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Miniature motorized microdrive and commutator system for chronic neural recording in small animals

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Abstract

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 g, including the headstage preamplifier. Use of the motorized microdrive has produced a ten-fold 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. We also describe a motorized commutator that actively tracks animal rotation based on a measurement of torque in the tether.

Keywords: Zebra finch; Chronic recording; Single-unit; Birdsong

1. Introduction

Studies of neural activity in behaving animals are essential to advancing our understanding of brain function. In some cases, head-restrained animals can be trained to perform a behavior of interest, permitting neural recordings to be made with electrodes that are inserted into the brain only during experiments, and then removed (Humphrey, 1970; Reitbock et al., 1981). However, many natural behaviors, such as exploration and locomotion (O'Keefe and Dostrovsky, 1971), song vocalizations (McCasland, 1987), and social interactions, are difficult to evoke in a head-restrained animal. In these cases, small electrodes are chronically implanted within the brain, thereby permitting the animal to move around with relatively little constraint while signals from individual neurons or clusters of neurons are recorded.

There are two broad approaches to chronic neuronal recording: First, electrodes may be surgically implanted into the area of interest and directly secured to the skull (Chapin and Woodward, 1982). This approach has the disadvantage that the recorded signals cannot be refined by moving the electrodes following implantation, but has the advantage of being lightweight and permitting more electrodes to be implanted. In the second approach, electrodes are mounted in a small positioning device, or microdrive, which is secured to the skull during the surgical procedure (Korshunov, 1995; Dave et al., 1999; Venkatachalam et al., 1999). The electrodes are advanced into the brain prior to an experimental session and then positioned within the brain region of interest to obtain useable signals. Fewer electrodes can be implanted with the microdrive technique, but the number of high-quality signals per electrode can be much higher because the electrodes can be moved during experiments, allowing the isolation of many different neurons.

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Chronic recording experiments are often carried out with small animals such as rats, mice (McHugh et al., 1996), or birds (Yu and Margoliash, 1996). As a result, a primary challenge to performing chronic recording experiments is that the apparatus must be compact and lightweight. Rats and mice can carry up to 25 and 5 g on the cranium, respectively, and zebra finches (*Taeneopygia guttata*, a small songbird) can carry up to 2 g. As a result of these weight constraints, the microdrives used in chronic recording have been limited to hand operated devices in which the electrodes are advanced or retracted by turning a small screw on the microdrive while the animal is restrained. Manually operated microdrives have several drawbacks. First, the controllability and repeatability of these microdrives are inferior to the sophisticated motorized manipulators used for acute experiments (Reitbock et al., 1981), which often include three independent axes and computer-controlled positioning (e.g. Sutter Instruments, Novato, CA). The lack of controllability afforded by chronic microdrives also influences the type of electrode commonly used in chronic recording experiments. Microwire electrodes, which give poor single-unit isolation, are favored since they appear to give more stable signals in chronic recordings compared to sharp tungsten electrodes. Unfortunately, in some brain areas, particularly those with small cells or high firing rates, microwire electrodes do not produce useable single-unit signals, even with stereotrodes or tetrodes (McNaughton et al., 1983).

Another difficulty encountered with manually operated microdrives is that the animal may resist restraint during the adjustment procedure, making single-unit isolation difficult. This was found to be a serious limitation to obtaining single-neuron recordings in the zebra finch. In addition, the handling required to manipulate the microdrive dramatically reduces some spontaneous behaviors, such as singing in the zebra finch. In response to these challenges, we have developed a miniature motorized microdrive capable of independent depth control of three electrodes. The entire device is roughly 6 mm in diameter, 17 mm high, and weighs less than 1.5 g including the headstage preamplifier.

Another technical difficulty encountered in chronic recording from freely behaving animals is transmitting the signals from the electrodes to the data acquisition system. This is accomplished either with radio or optical based telemetry systems, or more commonly, with a bundle of fine wires. If the latter technique is used, it is usually necessary to incorporate into the apparatus a commutator, a device that maintains multiple electrical connections while allowing complete rotational freedom of the animal. Commutators are available from a number of commercial sources, and operate by spring contacts sliding on a rotating shaft (Biela, Inc), or pins moving through concentric circular channels of liquid mercury (Dragonfly, Inc.). Mercury commutators have the advantage of requiring a lower torque to rotate but have the disadvantage of supporting fewer channels than the sliding-contact commutators. The torque required to rotate these devices can be difficult for a small animal to produce, and also tends to cause the cable to twist or kink. We also describe here a torque-feedback commutator, in which the rotation of the animal is actively tracked by a motorized commutator.

