of the motor, the cable connection to the motor is reinforced with a small application of Torr Seal.

Although there are only three connections to each motor, there are fifteen extremely fragile copper wires inside the motor cable. The end of the cable covering is removed by melting a ring in the plastic cover with the soldering iron and pulling the end off with forceps. The fifteen wires are bundled in three groups of five. One bundle of wires is labeled with a red enameled wire, one with a green wire and the other with no colored marking. The insulation at the end of the wires is removed by applying a small drop of methylene chloride based paint remover gel (Zip-Strip) to soften the enamel, which is then scraped off gently with sharp forceps. This process may require two applications of Zip-Strip. Each group of five wires is then twisted together and soldered to the connector. The plain bundle is connected to motor ground; the green bundle is connected to motor common; and the red bundle is connected to the individual motor drive signal.

The motors are pressed into the motor mounting plate and the shuttles are screwed all the way onto the lead screws. Construction of the motor subassembly takes place in two stages. In the first stage, the most crucial aspect of the construction process is that the alignment of the motors and lead screws with the shuttle channels in the microdrive body be as precise as possible. Although the output torque of the motors is greatly improved by the 47:1 planetary gear system, the torque (300 uNm) is just sufficient to drive the shuttles. Because the fit between the body of the microdrive and the electrode shuttles is precise, even a small misalignment of the motor shaft can produce sufficient friction to prevent movement. Careful alignment of the motor mounting plate on the microdrive body is required (see Figure A.2 b and c). The motor mounting plate is attached (with #0000-160 screws) to the microdrive body with the shuttles positioned in the shuttle channels. The shuttles are tested one at a time over their full travel range. If there is any binding, the motor mounting plate is loosened, repositioned and reattached. When all three shuttles are free to travel over the full range of motion, then the motor mounting plate and the microdrive body have been properly aligned.

The second stage of the construction of the motor assembly involves gluing the motor connector to the motor mounting plate and making the electrical connections from the motors to the motor connector. The mating part of the motor connector is inserted into the motor connector on the microdrive/connector assembly. This connector is then glued to the motor mounting plate (at the left in Figure A.2 b). It is wise to double-check for proper shuttle freedom of movement before the glue fully hardens since the motor connector strongly constrains the alignment of the motor mounting plate. At this point, the electrical connections from the motors to the motor connector are made, as described in the notes. Once this process is complete, the tops of each motor are linked to each other with a small bridge of epoxy (this more firmly secures the positions of the motors in the motor mounting plate; refer to Figure A.2 b and f).

Construction of the electrode array

The electrode bundle may be assembled directly onto the motor subassembly, or it may be constructed outside of the microdrive body by attaching the shuttles to a temporary mounting ring (identical to the bottom plate; see Figure A2 d). In either case, the bottom plate needs to be removed from the microdrive body to allow the shuttles to be inserted into the bottom of the microdrive and retracted upwards. Short lengths (1.0 mm) of 0.008" ID polyimide tubing (AM Systems, Inc.) are glued into the electrode holes in the shuttles to provide mechanical support for the electrodes. Three electrodes (~3 MOhm tungsten electrodes insulated with parylene; Microprobe, Inc. part #WE300312H3) are cut to the correct length and crimped (see Fig. 1) to provide the proper spacing of the tips. The end of each electrode shank is stripped of 1 mm of insulation. The electrodes are then inserted backwards into their shuttles, and secured in place with a drop of epoxy. The exact orientation of each electrode may now be fine tuned by carefully manipulating the crimp angle with a pair of forceps. A polyimide guide tube (0.004" ID , 2.5 mm) is placed over each electrode and the guide tubes grouped so that the electrode tips form a bundle with 100-200 um spacing. This spacing is constrained primarily by the diameter of the polyimide tubing. The guide tubes are tied with gold wire (0.003" OD) and secured with epoxy.

Once the electrode array is assembled, the motor controller is activated and the shuttles are moved to the top of the microdrive body. The electrode shanks are bent outward

between the motors, and the electrical connections are made from the electrodes to the main connector on the microdrive body using 0.005" teflon coated silver wire (AM Systems, Inc.) and silver epoxy (Epoxy Technology, Inc.). The bottom plate is reattached to the base of the microdrive, and a length of 0.005" bare silver wire is soldered onto the microdrive (to the hook-up wire) for animal ground. A differential ground wire (0.001" teflon coated platinum-iridium wire) is attached to the electrode bundle and aligned so it protrudes ~700 um into the brain near the implanted electrodes. The differential ground is essential to cancel out signal artifacts induced by bird movement. Finally, the electrode guide tube array and bottom plate of the microdrive body are coated with a thin layer of paraffin to protect the moving parts of the drive from the dental acrylic.

The design of the drive permits approximately 3.5 mm of movement in electrode depth through the brain. A second dimension of movement control can be added by the use of a lateral positioner coupled to the electrode bundle (see Figure A.1). A modified shuttle is epoxyed to the side of the microdrive body, perpendicular to the electrode bundle and oriented with the long axis of the target brain nucleus. A #0000-160 threaded rod is advanced through the shuttle until it is in contact with the electrode guide tube array. The threaded rod and the associated shuttle are coated with mineral oil to prevent binding to the acrylic used to cement the microdrive onto the skull. An eighth-turn of the threaded rod will shift the position of the electrode bundle by ~ 20 um. This manually operated positioner can be used periodically during the course of experimentation when the column of tissue associated with the current lateral position of the electrodes has become exhausted of isolatable cells. This typically occurs after a few days of motorized recordings throughout the moveable depth of the electrodes. At this point, the electrodes are retracted to their top position, the bird is restrained, and the lateral positioner is advanced by a small amount (20-50 um) suitable to move the electrodes into fresh tissue.