2. Methods

Subjects were adult male zebra finches (*Taeneopygia guttata*, 100–300 days old), 12–15 g in weight. Birds used in these experiments were selected on the basis of singing prolificacy. Microdrives were assembled as described below. Birds were anesthetized with 1–2% isoflurane and the electrodes were implanted to a depth 500 μm above nucleus RA (robust nucleus of the archistriatum; Vicario, 1991) using stereotaxic coordinates. After several days of recovery, the electrodes were advanced into RA for recording. At the end of each recording session, the electrodes were retracted to a position above RA. Retracting the electrodes to a position above the target nucleus produces slight positional changes in the location of the electrode tips such that new cells were encountered on subsequent recording sessions. At the conclusion of the experiments, the birds were deeply anesthetized with urethane (2 g/kg) and electrolytic lesions were made (10 μA for 15 s) to allow verification of electrode position. The animals were then perfused with saline followed by 4% paraformaldehyde. The brains were subsequently removed, and the lesion sites were identified using standard histological techniques. The care and experimental manipulation of these animals were in accord with the guidelines of the National Institutes of Health and have been reviewed and approved by the local Institutional Animal Care and Use Committee.

2.1. Motorized microdrive construction

The basic design of the microdrive mechanism is derived from one described previously (Venkatachalam et al., 1999). Electrodes are held by threaded shuttles that travel along small threaded rods (Fig. 1). Machine drawings of all components are shown in Fig. 2. 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 a miniature synchronous motor (see Appendix A). The motors are 1.9 mm in diameter and weigh approximately 100 mg. The motorized microdrive consists of three main subassem-

blies. The microdrive/connector assembly is constructed first, followed by the motor assembly, and finally, the electrode assembly (See Fig. 3B–D). In the following subsections we describe the construction of each of these components in detail.

2.1.1. 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 connector is positioned as desired. The ground lead of the motor connector is soldered to the hook-up wire on the opposite side and the connector is 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).

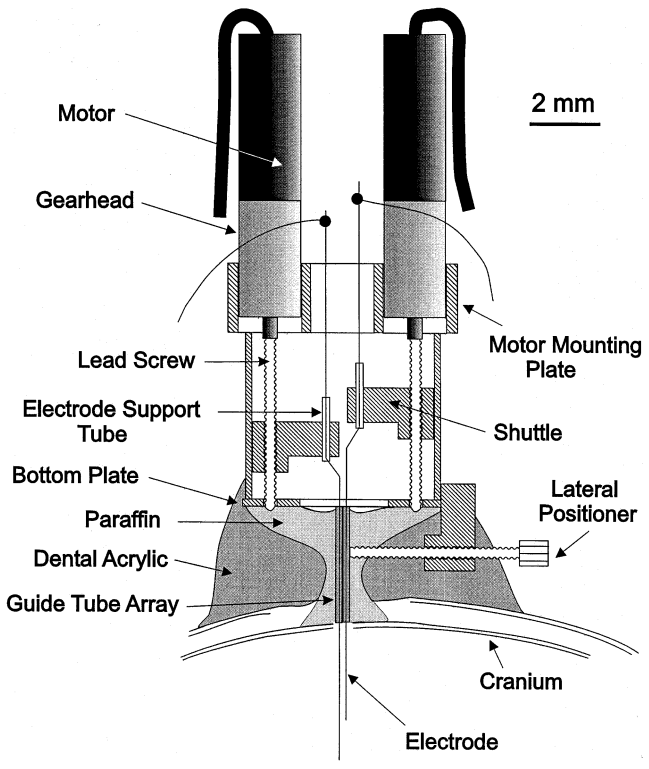

Fig. 1. Overview of motorized microdrive. Each electrode is held by a moveable shuttle that can be advanced and retracted by rotating a threaded lead screw. The shuttle moves in a cylindrical channel within the microdrive body. The lead screw is rotated by a small brushless DC motor that weighs ≈100 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.

2.1.2. Motor assembly

The motors are prepared and the threaded rods are connected to the motor shafts as described in Appendix A (see Fig. 3A). 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 μNm) 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 shuttle movement. Careful alignment of the motor mounting plate on the microdrive body is required (see Fig. 3B 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 in constructing 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 Fig. 3B). It is wise to double-check for smooth shuttle 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 Appendix B. Once this process is complete, the tops of each motor are linked to each other with a small bridge of Torr Seal (this more firmly secures the positions of the motors in the motor mounting plate; refer to Fig. 3B and F).

2.1.3. 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 Fig. 3D). 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

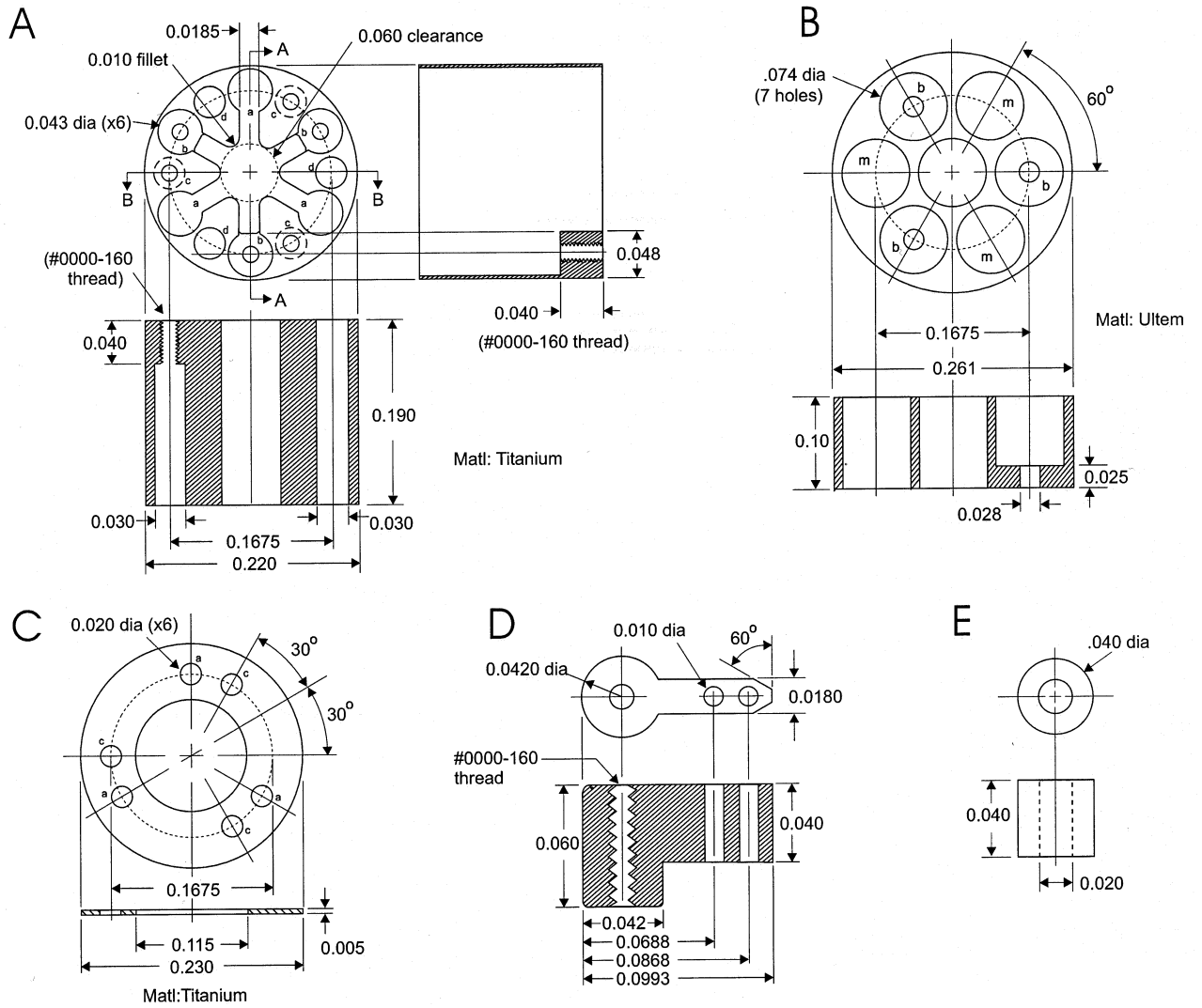

Fig. 2. Machine drawings of microdrive components. (A) Microdrive body. The shuttles move in the three channels marked 'a'. The motor mounting plate mates to the right-hand surface of the section drawing A–A, and is attached using the threaded holes marked 'b'. The bottom plate mates to the top surface of the section drawing B–B and is attached using the threaded holes marked 'c'. Holes marked 'd' (0.030 dia) are for weight reduction only and may be omitted. (B) Motor mounting plate. The motors are press fit into the holes marked 'm'. Holes marked 'b' align with the 'b' holes in the microdrive body. (C) Bottom plate. Holes marked 'a' and 'c' align with the same labels in the microdrive body. (D) Shuttle. (E) The shaft coupling is used to attach the threaded rod to the motor shaft.

provide mechanical support for the electrodes. Three electrodes ($\approx$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 μm 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 5-min 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 the animal ground. A differential ground wire (0.001″ teflon coated platinum–iridium wire) is attached to the electrode bundle and aligned so it protrudes $\approx$700 μm 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 Fig. 1). A modified shuttle is epoxyed to the side of the microdrive body, perpendicular to the