Motor control electronics

The brushless DC motors used in the microdrive have three windings, and are normally driven with three sinusoidal voltage inputs that have a 120° phase difference. Using this approach, a total of 9 wires are required to control three independent motors. An alternate technique was developed that requires only four motor control wires to be used in

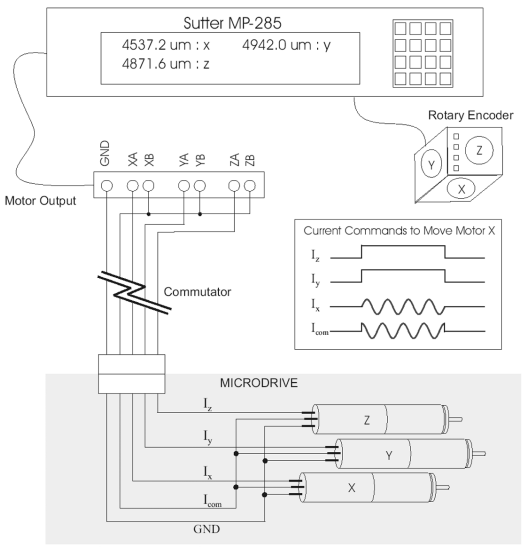

Figure A.3. Schematic of motor connections to the modified Sutter MP-285 controller. Each motor has three connections. These connections are normally driven by three sinusoidal voltage inputs with 120° phase shift. To reduce the number of connections required to drive the three motors, all motors were provided with one common ground and a common sinusoidal input current (I_{com}). The third input was controlled independently for each motor. The Sutter manipulator controller was modified to apply a sinusoidal input current (with a 90° phase shift) only to the motor being driven (e.g., I_x), and a constant bias current to the other inputs (e.g., I_y and I_z) to prevent uncommanded movement of the other motors.

addition to those required for recording neural signals: analog ground, + Vcc for the headstage preamplifier, and the four neural signals (three electrodes and a differential ground reference). In the alternate approach, the motors are driven by two sinusoidal current inputs, one at 0° and one at 90°, as a stepper motor is usually driven. The third connection on each motor is connected to analog ground. The wiring is reduced because all motors share one of these sinusoidal signals (e.g., the 0° signal), referred to as I_{com}, so that when any one motor is 'on', the 'off' motors also have one winding energized (Figure A.3). The second (i.e., 90°) current input is applied only to the motor selected to be 'on'. The 'off' motors are unlikely to turn with only one energized winding, but to prevent any possible spurious rotation, a constant DC current is applied to non-energized winding of the two 'off' motors, locking them in place.

The motor control was implemented using a modified commercial manipulator controller (Sutter Instruments, MP-285). As originally designed, the MP-285 is used to control a stepper-motor-driven, three-axis manipulator. Manipulator movements are computer-controlled in response to commands from a cluster of three rotary-encoded wheels. The embedded computer also keeps track of the current position of the manipulator axes and turns off power to the motors after some delay (t_i) during periods of inactivity. A serial port output allows the depth of the electrodes to be logged by custom-designed computer control

software used to record the neural data. These features make the MP-285 well suited to the control of the three-motor microdrive, with each axis controlling one electrode and motor.

Several modifications of the MP-285 were required. Modifications of the controller firmware were kindly provided by Sutter Instruments (Joe Immel, personal communication). One modification was a re-calibration of position display to reflect the electrode displacement per motor cycle of the motorized microdrive (which is different from the original manipulator). Another modification allows t_i to be user programmed. This delay is set short (<0.5 sec) so that high values of drive current may be used for transient motor movements without thermally overloading the motor.

A simple circuit was added internally to the MP-285 controller to detect command input from the rotary encoder and then perform two functions. First, since analog ground is used also for motor ground, the circuit connects the analog ground line to the controller power supply ground when any motor is activated. When the motors are not in use, the ground is automatically disconnected from the controller power supply to eliminate noise on the electrode signals. Second, the circuit applies the bias 'locking' current to the motors that are not in use. For example, if command input to the x-axis is detected, bias current is applied to the y- and z-axis motors. This design results in the constraint that only one electrode may be moved at a time. Circuit details are available from Sutter Instruments.

Microdrive reproducibility was limited on occasions in which the motors would briefly stall; this problem was minimized by careful attention to the construction process. However, it should be noted that since the microdrive control via the MP-285 is open-loop, any stalling of the motors will produce errors in the estimate of the electrodes depth. The motorized microdrive was found to be quite robust. Across all implants done to date, none of the motors appeared to suffer damage inflicted by the bird. In addition, because the motor subassembly detaches from the microdrive body, the process of reusing the microdrive after an experiment is straightforward. The motor subassembly is detached, the microdrive body is cleaned of acrylic, the motor unit is reattached and the drive is reloaded with electrodes to prepare for the next experiment. The motor or gearboxes occasionally fail for unknown reasons and must be replaced during the reconstruction process.