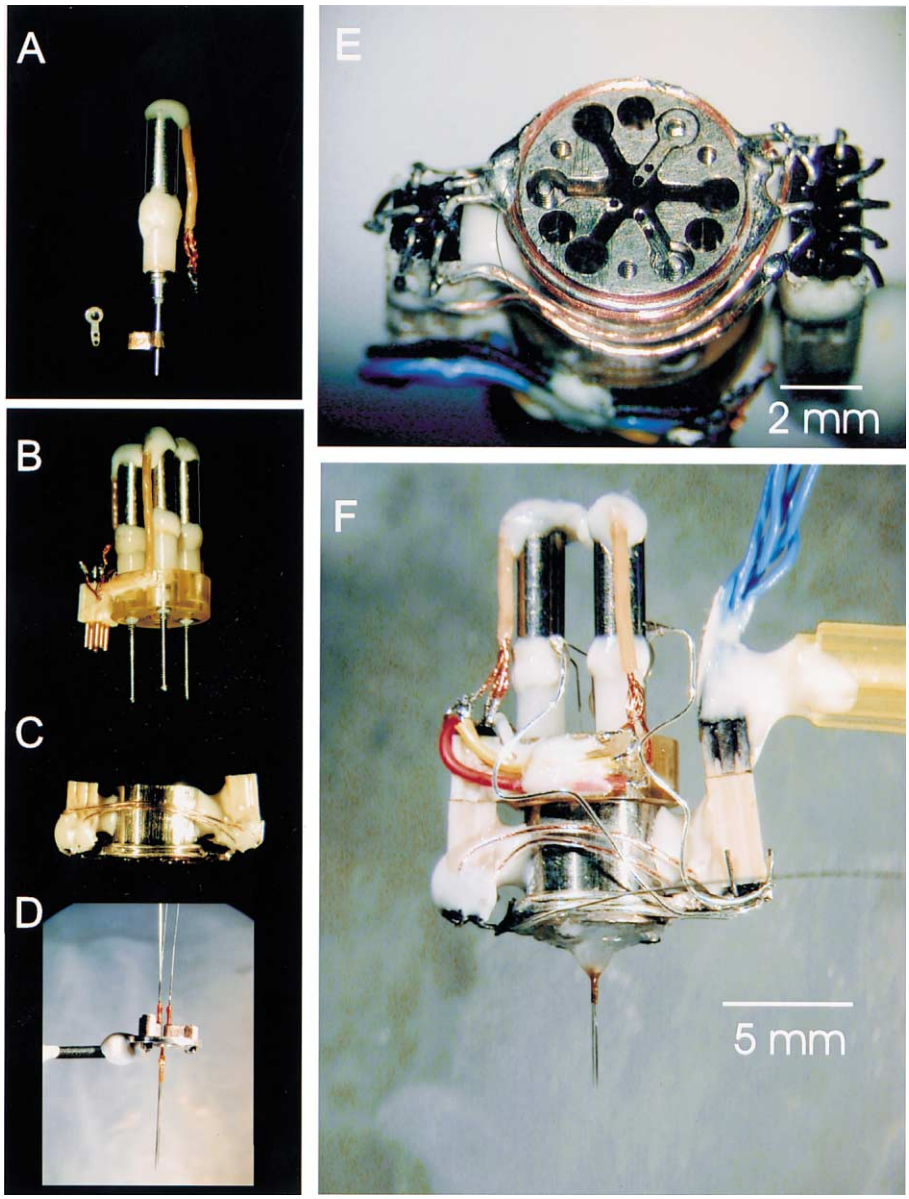

Fig. 3. Photographs of microdrive subassemblies and completed microdrive. (A) A single micromotor with attached lead screw and shuttle. Rotations of the motor shaft produce a translation of the shuttle. To the left is a top view of an individual shuttle. There are three main subassemblies for the microdrive. (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 into the shuttles 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. The electrodes were not inserted into the shuttles for this photograph. (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.

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 μm. 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 μm) suitable to move the electrodes into fresh tissue.

2.1.4. 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 nine wires are required to control three independent motors. An alternate technique was developed that requires only four motor control wires to be used in 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 (See Fig. 4). 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 (τ_i) during periods of inactivity. A serial port output allows the depth of the electrodes to be logged by the 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.

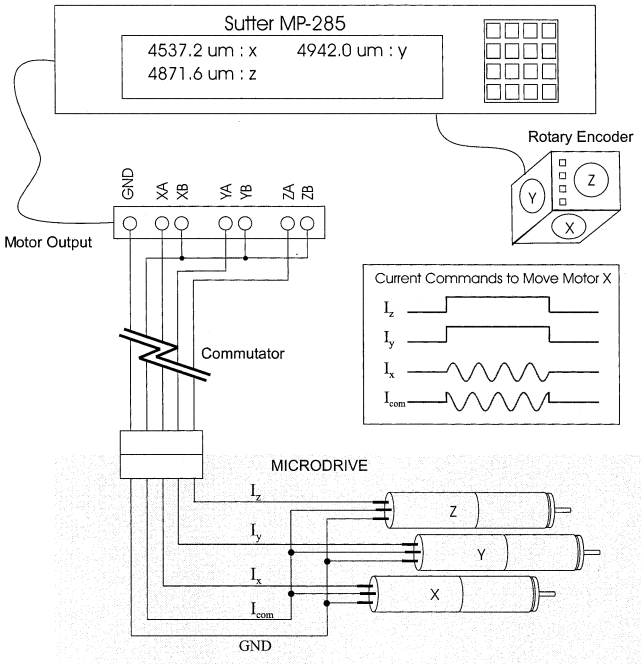

Fig. 4. Schematic of motor connections to the modified Sutter MP-285 controller. Each motor has three connections. These connections are normally driven by three sinusoidal voltage inputs with 120° phase shift. To reduce the number of connections required to drive the three motors, all motors were provided 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.

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 τ_i to be user programmed. This delay is set short (< 0.5 s) 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.

2.2. Torque-feedback commutator

For experiments with small animals, such as mice or zebra finches, the torque required to rotate the commutator must be kept as small as possible, not only to reduce the torque that the animal experiences as it moves around, but because the lightweight wires most often used for this purpose are poor transmitters of torque. For instance, the 20-cm long braided cable of ten wires (Cooner Wire, Inc. CZ-1187), used in the zebra finch experiments described here, transmits only 50 μNm of torque with one complete twist, after which the formation of a loop becomes likely. This is several hundred times smaller than the 10–20 mNm of torque required to rotate our 12-channel commercial mercury commutator (Dragonfly, Inc.).

One can dramatically reduce the torque seen by the animal and the cable by directly measuring the torque applied at the base of the commutator, and using this signal to control a motorized rotation of the commutator. Fig. 5 shows the overall design and performance of this system. The commutator is driven by a small, brushless DC servomotor (MicroMo Electronics, 2036-012B, with a 134:1 planetary gearhead). The motor speed and direction are controlled, via feedback electronics, by a signal generated from a torque sensor placed at the top of the animal tether (Fig. 5A). If the torque sensor indicates a rotation of the cable in the clockwise direction, then the commutator is driven clockwise to reduce the torque, and likewise for counterclockwise rotations.

2.2.1. Torque sensor

The torque sensor is essentially a swiveling link in the cable with a measurement of the rotation angle. The signals at the top (fixed) connector (Dale MMP22GS-14) are directly connected to the bottom (rotating) connector (Omnetics, Inc.) with fine wires. The wires pass through a short stainless steel tube to which the rotating connector is glued. The tube is seated in a ball bearing, allowing the bottom connector to rotate (Fig. 5A). As shown in Fig. 5B, a small Samarium–Cobalt disc magnet (0.2″ dia, 0.075″ thick) is glued to the tube; rotations of the magnet are sensed with a Hall generator (LakeShore Cryogenics, Inc., HGT-2100). The

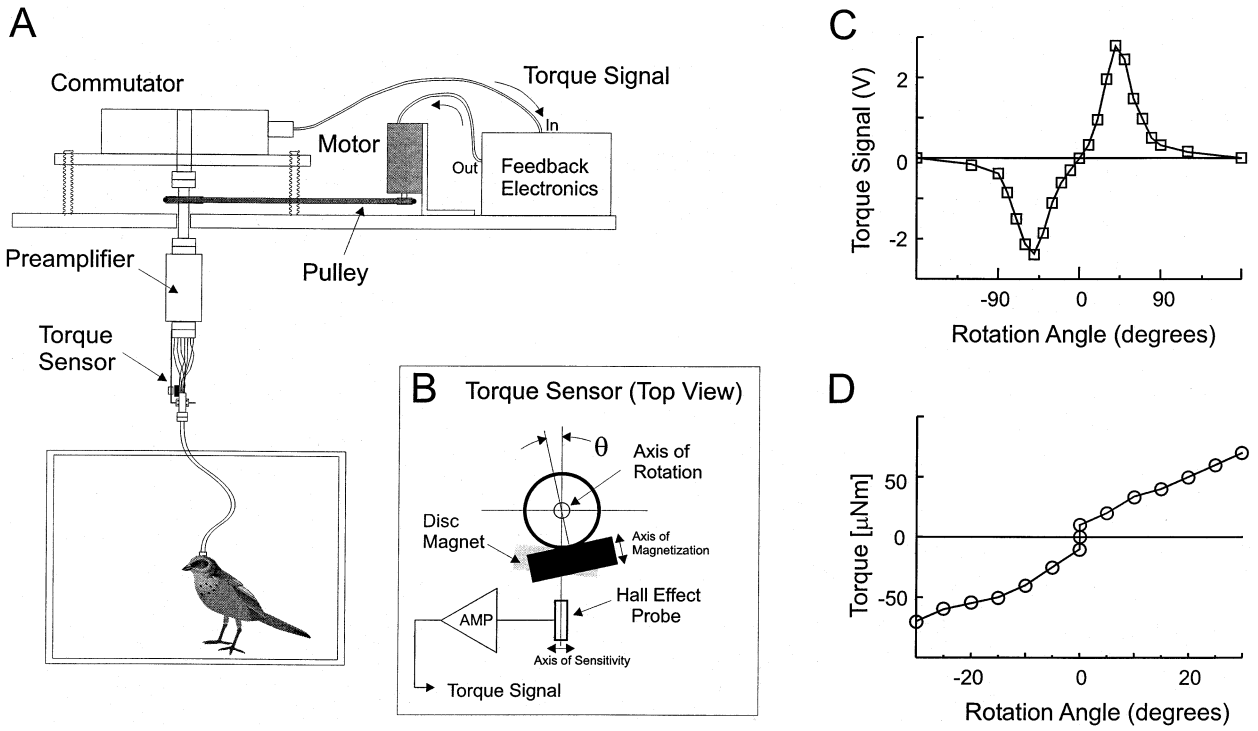

Fig. 5. Overview-drawing of torque-feedback commutator. (A) A commercial 12-channel commutator is connected to a brushless DC motor. A torque sensor is attached at the top of the wire bundle carrying electrical signals to and from the animal. The torque signal is returned through the commutator to the feedback electronics, which causes the motor to rotate the commutator so as to reduce the torque detected on the wire bundle. (B) The torque sensor operates by using a Hall effect probe to detect the rotation of a magnetic disc. (C) The magnet and the probe are oriented so that the signal from the Hall probe is zero when the magnet is centered and has an antisymmetric profile as the magnet is rotated to either side. (D) Measurement of the torque required to rotate the torque sensor. Static friction produces a response threshold of ≈ 10 μNm.

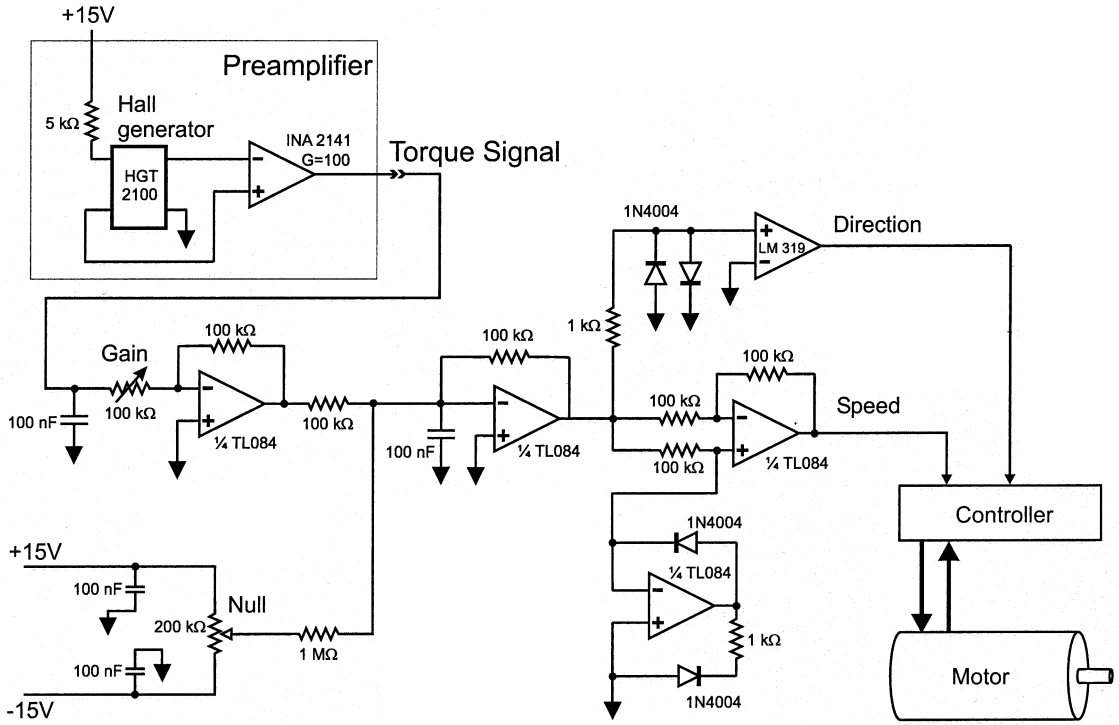

Fig. 6. Circuit diagram of torque feedback electronics. The circuit consists of several components: The magnet rotation is detected with a Hall generator and preamplified with a gain of 100. The signal is further amplified with a variable gain, and summed with an adjustable voltage to permit offsets to be nulled. The result is sent to an absolute value circuit to determine the required speed; the direction of rotation is determined using a comparator. The speed and direction signals are the inputs to the commercial motor controller.

voltage from the Hall generator is amplified and resulting torque signal is routed through the commutator to the feedback electronics and the motor controller. The disc magnet and Hall generator are oriented so that the torque signal is an antisymmetric function around some preferred angle (Fig. 5C), which should be close to the neutral, no-torque position of the rotating connector. The best results were found with the magnet attached at the top of the stainless steel tube with the plane of the disc parallel to the back of the torque sensor chassis. The surface mount Hall generator was glued to the back of the chassis near the center of the magnet with the plane of the chip perpendicular to the plane of the magnet (Fig. 5B).

Since rotations of the animal and cable are at low frequencies (<1 Hz), the overall performance of the motorized commutator is dominated by the static performance of the torque sensor. The torque required to produce various rotations of the torque sensor was measured and the results are shown in Fig. 5D. One point of interest is that there is a static component of friction, such that roughly 10 μNm of torque are required to produce any deflection of the torque sensor. Above this torque, there is a roughly linear relationship of torque to rotation angle. (Not shown in the figure is a roughly 10° hysteresis in the torque–rotation relationship produced by the static friction). The resulting torque sensitivity limit is roughly 10 μNm, with a slope of 0.5° rotation per μNm of additional torque. (1 μNm is equivalent to the weight of a 10 mg object acting on an arm of 1 cm.) Note that the torque sensor is more than 1000 times more responsive to static torque than the commercial mercury commutator.

2.2.2. Torque-feedback electronics

The feedback electronics is comprised of three components: the Hall generator and preamplifier (located below the commutator), the feedback amplifier, and the motor controller (Fig. 6). The Hall generator (LakeShore Cryogenics, HGT-2100) is biased with 2.5 mA current and the generated differential voltage is amplified with a Burr-Brown INA 2141 instrumentation amplifier with a gain of 100 to produce the torque signal. This signal is passed through the commutator to the feedback amplifier. The motor controller is a commercial brushless DC servomotor controller with a speed command and direction input (MicroMo Electronics, Inc. Type BLD-3502).

The feedback amplifier consists of an adjustable gain stage, a summation for an offset null, and a comparator and absolute value circuit to drive the motor controller. Since the amplified torque signal (representing a rotation angle) drives a speed input, the motor and controller act as an integrator, producing infinite gain at

DC and reducing the static torque error to zero. The gain of the feedback amplifier is set to produce a clockwise (viewed from the top) commutator rotation rate of 720°/s with a +1 V torque signal (and a CCW rotation for negative torque signals). The null control is adjusted so there is no commutator rotation with the cable removed from the torque sensor (Fig. 7).

3. Results

The motorized microdrive and torque-feedback commutator were used to record from neurons in premotor nucleus RA 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. As the weight and center-of-mass of the motorized microdrive are not substantially different than those of the manually operated microdrive, there was no obvious difference in the quantity of songs produced by birds using either method. However, 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. Furthermore, singing behavior seemed unaffected by the microdrive, tether, and commutator system. The temporal and spectral pattern of syllables retained the hallmark stability seen in normal adult zebra finches.

Neural recordings obtained in singing zebra finches with the motorized microdrive were of the same quality as those seen in head-fixed anesthetized animals. In three birds, we recorded 87 single units, 40 pairs, and five triplets. Fig. 8 shows an example of a triplet recording during singing. Because of the thread size used to drive the shuttles, and the computer-control of the MP-285, we were able to move the electrodes with a positional resolution of less than 1 μm. 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 reach 10 mV in amplitude. Good single-unit isolations with the motorized microdrive were often found to be stable for over an hour, since degradation in signal quality could be compensated for by readjustment of the electrode position. In contrast, readjusting the manually positioned electrodes for signal degradation was virtually impossible due to

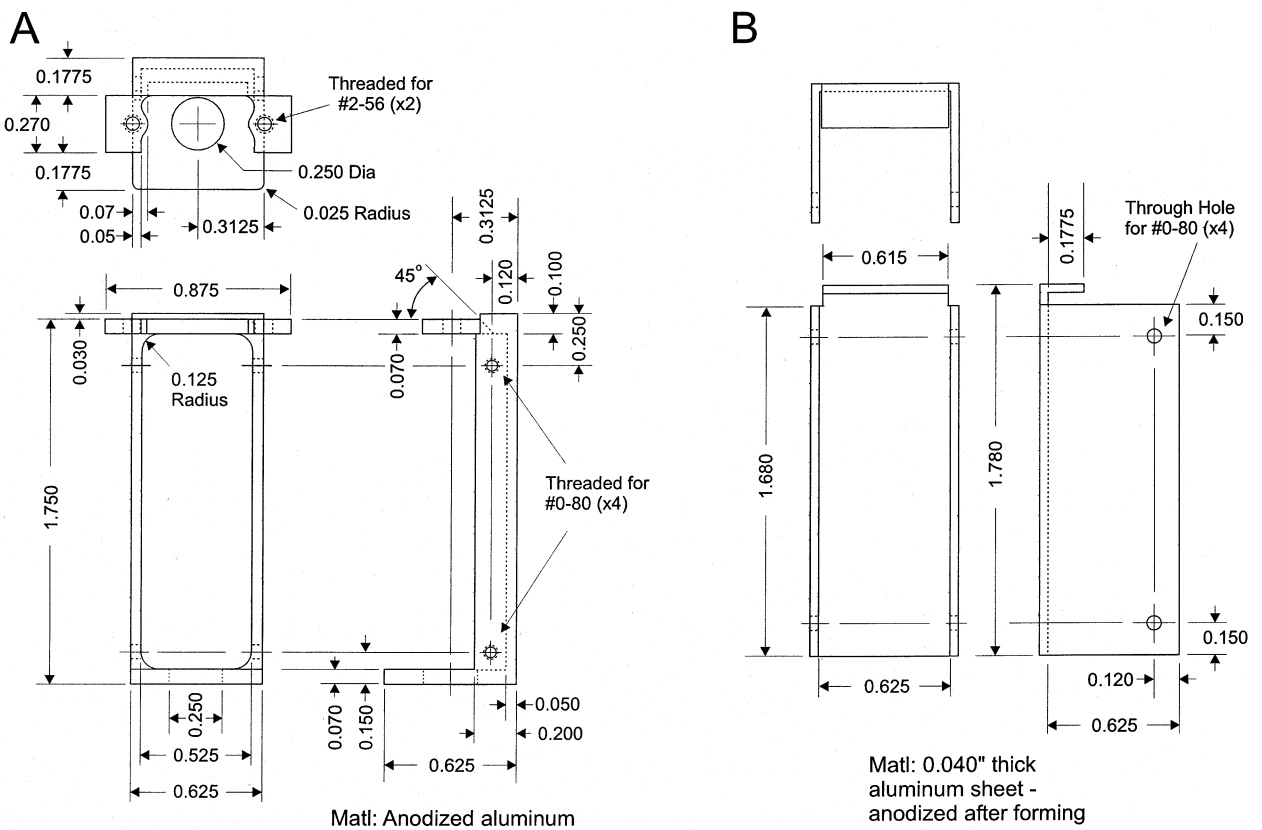

Fig. 7. Mechanical drawings of the torque sensor chassis. (A) The connector to the preamplifier and commutator mounts at the top of the front view drawing (bottom left) and a ball bearing (0.25″ O.D., 0.125″ I.D.) is glued into the hole at the left. (B) The cover is placed over the torque sensor after assembly.

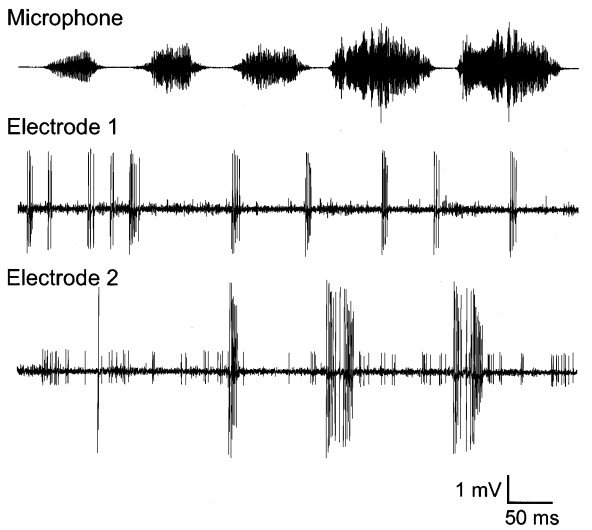

Fig. 8. Three neurons recorded simultaneously in song control nucleus RA of the zebra finch. Also shown is the simultaneously recorded song vocalization. There is a single neuron (≈ 3 mV) on electrode 1, and two separable neurons (≈ 6 and ≈ 1.5 mV) on electrode 2.

the need to catch and restrain the bird before initiating this process.

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 motors or gearboxes occasionally fail for unknown reasons and must be replaced during the reconstruction process.

4. Discussion

The advantages of using a motorized microdrive are seen clearly in the experiments to record simultaneously from multiple neurons in nucleus RA in singing zebra finches. 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 five triplets recorded in three birds with the motorized drive.

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. First, 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, since the bird would often bump into the side or top of the cage as it was released. Attempts to reduce the physical activity by releasing the bird in the dark resulted in little improvement. Even in the absence of sudden physical activity, cells were often lost during the release, perhaps as a result of postural shifts or blood pressure changes.

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 sometimes be isolated by dialing in a single-unit on one electrode and then dialing in a single-unit on another electrode. Often though, mechanical interaction between these closely spaced electrodes (150–200 μm tip separation) resulted in the need to switch back and forth between the two electrodes to optimize the signals. A few iterations of this process routinely yielded simultaneous units of high quality. 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.

Although the design of the motorized microdrive has been optimized for use with small birds, it would also be appropriate for chronic neurophysiological studies in behaving mice. In addition, the entire instrument can easily be scaled up in size for use with rats and larger animals. 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.

5. Note added in proof

We have recently designed a version of the motorized microdrive that may be better suited to recording in mice. This device is slightly larger (~ 3 g) and uses larger, more robust motors (www.smoovy.com, RMB, Inc. SPH39003). Design details are available from Advanced Machining and Tooling, Inc.

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Appendix A. Preparation of motors

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 (Fig. 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 ± 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 mate 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 pair 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.

Appendix B. Motor connections

Although there are only three connections to each motor, there are 15 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 15 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 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.

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